VASCULAR REGULATION OF NEOCORTICAL INTERNEURON PRODUCTION

A Dissertation

Presented to the Faculty of the Weill Cornell Graduate School

of Medical Sciences

in Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

by

Xin Tan

August 2015

VASCULAR REGULATION OF NEOCORTICAL INTERNEURON PRODUCTION

Xin Tan, PhD

Cornell University 2015

The neocortex contains glutamatergic excitatory neurons and GABAergic inhibitory interneurons. Extensive studies have revealed substantial insights into glutamatergic neuron production. However, our knowledge of the generation of GABAergic interneurons remains limited. Here we show that periventricular blood vessels regulate progenitor behaviour and neocortical interneuron production. Distinct from those in the dorsal telencephalon, radial glial progenitors (RGPs) in the ventral telencephalon responsible for producing neocortical interneurons progressively grow radial glial fibres anchored to periventricular vessels. Disrupting this robust progenitor-vessel interaction by selective removal of INTEGRIN β1 in RGPs leads to a decrease in progenitor division, a loss of PARVALBUMIN-, but not SOMATOSTATIN-, expressing interneurons, and defective synaptic inhibition in the neocortex. Moreover, endothelial cell-specific deletion of stem cell factor results in similar defects in progenitor division and neocortical interneuron production. These results highlight a prominent interaction between RGPs and vessels crucial for proper production and function of neocortical interneurons.

BIOGRAPHICAL SKETCH

Xin Tan was born on October 4th, 1987 in Chongqing, China. Since her high school, biology has been her favorite class. Always fascinated by the nature of the living, she went on to learn more about life science and majored in Biotechnology at the Zhejiang University, Hangzhou, where she gained her Bachelor's degree of Science. During sophomore, she joined Dr. Weijun Wang's lab investigating the role of AMPK in the post-diapause development of *Artemia franciscana*, a primitive crustacean capable of surviving hypoxia for years. After a year working on development, she chose to explore a new research field – molecular and cellular biology - in Dr. Tianhua Zhou's lab, which focused on the cell cycle and cytoskeleton. There, she proposed her own hypothesis and shaped the development of her own project – studying the role of NudC, a dynein-associated protein essential for cytokinesis, as a co-chaperone of HSP90 for LIS1. It was those benchwork experiences that further confirmed her passion and determination to continue the scientific training in the graduate school.

In the fall of 2009, Xin moved from China to United States, and enrolled in the BCMB graduate program at Weill Cornell Graduate School of Medical Sciences in New York City. That summer, she came across the book <In Search of Memory> by the Nobel Laureate Dr. Eric Kandel, which ignited her profound interest in neuroscience. In the first year of the graduate studies, she did rotations in three neuroscience labs and decided to do her dissertation work in Dr. Songhai Shi's lab at Memorial Sloan Kettering Institute, where she investigated the dynamic interaction between the two major cellular systems in the brain – the vascular and the neural systems, as well as the vascular regulation of neocortical interneuron production.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my mentor, Dr. Songhai Shi for his exceptional support and guidance through my Ph.D. His high standards on research inspired me to be stringent on my own work; his encouragement for never giving up led me through many difficult and challenging experiments; his generous support in the past five years, especially during my most stressful time, has been invaluable for me to carry on. It has been a fantastic journey not only to make exciting scientific discoveries, but also to discover myself and become a better person.

I would like to thank all the Shi lab members, past and present, for your constant help and extensive scientific discussions. Special thanks to Dr. She Chen and Dr. Ryan Insolera for teaching me lab techniques and helping me navigate the lab during my rotation, Dr. Lei Li and Zhizhong Li for the generous supply of viruses that moved my experiments faster, Dr. Xinjun Zhang and Wei Shi for your contribution to my dissertation work, and Dr. Keith Brown for starting the interneuron saga in the lab. A special thank you also goes to Lily Erdy for her help with all the small and big things through my Ph.D.

I want to acknowledge my thesis committee members, Dr. Betsy Ross and Dr. Julia Kaltschmidt, for their support on my thesis project. I was especially lucky to have the guidance of some of the most well-respected scientists in the world.

I would like to thank all my fellow classmates for making me feel not alone when I just moved to this foreign country and my friends (inside and outside the graduate school) for your invaluable support and help on all sorts of things through the years.

Last but not least, I would like to acknowledge my family for their unrestrained support. Thanks to the one person that I can never appreciate enough for everything you have given me – my Mom. Thanks for letting me travel so far away to pursue my dreams, and for always believing in me. I would not be where I am today without your unconditional giving and guidance.

TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER 1:

INTRODUCTION

1.1 Chapter overview

The cerebrum is the largest part of the brain, containing the cerebral cortex and several subcortical structures, including the hippocampus, basal ganglia and olfactory bulb. It is an extremely complex biological entity and is responsible for the higher brain functions of the central nervous system (CNS). The corresponding embryonic structure from which the mature cerebrum develops is called the telencephalon. In mammals, the dorsal telencephalon (pallium) develops into the cerebral cortex, one of the most pivotal structures and critical for memory, language, sensory perception, and consciousness, whereas the ventral telencephalon (subpallium) becomes the basal ganglia, which comprise multiple subcortical nuclei associated with various functions including control of motor movement and emotions.

The neocortex (also known as the isocortex or neopallium) is the newest but the most developed part of the cerebral cortex to evolve. As a result of pronounced expansion and development during evolution, the human neocortex accounts for nearly three-quarters of the brain volume and is responsible for our extraordinary cognitive functions (Mountcastle, 1997). A prominent trait of the neocortex is its complex yet well-organized cellular architecture, the formation of which relies on the production and positioning of its diverse neuronal populations. The mammalian neocortex is composed of six layers, which differ in neuronal cell type composition, cell density, and connectivity. The neurons of the neocortex are thought to be functionally organized into vertically arrayed radial units or columns that span the cortical layers and consist of two major classes: glutamatergic excitatory cells (pyramidal and spiny stellate neurons) and GABA (γ-aminobutyric acid)-ergic inhibitory interneurons (Hensch, 2005). Excitatory neurons constitute the vast majority (~70-80%) of neocortical circuit neurons. They project their axons within the cortex and to other distant brain regions generating cortical output. On the other hand, inhibitory interneurons are a diverse

group of local circuit cells responsible for providing a rich variety of inhibitions that shape the output of functional circuits. The proper function of neocortex critically depends on the production and positioning of a correct number of excitatory and inhibitory neurons, which largely occur during the embryonic stages (Parnavelas, 2002).

With the advent of improved fate-mapping tools, including genetically engineered mice, excitatory and inhibitory neurons were found to arise from different developmental lineages. In rodents, the progenitor cells of these two neuronal populations are fully segregated. Excitatory neurons are generated in the proliferative zone of the developing dorsal telencephalon (pallium) where they radially migrate into the cortical plate (Anderson et al., 2002; Anderson et al., 2001; Rakic, 1978). This region ultimately develops into the neocortex and hippocampus. In contrast, most neocortical interneurons are generated in the developing ventral telencephalon (subpallium), and migrate tangentially over long distances to reach the neocortex, where they co-assemble with excitatory neurons into functional circuits. The ventral telencephalon is composed of the ganglionic eminences (GEs) and the preoptic area (PoA), which are transitory structures that eventually give rise to the globus pallidus, striatum, and amygdala (Anderson et al., 1997; Batista-Brito and Fishell, 2009; Fogarty et al., 2007; Gelman and Marin, 2011; Gelman et al., 2009; Guillemot, 2005; Marin and Rubenstein, 2003; Wonders and Anderson, 2006). Disruption of the developing GABAergic neocortical inhibitory network has been implicated in several neurological disorders in humans, including schizophrenia, epilepsy, and autism.

Besides the neural system, the other major cellular component of the developing telencephalon is the vascular system. Prior to the neurogenesis, angiogenesis has started to propagate and expand the vascular network within the telencephalon (Hogan et al., 2004). The dynamic interaction between these two systems has been implicated to be crucial for both neurogenesis and angiogenesis (Devenport and Brown, 2004; Gerhardt et al., 2004; Vasudevan and Bhide, 2008).

In this chapter, I will present an overview of the development of both the neural

and vascular systems in the telencephalon, and the dynamic interaction between these two.

1.2 Development of the dorsal telencephalon and excitatory neurons

In the developing vertebrate nervous system, the neural tube is the embryo's precursor to the central nervous system (CNS). Early in development, the neural plate is formed along the dorsal midline of the ectodermal sheet, and folds into a tubular structure called the neural tube, a process known as neurulation. The most anterior region of the neural tube gives rise to the forebrain (prosencephalon) which consists of the diencephalon and telencephalic vesicles (Martynoga et al., 2005). The telencephalon is divided into two major regions: the dorsal and ventral telencephalon. Excitatory neurons in the neocortex are produced in the dorsal telencephalon (i.e. the developing neocortex) and they migrate radially from the birth place to constitute the future neocortex (Malatesta et al., 2003; Marinkovich, 2007; Noctor et al., 2001; Noctor et al., 2004; Rakic, 1988b). On the other hand, most if not all inhibitory interneurons are generated in the ganglionic eminence (GE) of the ventral telencephalon and they migrate tangentially over a long distance to reach the neocortex (Anderson et al., 1997; Miyoshi et al., 2007; Xu et al., 2004) **(Figure 1.1)**.

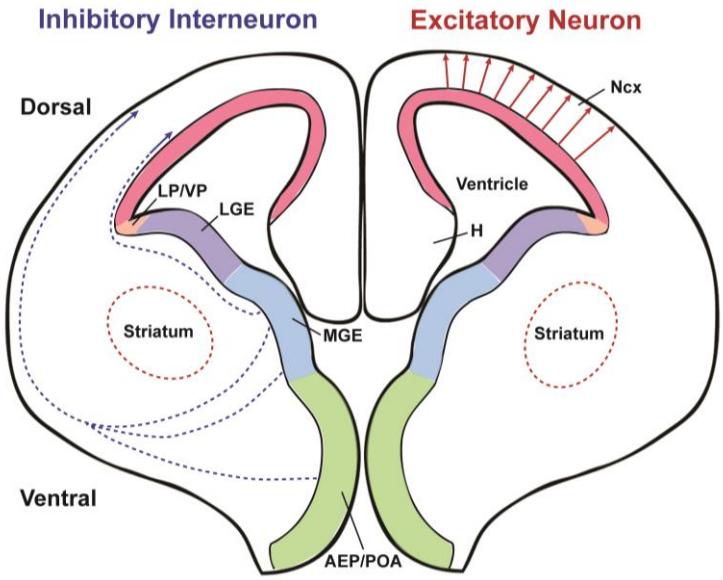


Figure 1.1: Distinct origins of excitatory neurons and inhibitory interneurons in the developing mouse neocortex. Excitatory neurons are generated in the proliferative zone of the dorsal telencephalon and then migrate radially to the cortical plate (red). In contrast, inhibitory interneurons are produced in the proliferative zone of the ventral telencephalon, especially the MGE and AEP/POA, and migrate tangentially to reach the neocortex following two major routes (blue). The colored regions indicate the proliferative zones across the embryonic brain expressing different transcription factors that are essential for proper neurogenesis of distinct neuronal populations. LGE: lateral ganglionic eminence; MGE: medial ganglionic eminence; POA: preoptic area; AEP: anterior entopeduncular area; LP: lateral pallium; MP: medial pallium; Ncx: neocortex; H: Hippocampus. Adapted from (Tan and Shi, 2013).

1.2.1 Neurogenesis of excitatory neurons

The neocortical primordium emerges from the dorsal telencephalic neuroepithelium composed of a single layer of neural precursor cells (NPCs), also referred to as neuroepithelial cells (NE), which are the precursors of all future neurons (Gotz and Huttner, 2005). These cells are defined by their epithelial features, including tight and adherent junctions in the apico-lateral plasma membrane. Additionally, NE cells are multipotent progenitors that express Nestin, an intermediate filament in mitotically active neural cells (Aaku-Saraste et al., 1996; Gotz and Huttner, 2005; Zhadanov et al., 1999). In the early stages of embryonic development, most NE cells proliferate through symmetric cell divisions – two NE cells are produced at each

division, thereby expanding the population of founder cells that will ultimately produce the CNS including the neocortex. Retrovirally-mediated lineage tracing demonstrated that many neuroepithelial cells are multi-potent progenitor cells (Williams and Price, 1995). They are capable of generating the first group of neurons in the neocortex and radial glial progenitors (RGPs), the major neural progenitor cells responsible for producing neurons and glia. In the mouse neocortex, the transition of NE cells to RGPs appears to occur around embryonic day 9.5-10.5 (E9.5-E10.5). This transition is characterized by the initiation of the expression of astroglial markers such as astrocyte-specific glutamate transporter (GLAST) and brain lipid binding protein (BLBP) (Hartfuss et al., 2001; Malatesta et al., 2003), and an alteration in tight junctions including loss of Occludin (Aaku-Saraste et al., 1996; Mollgoard and Saunders, 1975).

RGPs are a transient cell type in the developing brain and account for a major population of progenitor cells that give rise to neurons and glia in the neocortex and other brain structures. They are defined by their characteristic radial bipolar morphology and their astroglial properties. These long bipolar cells expand across the entire thickness of the developing neocortex with a long basal radial process pointing to the pial surface, a short apical ventricular endfoot reaching the ventricular zone (VZ) surface, and the soma located in the VZ (Bentivoglio and Mazzarello, 1999; Cameron and Rakic, 1991). During the early stages of neurogenesis, RGPs largely divide symmetrically to produce two RGPs after each division (Miyata et al., 2001; Miyata et al., 2004; Noctor et al., 2004). At later stages, they predominantly undergo interkinetic nuclear migration and divide asymmetrically at the ventricular zone surface to self-renew and, at the same time, to generate a differentiating daughter cell that is either a post-mitotic excitatory neuron or an intermediate progenitor cell (IPCs) (Bultje et al., 2009; Chenn and McConnell, 1995; Kosodo et al., 2004; Miyata et al., 2004; Noctor et al., 2001; Noctor et al., 2004; Noctor et al., 2008) **(Figure 1.2)**. IPCs accumulate in the subventricular zone (SVZ), a second proliferative region that lies above the VZ, where they divide symmetrically to generate two postmitotic neurons

(Haubensak et al., 2004; Miyata et al., 2004; Noctor et al., 2004). IPCs can also divide symmetrically to produce a pair of progenitors and each could subsequently generate a pair of neurons (Haubensak et al., 2004; Noctor et al., 2004). The generation of neocortical neurons through IPCs would therefore significantly increase the number of neurons produced by individual RGCs. Hence, it has been postulated that the abundance of IPCs may contribute to the evolutionary expansion of the neocortex (Martinez-Cerdeno et al., 2006). Notably, IPCs are molecularly distinguishable from RGCs by their expression of the transcription factors *Eomes* (Englund et al., 2005), *Cux1/Cux2* (Nieto et al., 2004; Zimmer et al., 2004), and *Satb2* (Britanova et al., 2005).

Individual RGPs must go through multiple rounds of asymmetric cell division to generate a correct number of excitatory neurons in the neocortex (Noctor et al., 2001; Rakic, 1988a; Yu et al., 2009). Proper control of neurogenesis is dependent upon Notch signaling (Lewis, 1998). Once activated by its ligands (e.g., Delta1) expressed by neighboring cells, Notch undergoes proteolysis and the resulting Notch intracellular domain translocates into the nucleus in RGPs. Notch intracellular domain acts as a transcriptional activator that turns on downstream genes including BLBP, and the transcriptional repressors Hes1 and Hes5 that suppress neurogenesis thereby maintaining the progenitor pool by inhibiting neuronal differentiation (Yoon and Gaiano 2005). Inactivation of Notch induces neuronal differentiation and depletes the neural stem cell population (Bertrand et al., 2002; Campos et al., 2001; Kageyama et al., 2008; Kopan and Ilagan, 2009; Ross et al., 2003).

Recently, another distinct type of self-renewing progenitor cell – the outer (sub)ventricular zone (OSVZ) progenitors (also called basal RGPs) – was discovered in developing human and ferret neocortices (Fietz et al., 2010; Hansen et al., 2010; Marthiens et al., 2010; Nikolova et al., 2007). Although they originate from and behave similar to RGPs, OSVZ progenitor cells retain only the basal process and lack the apical process necessary for anchoring to the VZ surface. They were initially recognized as a special population of neural progenitor cells as an evolutionary

consequence of neocortex expansion. Strikingly, a very small population of outer VZ (OVZ) progenitor cells, similar to OSVZ progenitor cells, has of late been observed in the developing mouse neocortex (Marinkovich, 2007; Shitamukai et al., 2011). They are likely generated by rare oblique RGP divisions (Shitamukai et al., 2011) **(Figure 1.2, see bRGC)**.

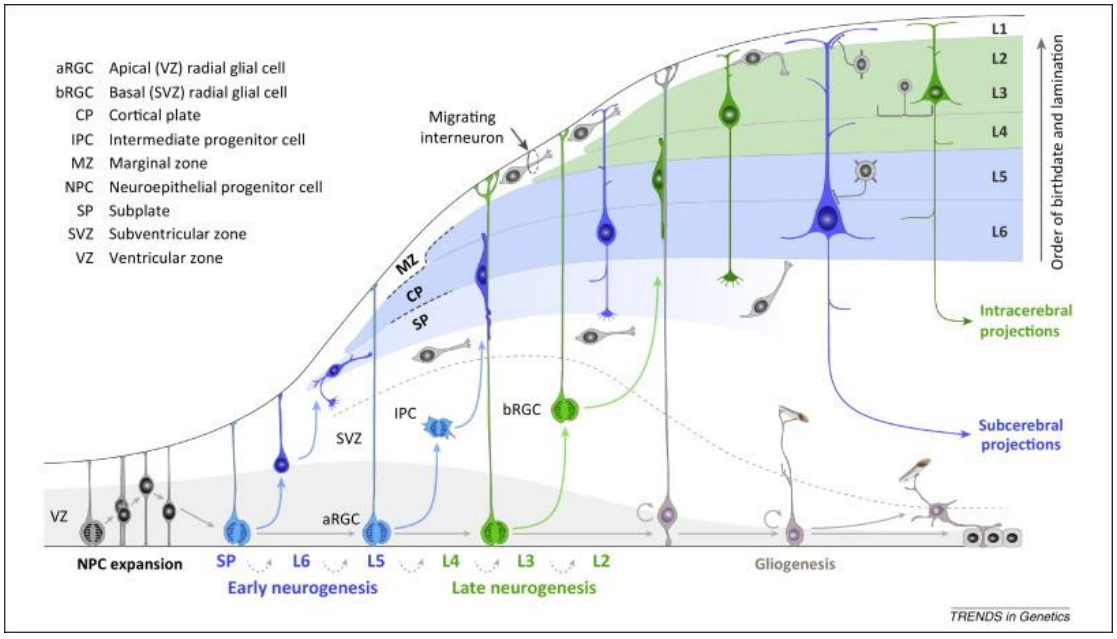


Figure 1.2: Generation, migration and differentiation of excitatory neurons in the mouse neocortex. Neuroepithelial cells (NPCs) undergo symmetric cell division to produce an initial pool of cortical progenitors that later transform into apical radial glia cells (aRGCs, the same as RGPs). Neocortical aRGCs line the ventricular zone from where they extend a long radial process stretching to the basal surface. aRGCs begin asymmetric cell division to generate another aRGC and a nascent projection neuron. The neuron then migrates radially from the ventricular zone (VZ) along the basal process of a RGC into the cortical plate (CP). The earliest-born neurons migrate to form the preplate. Later-migrating neurons split the preplate into the marginal zone (MZ) and subplate (SP). The MZ also consists of Cajal–Retzius cells originating from multiple sites in the forebrain including the cortical hem. As neurogenesis proceeds, diverse subtypes of neurons are generated through the successive asymmetric division of RGCs. Early-born nascent projection neurons settle in the deep layers (layers 5 and 6; red layers) and later-born projection neurons settle in towards the mid-neurogenesis stage. In addition, some populations of RGC daughter cells become intermediate progenitor cells (IPC) or basal radial glial cells (bRGC) in the subventricular zone (SVZ). After the neurogenic stages, the radial scaffold detaches from the apical surface and aRGCs become gliogenic, generating astrocytes, or transform into ependymal cells. Adapted from (Shibata et al., 2015).

1.2.2 Migration of excitatory neurons

After exiting the cell cycle, new-born excitatory neurons need to migrate out of the proliferative zone and into the cortical plate (CP), where they further differentiate and form the six-layered laminar structure of the future neocortex. Birth-dating studies have shown that layers II-VI of the neocortex are generated in an 'inside-out' fashion, such that neurons generated early reside in the deep layers, whereas later-born neurons migrate past the existing neurons to occupy more superficial layers (Angevine and Sidman, 1961; Berry and Rogers, 1965). Consequently, Layers V-VI excitatory neurons (born between E10.5 – E13.5, in mice) are the first to enter the cortical plate, while later born neurons (between E14.5- E17.5, in mice) migrate past them to inhabit more superficial locations, Layers II-IV (Bayer and Altman, 1991; Molyneaux et al., 2007). Proper lamination of the cortical structure is crucial for neocortical function.

In addition to their proliferative abilities, RGPs also serve as a scaffold to guide new-born neurons to the correct lamina of the neocortex defined as glial-guided neuronal migration (Rakic, 1988a). The dynamic cell-cell adhesions between radial glial fibres and migrating neurons are regulated by gap junctions (Hatten, 1990, 1999; Rakic, 1988a). At these contact points, the gap junction subunits connexin 26 (Cx26) and connexin 43 (Cx43) are expressed. Cx26 preferentially mediates contact between the RG fiber and the soma of the migrating neuron, whereas Cx43 colocalizes along the leading process (Elias et al., 2007). Acute downregulation of either protein results in impaired neuronal migration into the cortical plate (Elias et al., 2007). Although little is known about the molecular mechanisms for glial guided migration, recent evidence suggests that the C-terminal tail of Cx43 is required for this type of migration (Cina et al., 2009). Furthermore, focal adhesion kinase (FAK), an intracellular kinase and scaffold protein, regulates the assembly of the Cx26 contact points in migrating neurons. Loss of FAK increases the amount of tangentially dispersed cells (Valiente et al., 2011).

Many molecules have been implicated in the regulation of excitatory neuron radial migration in the developing neocortex. Of these, REELIN, an extracellular matrix protein secreted by Cajal-Retzius cells in the marginal zone, appears to be a key player. When mutated spontaneously in *reeler* mice, the birth date-dependent inside-out fashion of the positioning of excitatory neurons is reversed, such that the layers are generated in an 'outside-in' manner (Caviness and Rakic, 1978; D'Arcangelo, 2001; D'Arcangelo et al., 1995; Drakew et al., 1998; Forster et al., 2002; Magdaleno et al., 2002; Ogawa et al., 1995). Genetic analysis of mouse mutants and human neurologic disorders associated with disrupted cortical laminar organization has identified other molecules including cyclin-dependent kinase 5 (Cdk5) (Ohshima et al., 1996), p35 (Chae et al., 1997), transcription factor Pax6 (Caric et al., 1997; Schmahl et al., 1993), neurotrophin-4 (Brunstrom et al., 1997), integrins (Anton et al., 1999; Georges-Labouesse et al., 1998; Zhang and Galileo, 1998), and doublecortin (Gleeson et al., 1998) as critical regulators of neuronal migration, and thereby layer formation in the developing neocortex.

1.3 Development of the ventral telencephalon and GABAergic interneurons

Prior to the 1980's, it was widely believed that both excitatory and inhibitory neurons originated from germinal zones proximal to their final position in mature brain structures. In this model, akin to neocortical excitatory neurons, inhibitory interneurons are born in the ventricular zone of the dorsal telencephalon and migrate radially into the neocortex. However, numerous studies performed towards the end of the century challenged this concept. Using Bromodeoxyuridine (5-bromo-2-deoxyuridine, BrdU)-labeling and retroviral lineage analysis, a number of groups discovered that excitatory and inhibitory neurons originate from separate lineages and identified the ventral telencephalon as the primary source of cortical interneurons in rodents (DeDiego et al., 1994; Mione et al., 1994; Parnavelas et al., 1991).

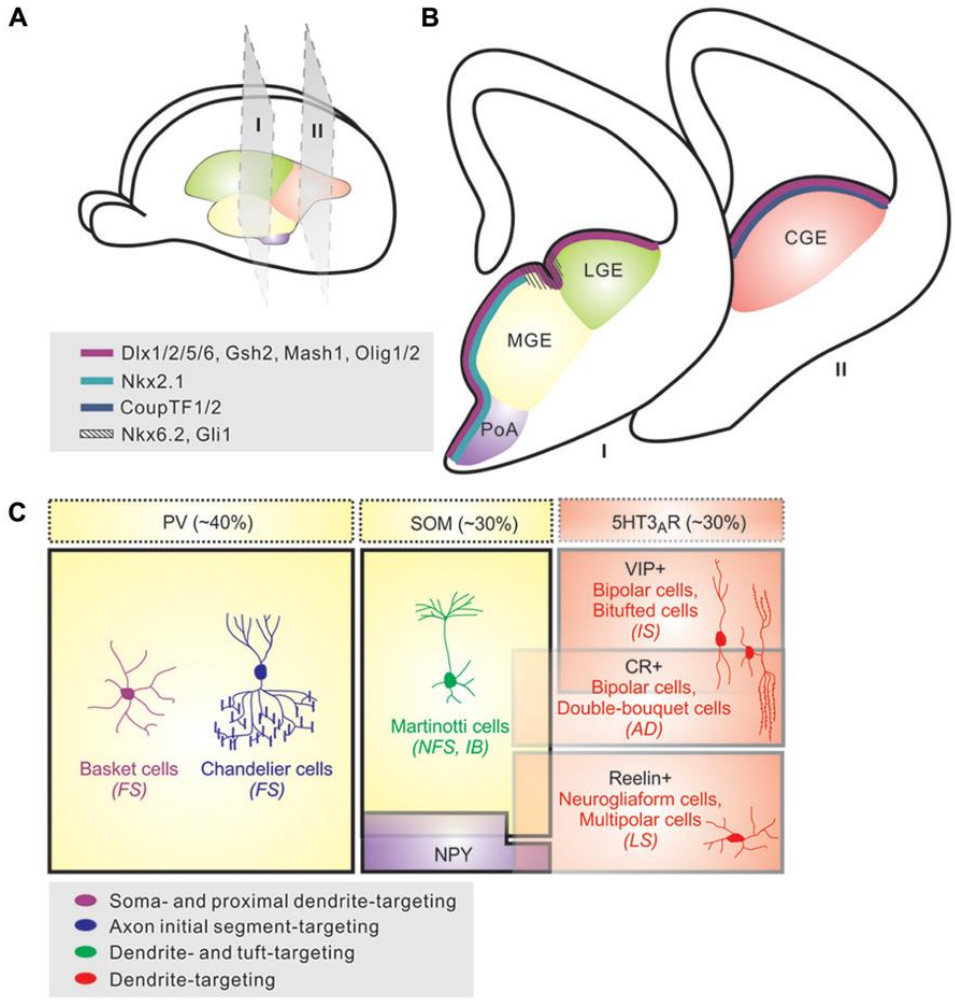
The developing ventral telencephalon is composed of the ganglionic eminences

and AEP (anterior entopeduncular)/PoA domains. First defined by its anatomy, the ganglionic eminence is subdivided into three discrete regions within the neuroepithelium: the medial (MGE), lateral (LGE), and caudal (CGE) ganglionic eminences (Smart, 1976; Sturrock and Smart, 1980). Appearing at the telo-diencephalic junction, the MGE is the earliest born eminence (E11.5, in mice) (Smart, 1976). At E12.5, a second protrusion (LGE) can be found that is separated from the MGE by a sulcus (Smart, 1976). The CGE develops later and is located at the posterior region where the MGE and LGE fuse (Anderson et al., 2001). Due to the lack of a physical border, there is some controversy of whether or not the CGE is a distinct entity or a caudalization of the MGE and LGE at later stages. The AEP/PoA is located in the subpallial domain, close to the telencephalic stalk **(Figure 1.3A, B)**. As embryonic development concludes, the morphological boundary between these regions recedes and is no longer recognizable in the postnatal brain. While originally proposed as a source of cortical interneurons, recent lineage analysis of the septum excludes its involvement in their generation (Rubin et al., 2010; Taglialatela et al., 2004).

Ventral telencephalic domains broadly express transcription factors that are crucial to cortical interneuron development. Expressed throughout the subpallial subventricular zone (SVZ), the *Dlx* family of homeobox transcription factors is of particular importance for GABAergic interneuron differentiation, migration and process formation. Specifically, *Dlx1* and *Dlx2* are functionally redundant genes required for GABAergic interneuron production and specification and are even capable of inducing glutamic acid decarboxylase (GAD 65/67) expression in pallial, glutamatergic neuron producing- progenitors (Anderson et al., 1997; Petryniak et al., 2007; Pleasure et al., 2000). *Dlx1/2*-null mutants die at birth and have pervasive defects in the development of interneurons, resulting in a 70% reduction of these cells in the neocortex (Anderson et al., 1997; Sussel et al., 1999). Moreover, these genes repress *Olig2*-dependant oligodendrocyte precursor cell (OPC) formation by acting on a common progenitor to determine neuronal versus oligodendroglial cell fate

acquisition (Petryniak et al., 2007). Working in concert with *Dlx1/2*, the proneural gene *Mash1* is expressed in the subpallial SVZ and is required for the production and differentiation of GABAergic interneurons (Long et al., 2009; Petryniak et al., 2007). Ablation of *Mash1* results in a marked decrease of GABAergic neocortical interneurons (Petryniak et al., 2007; Porteus et al., 1994). While *Dlx1/2* and *Mash1* are expressed throughout the subpallium, transcription factors that are intimately involved in interneuron fate-specification exhibit a more restricted expression pattern. As a result, the developing ventral telencephalon contains multiple progenitor pools, each of which gives rise to distinct interneuron subtypes **(Figure 1.3)**.

Figure 1.3: Origins and diversity of neocortical interneurons. (A) Neocortical interneurons are derived from progenitor cells located in the proliferative zones of the ventral telencephalon, specifically within the medial ganglionic eminence (MGE) and caudal ganglionic eminence (CGE). A small proportion is produced in the preoptic area (PoA). **(B)** Various transcription factors are expressed in distinct patterns throughout the saubpallial germinal zones; whereas Dlx1/2/5/6, Gsh2, Mash1, and Olig1/2 (magenta) are expressed in subventricular zone (SVZ) of the entire GE region, transcription factors such as Nkx2.1 (light blue) and CoupTF1/2 (dark blue) are expressed specifically within the MGE/PoA and CGE, respectively. Nkx6.2 and Gli1 (hashed lines) display a restricted expression pattern in the sulcus region between MGE and LGE. **(C)** Neocortical interneurons are highly diverse and can be defined based on morphology, neurochemical expression, electrophysiological properties, and subcellular synaptic targeting specificity. About 40% of neocortical interneurons exhibit fast-spiking (FS) electrophysiological profiles, and are comprised of basket and chandelier cells; these cells largely express parvalbumin (PV), although some chandelier cells are PV-negative. Cells expressing somatostatin (SOM or SST) account for ~30% of the neocortical interneurons that are morphologically heterogeneous (e.g., Martinotti cells) and typically exhibit non-FS physiological characteristics. The remaining ~30% of neocortical interneurons largely express the 5-hydroxytryptamine 3A receptor (5-HT3AR) and are comprised of vasointestinal peptide (VIP)-expressing and/or calretinin (CR)-expressing cells with bipolar or double-bouquet morphologies and fast adapting firing (AD) patterns, as well a group of Reelin-expressing, late-spiking (LS), neurogliaform cells. Additionally, a small population of cortical interneurons consists of multipolar cells that contain NPY and display irregular or fast AD firing properties. Adapted from (Sultan et al., 2013).



1.3.1 Neurogenesis of inhibitory interneurons

Even though the germinal zones of excitatory neurons and interneurons are spatially segregated, they both harbor RGPs in their VZ (Campbell and Gotz, 2002; Mori et al., 2005). However, the RGPs responsible for producing excitatory neurons and inhibitory interneurons are distinct. RGPs in the VZ of the neocortex express Pax6 and Emx1, two transcription factors essential for specifying neocortex features, without which the neocortex is converted into structures normally formed by the ventral telencephalon (Muzio et al., 2002). Conversely, RGPs in the ventral telencephalon express different sets of transcription factors such as Gsh1/2 and Oligo2 (Malatesta et al., 2003).

A recent study using retroviral labeling and live imaging analysis clearly demonstrated that RGPs in the VZ of the MGE undergo asymmetric cell division to produce neocortical interneurons (Brown et al., 2011). In addition, there are abundant IPCs in the SVZ that divide symmetrically to produce neocortical interneurons. In fact, the SVZ in the ventral telencephalon is substantially larger than that in the neocortex, indicating that symmetrically dividing IPCs represent an important source of neocortical interneurons (Malatesta et al., 2003). However, our knowledge about those progenitors in the ventral telencephalon is still very limited.

1.3.2 Migration of neocortical interneurons

In contrast to excitatory neurons, neocortical interneurons produced at the ventral telencephalon need to embark on a lengthy tangential migration to reach the dorsal neocortex, and then migrate radially to arrive at their final proper laminar positions (Anderson et al., 1999; Anderson et al., 2001; de Carlos et al., 1996; Jimenez et al., 2002; Lavdas et al., 1999; Miyoshi et al., 2010; Nery et al., 2002; Polleux et al., 2002; Tan et al., 1998; Ware et al., 1999; Wichterle et al., 2001; Yozu et al., 2005). Despite this arduous journey, neocortical interneurons integrate into the cortex in an 'inside-out' pattern where earlier born interneurons occupy deeper cortical lamina than their more superficial cohorts (Anderson et al., 2002; Cavanagh and Parnavelas, 1989). Interestingly, interneurons and projection neurons born at the same time often reside in the same cortical layers.

Previous studies have identified a diverse set of temporally and spatially distinct tangential migratory routes associated with different progenitor zones within the ventral telencephalon (Anderson et al., 2001; Marin and Rubenstein, 2001) **(Figure 1.4)**. Early during development (E11.5-E12.5), neocortical interneurons arise primarily from the MGE and the anterior entopeduncular area (AEP)/PoA, and follow a superficial route outside the developing striatum (Anderson et al., 2001). At mid-embryonic stages (E12.5-E14.5), the MGE appears to be the principal source of neocortical interneurons, which migrate either deeply or superficially, relative to the

developing striatum. Meanwhile, a population of MGE-derived cells also migrates to the developing striatum (Marin et al., 2000; Marin et al., 2001; Sussel et al., 1999). During this period, the LGE and CGE also start to produce interneurons that migrate to the neocortex (Miyoshi et al., 2010). At later stages (E15.5), the CGE reaches its peak of neurogenesis and becomes the more prominent/substantive source of cortical interneurons, which prefer a deeper migratory route (Marin and Rubenstein, 2001; Miyoshi et al., 2010).

After entering the neocortex, interneurons continue to disperse tangentially via highly stereotyped routes in the MZ, the subplate, and the lower intermediate zone/the SVZ (Lavdas et al., 1999). Eventually, interneurons switch from tangential to radial migration to adopt their final laminar position in the neocortex (Ang et al., 2003; Polleux et al., 2002; Tanaka et al., 2003).

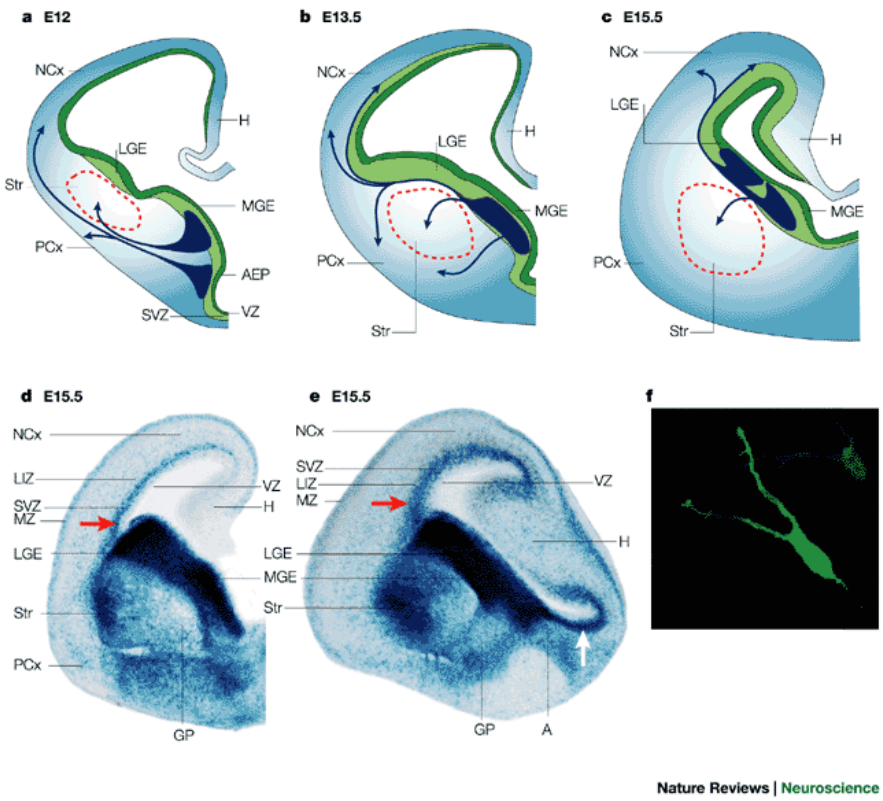


Figure 1.4: Tangential migration of immature interneurons from the ventral telencephalon to the dorsal cortex. At least three spatially and temporally distinct routes can be distinguished, as depicted here in schemas of transverse sections through the embryonic telencephalon. **(a)** Early during development (E12.5), interneurons that migrate to the cortex arise primarily from the medial ganglionic eminence (MGE) and the anterior entopeduncular area (AEP, also called PoA), and follow a superficial route. **(b)** At the peak of migration (E13.5), interneurons migrating to the cortex arise primarily from the MGE and follow a deep route to the developing striatum (Str). Some interneurons also migrate superficially. **(c)** At later stages (E15.5), cells migrating to the cortex might also arise from the lateral ganglionic eminence (LGE) and follow a deep route. **(d, e)** Analysis of both coronal **(d)** and sagittal **(e)** sections from mice in which β-galactosidase expression is driven by a *Dlx5*/*Dlx6* enhancer indicates that cell migration to the cortex by the deep route occurs primarily through the subventricular zone (SVZ), or in the boundary between the SVZ and the intermediate zone (red arrows). In addition, interneurons might reach the cortex by a caudal migratory stream that courses through the primordium of the amygdala (A; white arrow). **(f)** Migrating interneurons are typically bipolar or multipolar, with several leading processes, which are orientated in roughly the same direction, and a short trailing process. GP, globus pallidus; H, hippocampus; LIZ, lower intermediate zone; MZ, marginal zone; NCx, neocortex; PCx, piriform cortex; VZ, ventricular zone. Adapted from (Morrison and Spradling, 2008).

1.3.3 Different subtypes of GABAergic interneurons

GABAergic interneurons are local circuit neurons that constitute ~20% of the total neuronal population of the neocortex. Termed "short-axon" neurons by Ramon y Cajal, interneurons are key regulators of cortical output and plasticity. Interneurons regulate neuronal transmission via chloride permeable, ionotropic $GABA_A$ receptors to hyperpolarize or shunt the excitatory drive of their postsynaptic targets (DeFelipe and Jones, 1988). These cells also play a role in neuronal migration and proliferation during development, as well as the formation of cortical circuitry (Wang et al., 2004; Whittington and Traub, 2003). Neocortical interneurons are remarkably diverse and can be subdivided into distinct subgroups based on neurochemical marker expression, morphology, firing pattern and synaptic connectivity (Ascoli et al., 2008; Monyer and Markram, 2004). Current data suggest that nearly all of neocortical interneurons express one or more of these three markers: (1) the cytoplasmic calcium binding protein parvalbumin (PV), (2) the neuropeptide somatostatin (SST), and (3) the 5-hydroxytryptamine (serotonin) receptor 3A (5-HT3AR) (Rudy et al., 2010) **(Figure 1.3C)**.

PV-expressing interneurons represent ~40% of neocortical interneurons, most notably fast-spiking basket and chandelier cells. Cells expressing SST account for ~30% of GABAergic neurons are morphologically heterogeneous and typically exhibit non-fast spiking physiological characteristics. The $5HT3_AR$ group, which comprise ~30% of the total interneuronal population, is heterogeneous and includes all vasoactive intestinal peptide (VIP) expressing neurons, as well as an equally numerous subgroup of neurons that do not express VIP including neurogliaform cells (Lee et al., 2010). Other molecular markers such as, calretinin (CR), Kv3.1, cholecystokinin (CCK), and neuronal nitric oxide synthase (nNOS) are good indicators of subtype identity, while others, calbindin (CB), neuropeptide Y (NPY), and Kv3.2 are expressed in a variety of cell types (Cauli et al., 1997; Chow et al., 1999; DeFelipe et al., 1993; Garaschuk et al., 2000; Gonchar and Burkhalter, 1997; Gupta et al., 2000; Kubota and Kawaguchi, 1994, 1997; Monyer and Markram, 2004).

Although this classification system is largely accepted by the field, a more comprehensive analysis using genome-wide microarray of single cells and small subsets of interneurons may help reveal new populations (Kamme et al., 2003; Sugino et al., 2006).

1.3.4 Spatial specification of different interneuron subtypes

Genetic and transplantation studies have demonstrated that distinct regions of the GEs generate distinct interneuron subtypes (Butt et al., 2005; Flames et al., 2007; Miyoshi et al., 2007; Xu et al., 2004). The medial ganglionic eminence (MGE) is responsible for the vast majority (~60%) and most diverse subset of cortical interneurons. Transplantation experiments of MGE precursors have revealed that the majority of MGE-derived interneurons are a heterogeneous group that expresses either parvalbumin (PV) or somatostatin (SST) (Valcanis and Tan, 2003; Wichterle et al., 1999; Wichterle et al., 2001; Xu et al., 2004). The bulk of this domain expresses the homeobox transcription factor *Nkx2.1*, though the dorsal MGE expresses *Nkx6.2*, *Gli1* and is partially *Nkx2.1* negative (Fogarty et al., 2007; Rallu et al., 2002; Wonders et al., 2008). *Nkx2.1* is down-regulated in post-mitotic neocortical interneuron prior to their entry into the cortex, but remains in cells destined for other structures (i.e. the striatum) (Marin et al., 2003). *In vivo* loss of function experiments have identified that conditional removal of *Nkx2.1* at E10.5 results in a 50% decrease in cortical interneurons and an increase in striatal spiny neurons (Butt et al., 2008). Later removal of *Nkx2.1* at E12.5 in mice resulted in a switch of the MGE fate into CGE-derived cells (Anderson et al., 2001). Therefore, *Nkx2.1* plays a crucial role in the establishment and maintenance of MGE progenitors as well as the specification of MGE-derived interneuron subtypes located throughout the cortical laminae.

The diversity of subtypes within the MGE suggests the existence of potentially more restrictive subdomains that contain a bias towards the production of specific interneuron subtypes. A fate mapping study of the dorsal MGE, which expresses *Nkx6.2*, found that these progenitors appear to preferentially generate SST-expressing

cells (Flames et al., 2007). Consistent with this finding, *in vitro* transplantation studies of dorsal MGE and ventral MGE cultures revealed that, while both regions produce a mixed population of interneurons, there is a strong bias for the production of SST+ and PV+ cells in the dMGE and vMGE, respectively (Flames et al., 2007; Wonders et al., 2008). Together, these results suggest that MGE-derived subgroups are preferentially generated in spatially distinct regions along the dorsal/ventral axis within the MGE (Flames et al., 2007; Wonders et al., 2008; Xu et al., 2008).

The initial patterning and maintenance of Nkx2.1 protein expression in MGE progenitors requires the coordinated actions of several morphogens, most notably sonic hedgehog (*Shh*) (Fuccillo et al., 2004; Sussel et al., 1999; Xu et al., 2005). Several experiments have shown that depletion of Shh levels or inhibition of Shh signaling results in a large reduction of detectable NKX2.1 protein, effectively altering the specification of MGE-derived interneurons in some cases, to a CGE-derived CR+ bipolar fate (Gulacsi and Anderson, 2006; Xu et al., 2010). In addition, higher levels of Shh signaling promote the generation of SST-expressing interneurons and in doing so inversely affect the amount of PV-expressing cells (Wonders et al., 2008). Exposing vMGE progenitors to exogenous Shh in culture can also produce a similar effect on SOM+ fate, suppressing the generation of PV+ interneurons (Xu et al., 2010). Although Shh is secreted from the floor plate of the neural tube causing a ventral/high, dorsal/low gradient, dorsal MGE progenitors exhibit higher levels of Shh signaling due to enhanced expression of Shh effectors *Gli1* and *Gli2* (Wonders et al., 2008). Hence, these variations in Shh signaling along the dorsal/ventral axis are a potential molecular mechanism for the specification of interneuron subtypes.

Downstream of Nkx2.1 is the LIM-homeobox-transcription factor *Lhx6*, which specifies the fate of MGE-derived interneurons and is directly activated by Nkx2.1 (Alifragis et al., 2004; Lavdas et al., 1999; Liodis et al., 2007). It is expressed not in progenitors but post-mitotic MGE-derived interneurons soon after they leave the ventricular zone and persists into adulthood in most PV+ and SST+ cortical

interneurons (Lavdas et al., 1999). *Lhx6*-null mice exhibit loss of PV+ and SST+ interneurons in the cortex without any change in the total number of cortical interneurons. Transfection of MGE cells in slice cultures with an RNAi construct targeting *Lhx6* results in a reduction of interneuron migration into the cortex, but no alteration in GABA expression (Alifragis et al., 2004; Liodis et al., 2007). Moreover, *Lhx6* expression is sufficient to rescue PV and SST expression in *Nkx2.1*-null mice (Xu et al., 2008). Taken together, this evidence suggests *Lhx6* is necessary for proper specification and migration of MGE-derived interneurons, but not their GABAergic identity.

After the MGE, the caudal ganglionic eminence (CGE) is the next major source of cortical interneurons (Anderson et al., 2001; Nery et al., 2002), which contributes ~30% of the total cortical interneuron population (Miyoshi et al., 2010). The CGE generates a subset of diverse interneuron subtypes: CR or VIP-expressing, bipolar and double bouquet interneurons as well as late-spiking, reelin expressing neurogliaform cells (Miyoshi et al., 2010). These cells preferably inhabit the supragranular layers of the neocortex. Recent studies identified a large population of interneurons expressing the 5-HT3A (5-hydroxytryptamine) serotonin receptor and determined that the CGE as the predominant origin of these interneurons. This population of interneurons include neurogliaform cells and bipolar VIP-expressing interneurons but do not express PV or SST, excluding the MGE as a potential source (Butt et al., 2005; Cobos et al., 2007; Lee et al., 2010; Miyoshi et al., 2010; Nery et al., 2002; Xu et al., 2004). Unfortunately, while *Nkx6.2* and *CoupTF 1/2* are widely expressed in the CGE, no transcription factors specific to the CGE have been identified making it difficult to analyze the molecular mechanisms underlying CGE-derived interneuron specification. However, conditional loss of function of *COUP-TF1* in subventricular precursors and postmitotic cells leads to a decrease of late-born, CGE-derived, VIP- and CR-expressing bipolar cortical neurons, compensated by the concurrent increase of early-born MGE-derived, PV (parvalbumin)-expressing interneurons indicating that *COUP-TF1* is necessary for the specification of CGE-derived cortical interneurons

(Lodato et al., 2011).

The preoptic area (PoA) has been identified as a novel source of cortical interneurons (Bulfone et al., 1993; Cobos et al., 2007; Flames et al., 2007; Gelman et al., 2009; Puelles et al., 2000). Similar to the MGE, progenitors in this region express *Nkx2.1*, though PoA-derived cells do not appear to express Lhx6 (Gelman et al., 2009). A recent fate mapping study circumvented this issue by using *Nkx5.1*, a transiently expressed transcription factor by early post-mitotic PoA-derived cells, in order to permanently label these cells into adulthood. Here, the PoA was identified as a novel source of a relatively small population of GABAergic cortical interneurons with uniform properties - rapidly adapting low frequency firing, multipolar morphology, and 30% of which solely express NPY (Gelman et al., 2009).

Although some evidence suggests the LGE produces a small population of cortical interneurons, the fate of these cells is currently unknown (Anderson et al., 2001; Xu et al., 2004; Yang et al., 2011). Instead, the LGE appears to be a major source of calretinin-expressing olfactory bulb interneurons and striatal GABAergic projection neurons (Kohwi et al., 2007; Young et al., 2007). Akin to the MGE, this region can be divided along the dorsal/ventral axis into two progenitor domains. The dorsal LGE expresses genes characteristic of the pallium such as *Pax6*, *Ngn2*, and *Dbx1* and produces olfactory bulb interneurons (Flames et al., 2007; Puelles et al., 2001; Stenman et al., 2003). The ventral LGE expresses *Gsh2*, and low levels of *Pax6*, and primarily gives rise to the striatal projection neurons (Flames et al., 2007; Puelles et al., 2001; Stenman et al., 2003).

1.3.5 Temporal specification of interneuron subtypes

Similar to excitatory neurons, neocortical interneurons are specified in an ‘inside-out' manner and this laminar diversity is produced in a temporal sequence (Anderson et al., 2002; Cavanagh and Parnavelas, 1989). Furthermore, MGE and CGE derived cells are generated with different temporal profiles. While MGE-derived interneurons are mostly born between E12.5 to E16.5, CGE-derived interneurons are

produced at later developmental time points (E13.5-E15.5) and generate distinct interneuron subtypes suggesting time of origin may play a role in determining interneuron specification (Butt et al., 2005; Nery et al., 2002). Both *in vitro* culture assays and fate mapping experiments of temporal cohorts have revealed the competence of MGE progenitors to produce different interneuron subtypes changes over the course of neurogenesis (Miyoshi et al., 2007; Xu et al., 2004). Specifically, a high proportion of SST–expressing interneurons are born at early developmental stages, but are almost absent in E15.5, whereas PV-expressing interneurons are produced persistently throughout the MGE/PoA neurogenesis. Each temporal cohort exhibits unique physiological properties characteristic of their birthdate (Miyoshi et al., 2007; Xu et al., 2004) **(Figure 1.5).** Contrary to the MGE, interneuron subtypes generated within the CGE do not significantly change over time. CGE-derived cells typically inhabit the superficial layers of the neocortex, but there is no correlation between their temporal origin and specific layer destination (Miyoshi et al., 2010). Taken together, this suggests time of origin plays a role in the laminar positioning and specification of interneurons generated in the MGE, but not CGE.

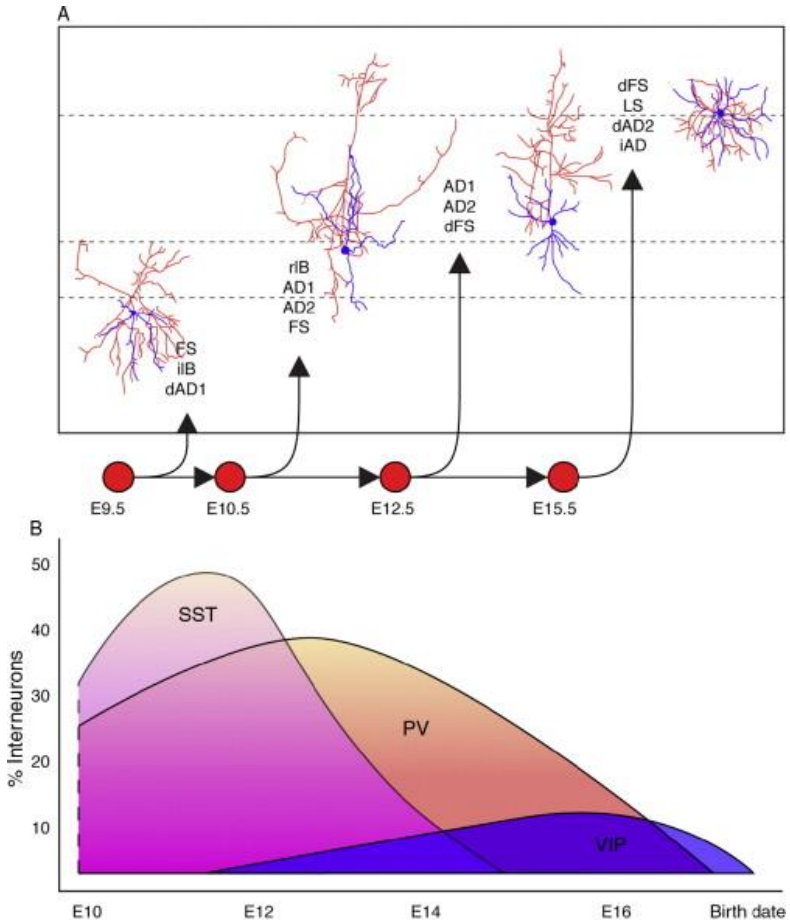


Figure 1.5: Temporal origins of cortical interneurons. (A) Examples of the morphological and electrophysiological diversity of cortical interneurons derived from the MGE at different times. Axons and dendrites are indicated in red and blue, respectively. **(B)** Diagram of temporal origin of three subtypes of MGE interneurons, the somatostatin (SST—pink), the parvalbumin (PV—orange), and a subtype of CGE-derived interneuron (VIP—blue). Adapted from (Batista-Brito and Fishell, 2009).

1.3.6 Specification of interneuron subtypes: nature or nurture?

The spatial and temporal mechanisms of interneuron diversity suggest specification is 'pre-programmed' in the germinal zone before a cell becomes post-mitotic. As evidence, transplantation of CGE precursor cells into the MGE has been shown not to adversely affect their ability to migrate and differentiate into subtypes typical of their origin (Butt et al., 2005; Nery et al., 2002). Furthermore, migrating embryonic interneuron precursors express a variety of transcription factors and mature interneuron subtype markers (Batista-Brito and Fishell, 2009). This supports the notion that significant aspects of interneuron identity are already

acquired during embryonic stages and potentially prior to their birth, though the possibility remains that some mature characteristics may by strongly influenced by their surrounding environment.

Intrinsic programming is likely initiated by the combinatorial expression of transcription factors (both homeodomain and bHLH) required for interneuron development that endow progenitor pools with the potential to produce distinct subtypes (Briscoe et al., 2000; Lu et al., 2002; Sugimori et al., 2007; Zhou and Anderson, 2002). A large amount of progenitor diversity can be produced by a relatively small number of genes; therefore, it is possible that multiple subdomains exist within distinct spatially separate regions. Fate mapping of more restricted transcriptional domains have revealed a strong bias for the generation of interneuron subtypes, yet a mixed population of subtypes was still produced (Fogarty et al., 2007). Future identification of neural progenitors that are molecularly distinct within the ventral domains would be essential to advance our understanding on the generation of the diverse group of interneuron subtypes.

1.4 Development of the vascular network in the developing telencephalon

Besides the neural system, the other major cellular component of the developing telencephalon is the vascular network. The brain is one of the most energy-consuming organs. It represents only 2% of the weight of an adult human but it uses 20% of the energy produced by the body. Efficient energy supply (e.g. glucose and oxygen), provided by the blood flows in the vessels, is crucial for the brain so that our memory, mobility and senses can function normally. The generation of blood vessels in the mammalian embryo begins with vasculogenesis when a subset of splanchnopleuric mesodermal cells gives rise to blood islands. The cells at the periphery of the blood islands are endothelial cell precursors called angioblasts, whereas cells at the center are hematopoietic precursor cells (Tsao et al., 1998). The central nervous system (CNS) acquires its vasculature by angiogenesis, a process consisting of proliferation of endothelial cells in existing blood vessels or vascular plexuses and leading to

formation of new blood vessels.

1.4.1 Angiogenesis in the developing telencephalon

Angiogenesis begins early in CNS development and continues throughout life. It occurs through the coordinated activity of two distinct endothelial cell types: tip cells and stalk cells (Gerhardt et al., 2003). Endothelial tip cells are specialized non-mitotic migratory cells with many highly dynamic filopodia, which navigate in response to local depots or gradients of vascular endothelial growth factor (VEGF-A). Tip cells are only present during angiogenesis and are responsible for vascular patterning. Endothelial stalk cells divide in the wake of the tip cells to generate the tubular structure of blood vessels. Endothelial tip cells have been observed to extend filopodia to the ventricular surface, where radial glia divide, and to interact with the fibers of radial glia in the hindbrain, suggesting communication between progenitors of the vasculature and central nervous system (Gerhardt et al., 2004). It has also been indicated that endothelial cells in the brain share a similar molecular profile with neighboring neural stem cells (i.e., ventral endothelial cells express Dlx1/5 and Nkx2.1, whereas dorsal endothelial cells express Pax6), strongly suggesting a relationship between the mechanism of patterning during angiogenesis and neurogenesis in the brain (Vasudevan et al., 2008).

Based on anatomical location, independent growth patterns and developmental regulation, telencephalic vasculature fall into two categories: pial vessels and periventricular vessels. In the mouse, the pial vessels are directed by and envelop the neural tube by embryonic day 9.5 (E9.5) prior to the neurogenesis, without any apparent spatial or temporal gradients in their development (Hogan et al., 2004). Pial vessels are surrounded by cerebrospinal fluid (CSF) and give rise to smaller arteries that eventually penetrate into the brain tissue. On the other hand, the periventricular vessels, which form the bulk of the telencephalic vasculature, arise as branches of the basal vessel located on the telencephalic floor of the basal ganglia primordium. Firstly, the periventricular vessel branches form an orderly lattice in the ventral telencephalon.

Later, the periventricular vessel network propagates into the dorsal telencephalon as a result of migration of endothelial cells. By E11.5, they establish an orderly, ventral-to-dorsal gradient of telencephalic angiogenesis (Vasudevan and Bhide, 2008; Vasudevan et al., 2008) **(Figure 1.6)**. An elegant study using scanning electron microscopy of vascular corrosion casts and 3D reconstruction of serial 1 μm-thick sections to study the arterial network of the embryonic mouse brain confirms the origin of the periventricular vessel network from the basal vessel (Morrison and Spradling, 2008). It is possible that the periventricular vessels develop into arterial networks and pial vessels into venous sinuses (Morrison and Spradling, 2008).

Prevailing notions depict CNS angiogenesis as a passive process driven primarily by demands for oxygen and other nutrients by the growing neuronal populations. However, recent studies indicate that the periventricular vessels are the major population of vessels in the parenchyma of the embryonic telencephalon. The ventral-to-dorsal gradient of the periventricular angiogenesis matches that of the telencephalic transverse neurogenetic gradient, although the angiogenesis gradient is in advance of the neurogenetic gradient temporally, by about a day. Within the dorsal telencephalon, the periventricular vessel gradient and the gradient of appearance of tangentially migrating interneurons overlap spatially as well (Vasudevan and Bhide, 2008). The same research group also further indicated that these periventricular vascular networks are strategically positioned to fulfill the task of providing support and critical guidance cues (i.e. endothelial $GABA_A$ receptors) that regulate the divided migratory routes of ventral-originated neocortical interneurons (Won et al., 2013). Altogether, these studies implicated that CNS angiogenesis is not a passive but an interactive process: the pioneering periventricular vessels of the telencephalon, which develop in advance of and free of neuronal development, hold a valuable key for the establishment of the later forming neuronal networks and point to a novel paradigm of endothelial cell–neuron interactions in the embryonic forebrain.

Several well-characterized molecular pathways are implicated in vascular patterning, including the vascular endothelial growth factor (VEGF), Notch, ephrin

and EDG pathways, as well as unidentified genes such as out-of-bounds (Hogan and Kolodziej, 2002; Lawson et al., 2001; Zhong et al., 2001). There is overwhelming evidence for a strong instructive influence of the VEGF signaling system on endothelial cell growth and patterning (Gerhardt et al., 2003; Holmes and Zachary, 2005; Kearney et al., 2004; Tammela et al., 2008). The expression of VEGF inside the telencephalon is highest in the ventricular zone and the marginal zone in E9.5 mice, which could attract endothelial cells to form the pial and periventricular vascular networks (Breier et al., 1992; Hogan et al., 2004). VEGF knockout mice showed a vastly reduced numbers of intraneural endothelial cells already at E10.5, and concomitant defective CNS development then eventually follows from insufficient vasculature (Raab et al., 2004). However, it is still unclear how exactly the ventral-to-dorsal gradient of the periventricular vessels is formed. It is possible that there are other gradient molecules yet to be uncovered that guide the ventral-to-dorsal migration of the endothelial cells.

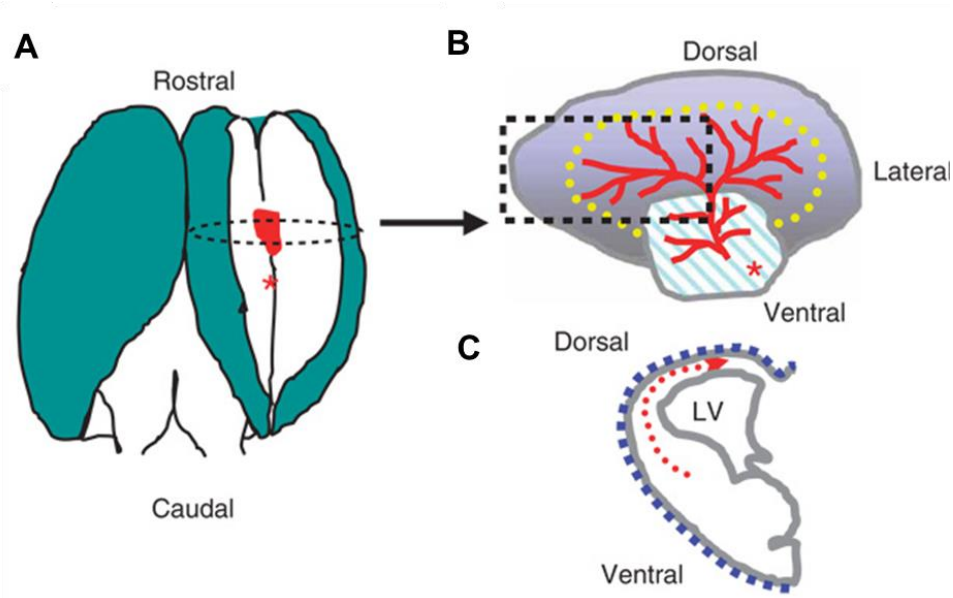


Figure 1.6: Diagramatic representation of the angiogenesis gradients in the embryonic telencephalon. The periventricular vessel network (red) originates from the basal vessel (red asterisk in **A**) in the telencephalon (peacock green) and grows in ventral-to-dorsal and lateral-to-medial directions. Dotted circle in **A** is expanded in **B** (purple, telencephalon; blue cross-hatching, basal forebrain) for a two-dimensional view of the periventricular network (yellow dotted circle) and the basal vessel (red asterisk in **B**). The boxed area in **B** represents **C**, with the medial aspects of the telencephalon devoid of periventricular vessels. (**C**) Ventral-to-dorsal and lateral-to-medial gradients of periventricular angiogenesis (broken red line with directional arrow). Blue dotted line, pial vessels. Adapted from (Vasudevan et al., 2008).

1.4.2 Blood Brain Barrier (BBB)

The blood-brain barrier (BBB) is a highly specialized brain endothelial structure of the fully differentiated neurovascular system, which makes blood vessel structure in the brain unique compared to other organs. In concert with pericytes, astrocytes, and microglia, the BBB separates components of the circulating blood from neurons. Neural cells in the CNS need to be protected against invasion, not only because infection might ensue, but also because their charge may be threatened by ionic compounds that exist in extracelluar space. Endothelial cells and pericytes are encased by basement membrane (~30–40 nm thick) containing collagen type IV, heparin sulfate proteoglycans, laminin, fibronectin, and other extracellular matrix proteins. The basement membrane of the brain endothelium is continuous with astrocytic

end-feet that ensheath the cerebral capillaries (Zlokovic, 2008). Astrocytes have a significant influence on capillary function, including regulating cerebral blood flow, upregulating tight junction proteins, contributing to ion and water homeostasis, and interfacing directly with neurons **(Figure 1.7).** Although the barrier properties of the BBB are at the level of the tight junction in endothelial cells, there is an important role for other components of the BBB, including the basement membrane, pericytes, astrocytes, and neurons. There is complex cross-talk between all entities and cell types, collectively known as the "neurovascular unit." Consideration of the neurovascular unit is important for disease processes that induce hemorrhage, vasogenic edema, infection, and inflammation (Zlokovic, 2008).

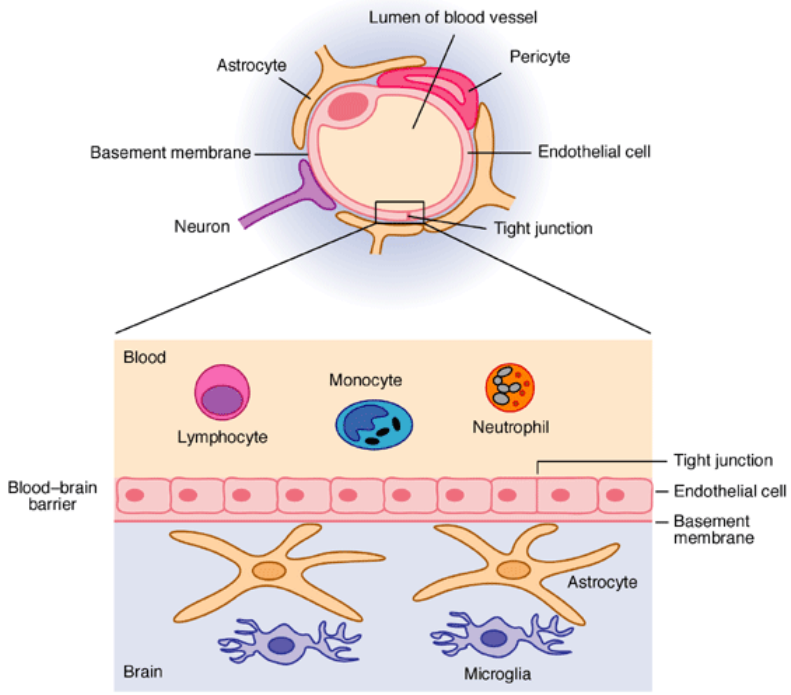


Figure 1.7: The blood–brain barrier (BBB). The BBB is created by the tight apposition of endothelial cells lining blood vessels in the brain, forming a barrier between the circulation and the brain parenchyma (astrocytes, microglia). Blood-borne immune cells such as lymphocytes, monocytes and neutrophils cannot penetrate this barrier. A thin basement membrane surrounds the endothelial cells and associated pericytes, and provides mechanical support and a barrier function. Thus, the BBB is crucial for preventing infiltration of pathogens and restricting antibody-mediated immune responses in the central nervous system, as well as for preventing disorganisation of the fragile neural network. This, together with a generally muted immune environment within the brain itself, protects the fragile neuronal network from the risk of damage that could ensue from a full-blown immune response. On rare occasions, pathogens (e.g. viruses, fungi and prions) and autoreactive T cells breach the endothelial barrier and enter the brain. A local innate immune response is mounted in order to limit the infectious challenge, and pathogens are destroyed and cell debris is removed, a vital process that must precede tissue repair. Adapted from Expert Reviews in Molecular Medicine © 2003 Cambridge University Press.

1.5 Vascular niche for neural stem cells (NSCs)

With the introduction of the ‘stem cell niche’ concept in 1978, Shofield described the potential for the existence of an extracellular environment that would be able to maintain a stem cell in its undifferentiated, proliferating state (Schofield, 1978). Through the years, this concept has been supported by many other literatures

reporting the specialized niche microenvironments supporting lifelong self-renewal and production of differentiated cells in various organs (Fuchs et al., 2004). For example, within the adult mammalian brain, stem cells harvested from non-neurogenic regions can generate neurons and astrocytes when cultured in vitro, but only make glia *in vivo* (Gage, 2000). Moreover, primary cells from neurogenic areas transplanted into non-neurogenic regions exhibit very limited neurogenesis (Temple, 2001). In contrast, upon transplantation into the SVZ, RMS or SGZ, cultured neural stem cells derived from non-neurogenic regions can generate neurons appropriate to the region (Temple, 2001). All these previous observations have indicated the existence of adult neurogenic niches, which have an instructive role in directing neuronal production and stem cell maintenance as well as shield ongoing neurogenesis from possible external inhibitory influences.

The identification of the vasculature as a prominent feature across the several stem cell niches, as well as the recent *in vivo* observations within the adult germinal regions (Shen et al., 2008; Tavazoie et al., 2008), suggest that it plays a critical role in niche regulation and maintenance. The inter-relationship between the vascular and nervous system has been further highlighted in the past decade as the diverse roles of vasculature in regulating the proliferation and differentiation of NSCs are gradually uncovered. Rather than being randomly distributed throughout the brain, NSCs in the SVZ and SGZ are found to be enriched around the blood vessels, which in fact has led to the discovery of vascular niche for the NSCs (Palmer et al., 2000; Shen et al., 2008). Blood vessels are formed by endothelial cells and perivascular support cells such as pericytes and smooth muscle cells, which, together with laminin-rich basal lamina surrounding the blood vessels, could create a vascular niche within these neurogenic regions. Vasculature in the SVZ shows specialized features including an altered BBB and easier access to blood-derived signals that functionally distinguish blood vessels in the SVZ niche from vessels in nonneurogenic brain regions (Tavazoie et al., 2008). Specifically within the SGZ, there exists an anatomical relationship between

proliferating hippocampal neural progenitors and proliferating endothelial cells (Palmer et al., 2000). In contrast to the SVZ where angiogenic sprouting and division of endothelial cells seems to be absent (Tavazoie et al., 2008), surges of endothelial cell division within the SGZ are spatially and temporally coordinated with clusters of neurogenesis (Palmer et al., 2000). The high level of VEGF and VEGFR, as well as the shared responsiveness to similar growth factors including neurotrophins, neurooilins, semaphorins and ephrins, strongly suggest that angiogenesis and neurogenesis are coupled within the SGZ of the hippocampus. A mechanism illustrating this bidirectional communication has been proposed within the HVC of the songbird brain, where testosterone-induced up-regulation of VEGF and VEGFR2 in neurons and astrocytes, respectively, increases angiogenesis. The newly generated capillaries produce BDNF that subsequently promote the recruitment and migration of new-born neurons (Louissaint et al., 2002).

Endothelial cells are a source of various diffusible signals, such as vascular endothelial growth factor (VEGF), fibroblast growth factor 2 (FGF2), insulin-like growth factor 1 (IGF1), pigment epithelium-derived factor (PEDF), brain-derived neurotrophic factor (BDNF) and other unidentified factors that locally regulate the proliferation and differentiation of NSCs. For example, VEGF is a hypoxia-inducible protein that can promote angiogenesis through receptor tyrosine kinases activation on endothelial cells. Recent evidence indicates that VEGF can also act as a directly-acting neurotrophic factor and is even required for the adult neurogenesis in the hippocampus, which is independent of its effects on endothelial cells (Cao et al., 2004). PEDF is the first soluble factor shown to selectively activate SVZ astrocytes, whose expression is restricted to endothelial and ependymal cells, suggesting that PEDF is a niche-derived signal for NSC self-renewal (Ramirez-Castillejo et al., 2006). FGF2 was first found to be secreted by endothelial cells and regulate their proliferation, migration and differentiation via an autocrine manner. Later studies further suggest that adult NSCs also respond to this potent angiogenic factor, which

can affect neurogenesis and proliferation of NSCs (Gritti et al., 1996). Given that VEGF, PEDF and FGF2 are involved in the regulation of both neurogenesis and angiogenesis, the link between these two processes within the NSC niche is further strengthened.

Vascular niches for adult NSCs are complex and encompass diverse aspects of the vascular system, which include diffusible signals derived from endothelial cells and direct contact with endothelial and perivascular cells as well as the vascular basement membrane (BM). The LAMININ receptor INTEGRIN α6β1 is important for tethering SVZ progenitors to the vasculature niche through the binding of LAMININs that are highly expressed on the outer surface of blood vessels, and perturbation of this interaction affects adhesion and proliferation (Shen et al., 2008). In addition, radiation-induced disruption of blood vessel-SGZ progenitor cell interaction results in a loss of neurogenic potential. Even SGZ progenitor cells from a non-irradiated brain transplanted into an irradiated host adopt a glial fate and lose their neurogenic capability, which further demonstrates the importance of the normal association between NSCs and vasculature, likely via the anchoring by the basal lamina (Monje et al., 2002).

Understanding the unique features of vascular niches that regulate adult neural stem cell self-renewal and differentiation is the key to understanding the *in vivo* stem cell regulation and will lead to insights into brain repair and future stem cell transplantation work for the adult neural diseases such as Alzheimer and Parkinson diseases. Although holding comparable promises on advancing our knowledge in order to treat neurological diseases, very little has been known about the vascular niche in the developing brain.

1.6 Concluding remarks

GABAergic neocortical interneurons are a functionally heterogeneous population whose specification and migration are governed by tightly regulated genetic cascades.

Although extensive studies in the past have revealed key insights on the neurogenesis and neuronal migration of the excitatory neurons in the dorsal telencephalon, our understanding on the neural progenitors that produce neocortical interneurons is still limited. Minor disruption of the developmental processes that generate these two neuronal groups upsets the precise balance of excitation and inhibition in the neocortex and can lead to a variety of neurological consequences in humans. Besides the neural system, the vascular network also actively develops in the embryonic brain and dynamically interacts with the neural system. Therefore, it is imperative to further elucidate the mechanisms regulating the production and organization of GABAergic interneurons in the neocortex, and investigate the potential role of blood vessels in this process.

CHAPTER 2:

MATERIALS AND METHODS

2.1 Animals

Itgβ1$^{fl/fl}$ (stock number 004605), *Tek-Cre* (stock number 008537) and *Ai14-tdTomato reporter* (stock number 007914) mouse lines were obtained from The Jackson Laboratory. *Scf*^{gfp} and *Scf*^{fl} mouse lines were kindly provided by Dr. S.J. Morrison (HHMI/University of Texas Southwestern Medical Centre) and *Nkx2.1-Cre* mouse line was kindly provided by Dr. S.A. Anderson (University of Pennsylvania). CD-1 mice were obtained from Charles River Laboratory. For timed pregnancies, the plug date was designated as E0 and the date of birth was defined as P0. All mouse colonies were maintained at Memorial Sloan Kettering Cancer Centre (MSKCC) and handled according to the protocols approved by the Institutional Animal Care and Use Committee (IACUC).

2.2 Retrovirus production

Replication-incompetent retrovirus expressing EGFP was produced from the 293gp NIT-EGFP (generous gift from Drs. F. Gage, Salk Institute) retrovirus packaging cell line. These cell lines were plated on BioCoat (BD Bioscience) plates and transfected with 15μg pVSV-G/10mm plate using FuGene6 (Roche). The culture supernatant containing viral particles were collected from 12 to 24 plates, 24 hours after 100% cell confluency. The virus-containing media was spun down at 3,000 RPM for 5 minutes, and then filtered through a 0.22um vacuum filter. To concentrate the virus, the media was centrifuged at 27,000RPM for 2 hours, resuspended in 200 μL Phosphate Buffered Saline (PBS, pH 7.4), and stored in 5uL aliquots at -80˚C for titre determination and infection of cells and animals. Viral titres were determined by limiting dilution on DF1 and NIH-3T3 cells, respectively. The viruses were serially diluted when used *in vivo* until the desired amounts of cells

were labeled.

2.3 *In utero* intraventricular injection

The uterine horns of different gestation stage pregnant mice were exposed in a clean environment. EGFP-expressing retrovirus solution (~1.0 μL) with Fast Green (2.5 mg/ml, Sigma) was injected into the embryonic cerebral ventricle through a bevelled, calibrated glass micropipette (Drummond Scientific). After injection, the peritoneal cavity was lavaged with ~10 mL warm PBS (pH 7.4) containing 1% Penicillin/Streptomycin, the uterine horns were replaced, and the wound was closed. The mice were monitored at 12, 24, and 48 hours post-surgery to ensure they remained healthy.

2.4 Immunohistochemistry

Mouse embryos were transcardially perfused with ice-cold Phosphate Buffered Saline (PBS, pH 7.4) followed by 4% paraformaldehyde (PFA) in PBS (pH 7.4). Postnatal mice were also transcardially perfused with ice-cold PBS (pH 7.4) followed by 4% paraformaldehyde (PFA) in PBS (pH 7.4). P21 and older brains require a minimum of 10mL and 25mL of each solution, respectively. Brains were then dissected out and post-fixed in the same fixation solution at 4℃. Coronal sections (~60 μm) were prepared using a vibratome (Leica Microsystems). For cryosections, fixed brains were placed in 30% sucrose in PBS overnight at 4℃ until submerged. After embedding in O.C.T. tissue-freezing medium, coronal cryosections (16-20 μm for embryonic brains; 40 μm for postnatal brains) were prepared and mounted onto 2% gelatin-coated slides (Lab Scientific).

Sections were incubated for 1 hour at room temperature in a blocking solution (5% normal goat serum, 0.5% Triton X-100 in PBS), followed by incubation with the primary antibodies overnight at 4℃. Sections were then washed in 0.5% Triton X-100 in PBS and incubated with the appropriate secondary antibodies for 2 hours at room temperature.

The primary antibodies used were: rabbit polyclonal anti-GFP (Invitrogen, 1:2,000), rat monoclonal anti-GFP (Nacalai, 1:1,000), rat monoclonal anti-PECAM-1 (clone MEC13.3, Biolegend, 1:200), rabbit polyclonal anti-phosphorylated Histone H3 (Upstate, 1:300), rabbit polyclonal anti-OLIG2 (Millipore, 1:1000), rabbit polyclonal anti-GABA (Sigma, 1:1,000), mouse monoclonal anti-PARVALBUMIN (Millipore, 1:1,000), rat monoclonal anti-SOMATOSTATIN (Millipore, 1:500), mouse monoclonal anti-NESTIN (Iowa Developmental Hybridoma Bank, 1:200), rabbit polyclonal anti-BLBP (Abcam, 1:200), rabbit monoclonal anti-NKX2.1 (Epitomics, 1:200), biotin-conjugated ISOLECTIN B4 (Sigma, 1:500), rabbit polyclonal anti-LAMININ (Abcam, 1:1000), rat monoclonal anti-INTEGRIN α6 (Millipore, 1:100), rat monoclonal anti-INTEGRIN β1 (Millipore, 1:100), and rabbit anti-Cleaved Caspase-3 (Cell Signaling Technology, 1:500). Secondary antibodies were Alexa Fluorophore conjugated (Life Technologies, 1:1000). 4',6-diamidino-2-phenylindole (DAPI, 300 nM) was applied together with the secondary antibodies as a nuclear marker. Immunostaining for phosphorylated Histone H3, INTEGRIN α6, INTEGRIN β1, Cleaved Caspase-3, PARVALBUMIN, and SOMATOSTATIN were carried out on cryosections.

2.5 BrdU labelling

For BrdU labelling, a single injection of BrdU (25 mg per kg body weight) was administrated to timed pregnant mice via intraperitoneal injection at different embryonic stages, 30 minutes prior to sacrifice. For BrdU detection, sections were treated with 1 N HCl for 1 hour at 37℃, and then washed in 0.1M Borate Buffer 3 times for 10 minutes. They were then immunostained with rat monoclonal BrdU antibody (Accurate, 1:200).

2.6 DiI labeling

For lipophilic tracer 3H-Indolium,

5-[[[4-(chloromethyl)benzoyl]amino]methyl]-2-[3-(1,3-dihydro-3,3-dimethyl-1-octadecyl-2H-indol-2-ylidene)-1-propenyl]-3,3-dimethyl-1-octadecyl-, chloride (CM-DiI) labelling, mouse embryonic brains were dissected in ice-cold PBS (pH 7.4) and fixed with 4% PFA in PBS (pH 7.4) for 4-6 hours. CM-DiI (Invitrogen, 1 mg/mL dissolved in DMSO) was intraventricularly injected into the lateral ventricles. Brains were incubated in PBS at 37℃ for 2 days and sectioned using a vibratome. Sections were washed in 0.3% Tween 20 in PBS 3 times and incubated with biotin-conjugated ISOLECTIN B4 (Sigma, 1:500) overnight at 4℃. Sections were then washed in 0.3% Tween 20 in PBS and incubated with Alexa Fluor® 488 Streptavidin (Life Technology, 1:1000) and DAPI for 2 hours at room temperature.

2.7 Confocal imaging

Images were acquired with a laser scanning confocal microscope (Olympus FV1000), and analyzed with FluoView (Olympus) and Photoshop (Adobe Systems). Three-dimensional reconstruction was performed using Imaris.

2.8 Organotypic slice culture and time-lapse imaging

Two days after *in utero* retrovirus injection, live brains were recovered and coronal telencephalic slices (~ 250 μm) were prepared. Slices were maintained on a Millicell-CM insert (Millipore) in culture medium containing 65% MEM (Gibco), 25% Hanks balanced salt solution, 5% heat-inactivated horse serum, 1x N2 supplement (Gibco), 0.66% glucose, 0.0125ug/mL ascorbic acid, and 1x Pen/Strep/L-glutamine. One drop of collagen gel mix (Millipore) was added on top of the slices before transferring them to a 37℃ incubator. After recovering for at least 4 hours, EGFP-expressing cells in slices were imaged on an inverted confocal microscope (SP5, Leica) every 30 minutes for up to 48 hours. Projection images were generated from Z-stacks. Montages were assembled and time-lapse sequences were arranged and analyzed using Imaris software.

2.9 Serial sectioning and three-dimensional reconstruction

For three-dimensional reconstruction of embryonic brains, serial sections of the brains (60 μm) were prepared using a vibratome and processed for immunohistochemistry. Each section was traced in sequential order from rostral to caudal using Neurolucida and Stereo Investigator software (MBF Bioscience) installed in an upright microscope (Zeiss, Axio Imager). The outer boundary of the entire section area and the inner boundary of the neocortex (i.e. the ventricular surface) from the midline to the perihinal cortex were traced. The cell body and basal endfoot of each labelled radial glial progenitor cell in the MGE/PoA were identified using a distinctive marker. The basal process of each labelled radial glial progenitor cell was traced using green-coloured lines. After the completion of one section, the trace was lined up with the next section to recover the whole radial glial progenitor cell.

2.10 Stereological cell counting and statistical analysis

For each postnatal brain, the number of interneurons in the somatosensory cortex was stereologically analyzed on both hemispheres from four consecutive coronal sections on an upright microscope (Zeiss) equipped with Neurolucida and Stereo Investigator (MicroBrightField, Inc.). Three to five brains were examined for each group. Cortical laminar position was determined by cell packing density revealed by DAPI staining.

2.11 Electrophysiology

The brains of wild type and mutant mice were removed at P21 and acute coronal cortical slices were prepared at ~350 μm with a Vibratome (Leica Microsystems) in ice-cold choline chloride-based cutting solution containing (in mM): 120 Choline Chloride, 26 $NaHCO_3$, 2.6 KCl, 1.25 NaH_2PO_4, 7 $MgSO_4$, 0.5 $CaCl_2$, 1.3 Ascorbic Acid and 15 D-glucose, bubbled with 95% O_2 and 5% CO_2. The slices were then transferred into artificial cerebral spinal fluid containing (in mM): 126 NaCl, 3 KCl, 1.2 NaH_2PO_4, 1.3 $MgSO_4$, 4 $CaCl_2$, 26 $NaHCO_3$ and 10 glucose, bubbled with 95%

O2 and 5% CO2, and recovered in an interface chamber at 32℃ for at least 1 hour and then kept at room temperature before being transferred to a recording chamber at 34℃ in a microscope equipped with infra-red differential interference contrast (IR-DIC) and epi-fluoresencence illumination (BX51, Olympus). Glass recording electrodes (7~9 MΩ resistance) were filled with an intracellular solution consisting (in mM): 147 CsCl, 1 MgCl2, 1 EGTA, 10 HEPES, 4 Na2ATP, 0.4 Na2GTP and 2 QX-314. Tetrodotoxin (1 μM), D-AP5 (50 μM) and NBQX (10 μM) (Tocris Biosciences, MI) were included to isolated miniature inhibitory synaptic currents (mIPSCs). Recordings were collected and analyzed using Axopatch 700B amplifier and pCLAMP10 software (Molecular Devices). Miniature IPSCs were analyzed using mini Analysis Program (Synaptosoft Inc). Data were presented as mean ± s.e.m. and statistical significance was determined using Student's t-test.

CHAPTER 3:

THE INTERACTION BETWEEN NEURAL PROGENITORS AND THE VASCULATURE IN THE MAMMALIAN BRAIN

The mammalian neocortex contains two major classes of neurons: glutamatergic excitatory neurons and GABAergic inhibitory interneurons (Bayer and Altman, 1991). Excitatory neurons constitute the majority of neurons that make up functional circuits and are responsible for generating output, whereas inhibitory interneurons provide a rich variety of inhibitions that shape the output of functional circuits (Ascoli et al., 2008; Huang et al., 2007; Markram et al., 2004). Proper functioning of the neocortex critically depends on the production of a correct number of excitatory and inhibitory neurons, which largely occurs during the embryonic stages (Parnavelas et al., 2002). Extensive studies over the past decade have provided a comprehensive view of excitatory neuron neurogenesis in the developing neocortex. Radial glial progenitors (RGPs) in the ventricular zone of the developing dorsal telencephalon account for a major population of neural progenitor cells (Anthony et al., 2004; Malatesta et al., 2000; Miyata et al., 2004; Noctor et al., 2001; Noctor et al., 2002; Noctor et al., 2004; Tamamaki et al., 2001). During the peak phase of excitatory neuron neurogenesis (embryonic day 13.5 to 18.5, E13.5-E18.5, in mice), they undergo asymmetric cell division at the ventricular zone surface to self-renew and to simultaneously produce postmitotic excitatory neurons or intermediate progenitor cells that divide symmetrically in the subventricular zone (SVZ) to produce postmitotic excitatory neurons (Bultje et al., 2009; Chenn and McConnell, 1995; Kosodo et al., 2004; Miyata et al., 2004; Noctor et al., 2001; Noctor et al., 2004; Noctor et al., 2008). Moreover, individual RGPs go through multiple rounds of asymmetric cell division and generate a number of neocortical excitatory neurons that are spatially organized into ontogenetic radial units in the neocortex (Denaxa et al., 2001; Noctor et al., 2001; Rakic, 1988a; Yu et al., 2009). In contrast, our knowledge of neocortical interneuron

neurogenesis remains sparse.

It is widely accepted that most, if not all, neocortical interneurons are generated in the developing ventral telencephalon, including the ganglionic eminences (GEs) and the preoptic area (PoA), and they migrate tangentially over long distances to reach their destination in the neocortex (Anderson et al., 1997; Batista-Brito and Fishell, 2009; Fogarty et al., 2007; Gelman and Marin, 2011; Gelman et al., 2009; Guillemot, 2005; Marin and Rubenstein, 2003; Wonders and Anderson, 2006). Similar to those in the VZ of the dorsal telencephalon, progenitors in the VZ of the MGE and PoA are RGPs in nature (Anthony et al., 2004; Brown et al., 2011). They divide asymmetrically at the VZ surface to produce neocortical interneurons either directly or indirectly through intermediate progenitors that divide symmetrically in the subventricular zone (SVZ) (Brown et al., 2011). Yet, little has been known about the organizations and cellular behaviors of these RGPs in the ventral telencephalon.

Besides the neural system, the other major cellular component of the developing telencephalon is the vascular network. Studies in other systems have shown that vasculature can provide a proliferative niche for stem cells (de Rooij et al., 2008; Devenport and Brown, 2004; Ding et al., 2012; Doetsch, 2003; Emsley and Hagg, 2003). Within the dentate gyrus of the adult hippocampus, progenitors are often found in proliferative clusters in close proximity to blood vessels (Devenport and Brown, 2004). Blood vessels and its basal lamina, a unique extracellular matrix (ECM) structure rich in laminin and collagen-1, also play a role in adult SVZ progenitors (Fuchs and Whartenby, 2004; Gage, 2000). However, it remains unclear regarding the relationship between neural and vascular system in the developing brain. Based on anatomical location, independent growth patterns and developmental regulation, telencephalic vasculature fall into two categories: pial vessels and periventricular vessels. The pial vessels are directed by and envelop the neural tube by embryonic day 9.5 (E9.5) prior to the neurogenesis (Miyoshi et al., 2007). The periventricular vessels, originating from a basal vessel on the telencephalic floor of the basal ganglia primordium, actively develop in the ventral telencephalon and form an elaborated

network that progressively propagates into the dorsal telencephalon by E11.5 (Abbott et al., 2006; Alvarez-Buylla et al., 2008). Their early presence in the ventral telencephalon raises the intriguing possibility that periventricular vessels may regulate the behaviour of ventral telencephalic RGPs and neocortical interneuron production.

3.1 Retrovirus-mediated labelling of individual radial glial progenitors (RGPs)

Previous studies using retrovirus-mediated labelling of the progenitor cells in the ventricular zone of the developing dorsal telencephalon have provided unprecedented insights into the cellular processes underlying the production of neocortical excitatory neurons (Kornack and Rakic, 1995; Noctor et al., 2001; Noctor et al., 2004; Price et al., 1991; Ryder et al., 1990; Walsh and Cepko, 1992, 1993). To reveal the nature and cellular behaviors of the RGPs in the MGE/PoA that are responsible for producing a majority of neocortical GABAergic interneurons, we performed *in utero* intraventricular injection of low-titre Moloney murine leukemia retrovirus expressing enhanced green fluorescence protein (EGFP) into the lateral ventricle of the developing mouse neocortex at E12.5, the peak phase of neocortical interneuron neurogenesis in the MGE/PoA **(Figure 3.1a)**. Retrovirus infection of dividing cells leads to the integration of the viral genome into the daughter cell genome. Brains were collected at E14.5, sectioned and stained for EGFP (green) and with DNA dye DAPI **(Figure 3.1b)**. We found that in the MGE/PoA, a significant subpopulation of RGPs developed a basal process (also called radial glial fibre) with an enlarged and/or branched endfoot that terminates within the mantle region, without reaching the pial surface, in contrast to those RGPs previously observed in the dorsal telencephalon (Noctor et al., 2001).

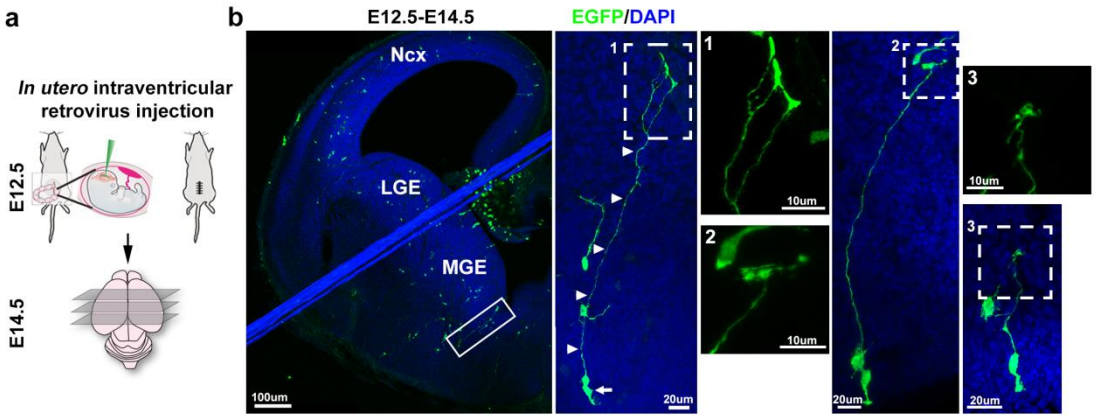
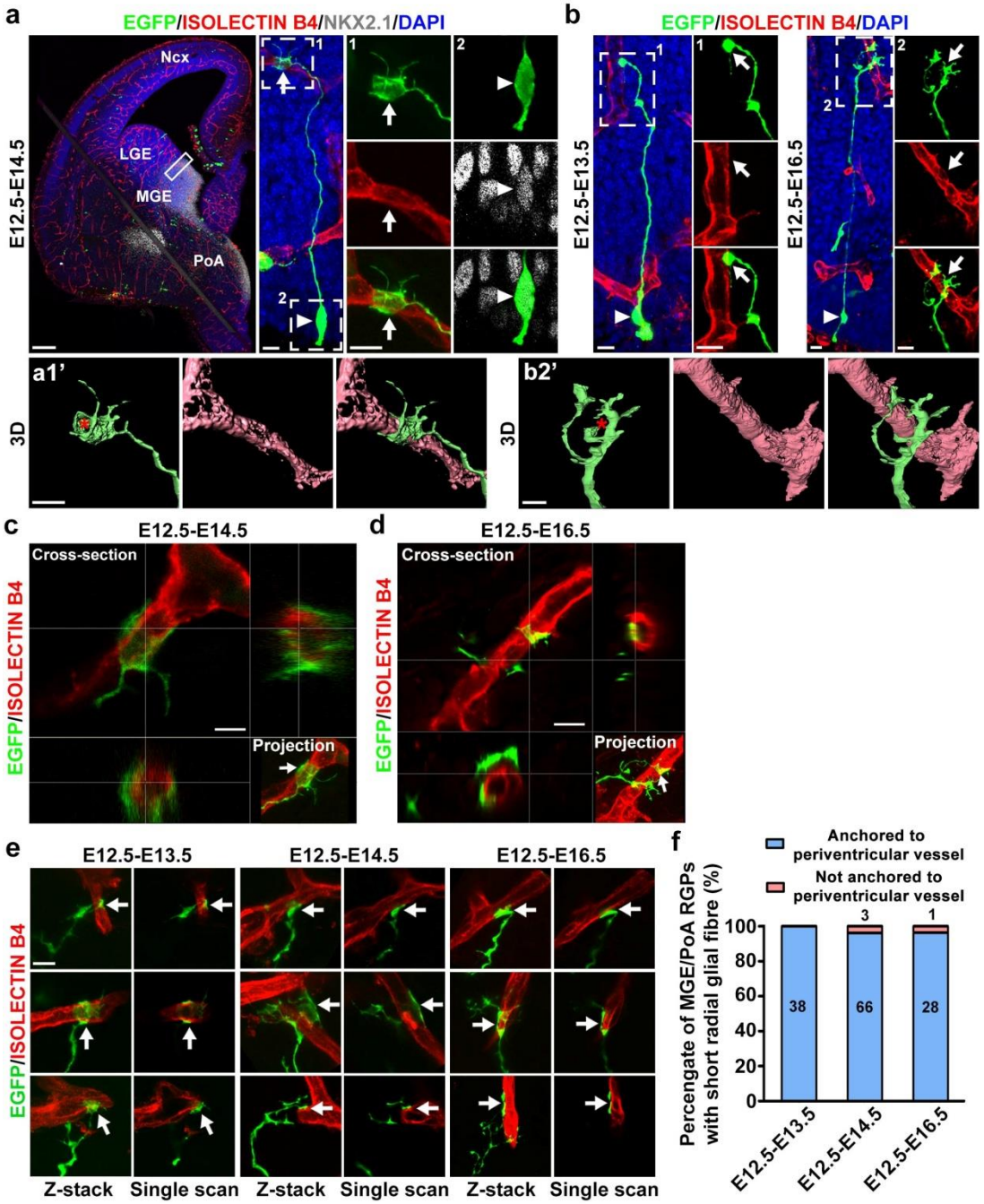


Figure 3.1: Retrovirus-mediated labelling of mitotic progenitor cells in the MGE. **(a)** Schematic diagram of *in utero* intraventricular injection of retrovirus for labelling mitotic progenitor cells in the MGE. **(b)** Image of a section of E14.5 mouse brain injected with low-titre EGFP-expressing retrovirus at E12.5 and stained with DAPI (blue). High magnification images of an EGFP-expressing RGP in the MGE (white box) are shown to the right. Note its enlarged and branched radial glial fibre end (area 1) that terminated within the mantle region, without reaching the pial surface. To the right are two more EGFP-expressing RGPs in the MGE/PoA observed in other brain samples, which also possess enlarged endfoot structure within the mantle region (areas 2 and 3).

3.2 Vascular anchorage of individual RGPs in the MGE/PoA

Since RGPs in the dorsal telencephalon are typically anchored to pial surface with their basal endfeet, we wondered what the specialized endfoot structure of these RGPs in the MGE/PoA could be anchored to within the mantle region. To determine that, we stained those brain sections for NKX2.1 (white), a transcription factor specifically expressed in the MGE/PoA progenitor of the developing telencephalon (Butt et al., 2008; Fogarty et al., 2007; Sussel et al., 1999; Xu et al., 2008), ISOLECTIN B4 (red)**,** a glycoprotein marker that reliably labels blood vessels in the developing brain (Vasudevan et al., 2008), and with DAPI (blue) **(Figure 3.2a)**. Interestingly, we observed that those basal endfeet closely wrapped around the periventricular vessels (red), as revealed by three-dimensional (3-D) reconstruction **(Figure 3.2a1' and b2')** and cross-sectional imaging analyses **(Figure 3.2c, d)**. Intact RGPs were distinguished from RGPs with a truncated radial glial fibre by the existence of complex ending structures, serial sectioning and 3-D reconstruction.

Figure 3.2: RGP anchorage to periventricular vessels in the MGE/PoA. (a) An E14.5 brain section (with EGFP-expressing retrovirus injected at E12.5) stained for blood vessel marker ISOLECTIN B4 (red), and MGE/PoA-specific transcription factor NKX2.1 (white), and with DAPI (blue). High magnification images of an EGFP-expressing RGP in the MGE (white box) positive for NKX2.1 (area 2, arrowhead) with its radial glial fibre end wrapped around a periventricular vessel (area 1, arrows) are shown to the right. Three-dimensional (3D) reconstruction images are shown at the bottom (a1'). Ncx, neocortex; LGE, lateral ganglionic eminence; MGE, medial ganglionic eminence; PoA, preoptic area. Scale bars: 200 μm, 10 μm, 10 μm (from left to right); 10 μm (a1). **(b)** E13.5 and E16.5 MGE RGPs labelled by EGFP-expressing retrovirus (green) at E12.5 and stained for ISOLECTIN B4 (red) and with DAPI (blue). High magnification of the radial glial fibre end (areas 1 and 2) are shown to the right. 3D reconstruction images of area b are shown at the bottom (b2'). Scale bars: 10 μm. **(c, d)** Cross-sectional images of the radial glial fibre end and vessel association in the MGE. The examples are the same as those shown in Figure 3.2a1 and b2. Projection images are shown at the bottom right corner. Note the wrapping of radial glial fibre end (green, arrows) around the vessel (red). Scale bars: 5 μm. **(e)** The dynamic interaction between radial glial fibre end and periventricular vessels (arrows) in the MGE/PoA at different embryonic stages. Projection images are shown to the left and single-section images to the right. Scale bar: 10 μm. **(f)** Percentage of RGPs with a short radial glial fibre that were or were not anchored to the periventricular vessel.


a
EGFP/ISOLECTIN B4/NKX2.1/DAPI
E12.5-E14.5
Ncx
LGE
MGE
PoA
1
2
b
EGFP/ISOLECTIN B4/DAPI
E12.5-E13.5
E12.5-E16.5
a1'
b2'
3D
c
E12.5-E14.5
Cross-section
Projection
EGFP/ISOLECTIN B4
d
E12.5-E16.5
e
E12.5-E13.5
E12.5-E14.5
E12.5-E16.5
Z-stack
Single scan
f
Anchored to periventricular vessel
Not anchored to periventricular vessel
Percengate of MGE/PoA RGPs with short radial glial fibre (%)
100
80
60
40
20
0
38
66
28
3
1
E12.5-E13.5
E12.5-E14.5
E12.5-E16.5

Since this intimate interaction between RGPs and vessels has never been reported before, systematic characterization of the RGP basal endfoot structure and its association with periventricular vessels was carried out from E13.5 to E16.5, with retroviruses injected at E12.5. Similar tight association between RGPs and periventricular vessels were found in the MGE/PoA at different embryonic stages when neocortical interneurons were produced **(Figure 3.2e)**. Notably, the endfoot structure associated with the vessel appeared diverse, ranging from simple club-shaped **(Figure 3.2b1)** to complex claw-shaped with numerous branches **(Figure 3.2b2)**. Virtually all non-pia-reaching RGPs in the MGE/PoA were associated with the periventricular vessel **(Figure 3.2f)**, indicating that the vascular interaction is highly significant during the embryonic development of ventral telencephalon. The staining pattern of ISOLECTIN B4 was also further confirmed by a specific marker for endothelial cells, Platelet endothelial cell adhesion molecule-1 (PECAM-1, also known as cluster of differentiation 31, CD31) (Williams et al., 1996) **(Figure 3.3)**. Notably, ISOLECTIN B4 stains not only the endothelial cells but also pericytes (white arrowheads) at the embryonic stage, but the RGP basal endfeet appear to interact with the endothelial cells (white arrows) instead of the pericytes.

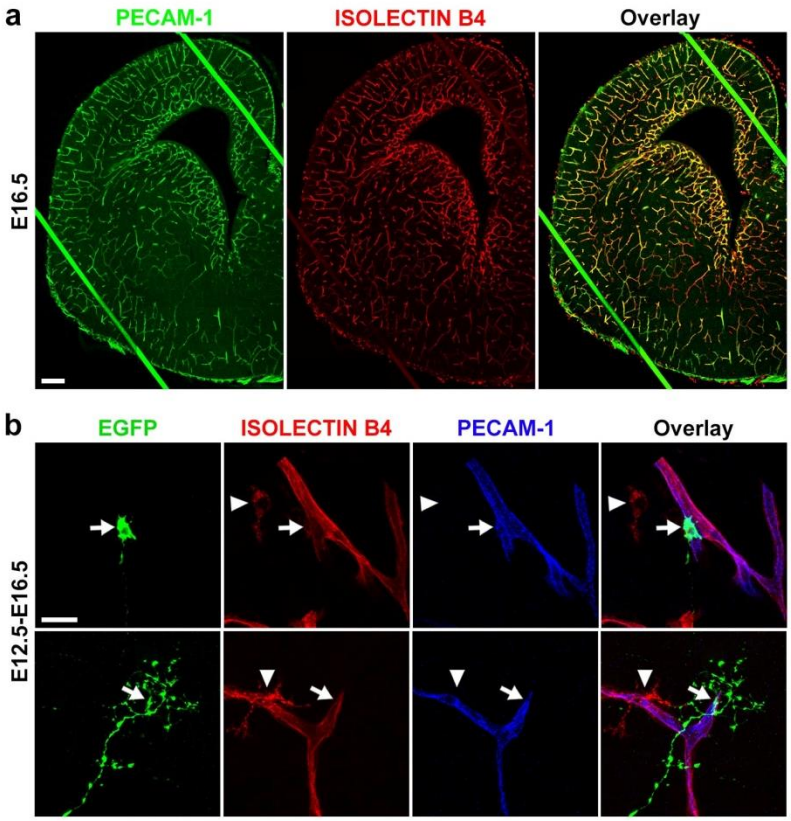


Figure 3.3: Anchorage of the RGP basal endfoot to the periventricular vessel in the MGE/PoA. (a) E16.5 brain sections stained for Platelet endothelial cell adhesion molecule-1 (PECAM-1, green), an endothelial cell specific marker, and ISOLECTIN B4 (red), another blood vessel marker. Note the co-labelling of the vessels by PECAM-1 and ISOLECTIN B4. Scale bar: 200 μm. **(b)** Representative images showing the association between the radial glial fibre end labelled by EGFP-expressing retrovirus (green, arrows) and the periventricular vessel labelled by ISOLECTIN B4 (red) and PECAM-1 (blue) in the MGE/PoA. Arrowheads indicate the pericytes labelled by ISOLECTIN B4, but not by PECAM-1. Scale bar: 20 μm.

3.3 Pial anchorage of individual RGPs in the dorsal neocortex

RGPs are a widespread non-neuronal cell type in the developing CNS of all vertebrates examined so far, although only those in the dorsal telencephalon that produce excitatory neurons have been extensively studied. Their characteristic radial bipolar morphology and their astroglial properties were also first defined in the dorsal telencephalon. These long bipolar cells expand across the entire thickness of the developing neocortex with a long basal radial process pointing to the pial surface, a

short apical ventricular endfoot reaching the ventricular zone (VZ) surface, and the soma located in the VZ (Bentivoglio and Mazzarello, 1999; Cameron and Rakic, 1991). Given that the ventricular zone of both the developing dorsal and ventral telencephalon is exposed to the retrovirus injected into the lateral ventricle, the injections we have performed above resulted in EGFP labelling of dividing progenitor cells in the ventricular zone of the dorsal telencephalon and their daughter cells **(Figure 3.4a, arrowhead)**, as previously reported (Noctor et al., 2001; Noctor et al., 2004). In contrast to those RGPs in the ventral telencephalon, the radial glial endfeet of RGPs in the dorsal telencephalon were rarely anchored to the vessel **(Figure 3.4a, b)**; instead, they were anchored to the pial basement membrane labelled by LAMININ, which is a thin layer of extracellular matrix proteins (Haubst et al., 2006) **(Figure 3.4c)**.

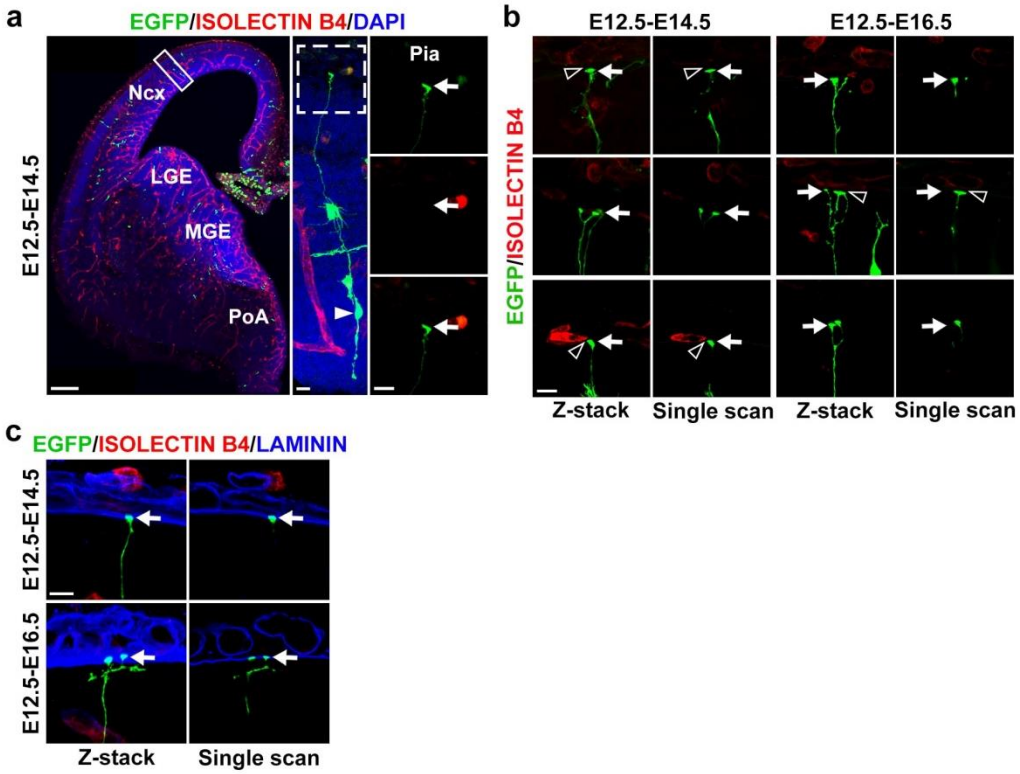


Figure 3.4: RGPs in the dorsal telencephalon predominantly possess a radial glial fibre attached to the pial basement membrane, but not the vessel. (a) An E14.5 brain section (with retrovirus injected at E12.5) stained for ISOLECTIN B4 (red) and with DAPI (blue). High magnification images of an EGFP-expressing clone containing an RGP (arrowhead) with a radial glial fibre reaching the pia (broken lines and arrows) in the developing neocortex (Ncx) are shown to the right. Scale bars: 100 μm, 10 μm and 10 μm (from left to right). **(b)** Radial glial fibre ends (arrows) in the neocortex stained for ISOLECTIN B4 (red). Projection images are shown to the left and single-section images are shown to the right. Note that the radial glial fibre ends are separated from the vessel (open arrowheads). Scale bar: 10 μm. **(c)** Radial glial fibre ends in the neocortex stained for ISOLECTIN B4 (red) and LAMININ (blue). Projection images are shown to the left and single-section images are shown to the right. Scale bar: 10 μm.

Recently, one research group reported that there are a subpopulation of VZ dividing cells in the dorsal telencephalon that either possess a short basal process or lack the basal process altogether (‘club-shaped’), which are morphologically, ultrastructurally and molecularly distinct from typical RGPs and are named as short neural precursors (SNPs) (Gal et al., 2006). This observation raises the possibility that those SNPs may be anchored to blood vessels since they do not extend their basal

processes to the pial surface. However, using our retroviral labeling approach, we rarely observed those SNPs in the dorsal telencephalon and therefore did not be able to test this possibility.

Altogether, these results suggest that periventricular vascular anchorage is a prominent and distinct property of ventral telencephalic RGPs that produce neocortical interneurons, and is rarely seen in the dorsal telencephalon that generate excitatory neurons.

3.4 Progressive generation of vessel-anchored RGPs in the MGE/PoA

To visualize the interaction between the radial glial fibre of RGPs and the vessel at the population level, we performed intraventricular injection of the lipophilic tracer 3H-Indolium,5-[[[4-(chloromethyl)benzoyl]amino]methyl]-2-[3-(1,3-dihydro-3,3-dimethyl-1-octadecyl-2H-indol-2-ylidene)-1-propenyl]-3,3-dimethyl-1-octadecyl-, chloride (CM-DiI) into the lateral ventricles at E10.5, E11.5 and E14.5 to label the VZ RGPs. Brains were later sectioned and stained with ISOLECTIN B4 **(Figure 3.5)**. At E11.5, the organization of RGPs in the MGE was similar to that in the neocortex; most if not all the DiI-labelled radial glial fibres projecting outside the VZ reached the pial surface in straight bundles **(Figure 3.5a, left, areas 1-3, red)** and did not obviously associate with the vessels, despite their presence in the ventral telencephalon **(Figure 3.5a, left, area 1 and 2, green)**. Notably, at E10.5 and E11.5, a ventral-to-dorsal gradient of periventricular vessels could be observed in the developing telencephalon - the periventricular vessels are very sparsely distributed in the dorsal telencephalon, in contrast to the elaborate vascular network that is already formed in the MGE. At E14.5, while the radial glial fibres in the neocortex remained extended to the pial surface **(Figure 3.5b, right)**, only a small proportion of the radial glial fibres in the MGE reached the pial surface **(Figure 3.5a, right, area 6)**. Interestingly, they extensively covered the periventricular vessels **(Figure 3.5a, right, areas 4 and 5, arrows)**, indicating a robust interaction between radial glial fibres and periventricular vessels. Moreover, these results suggest that the association between

RGPs and periventricular vessels in the MGE occurs progressively as development proceeds.

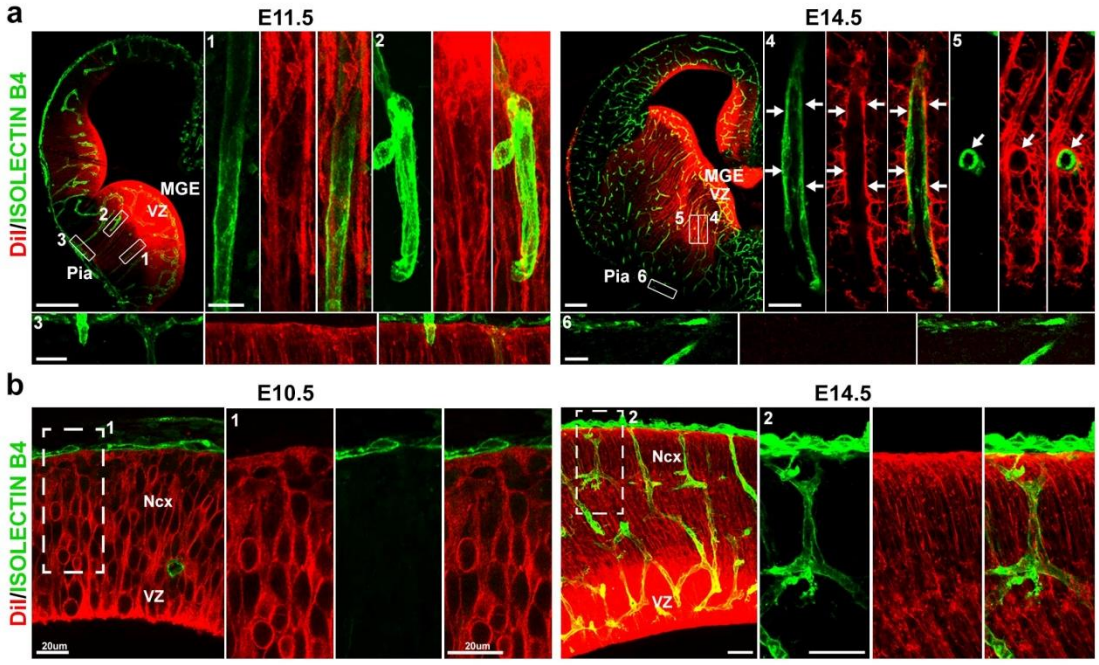
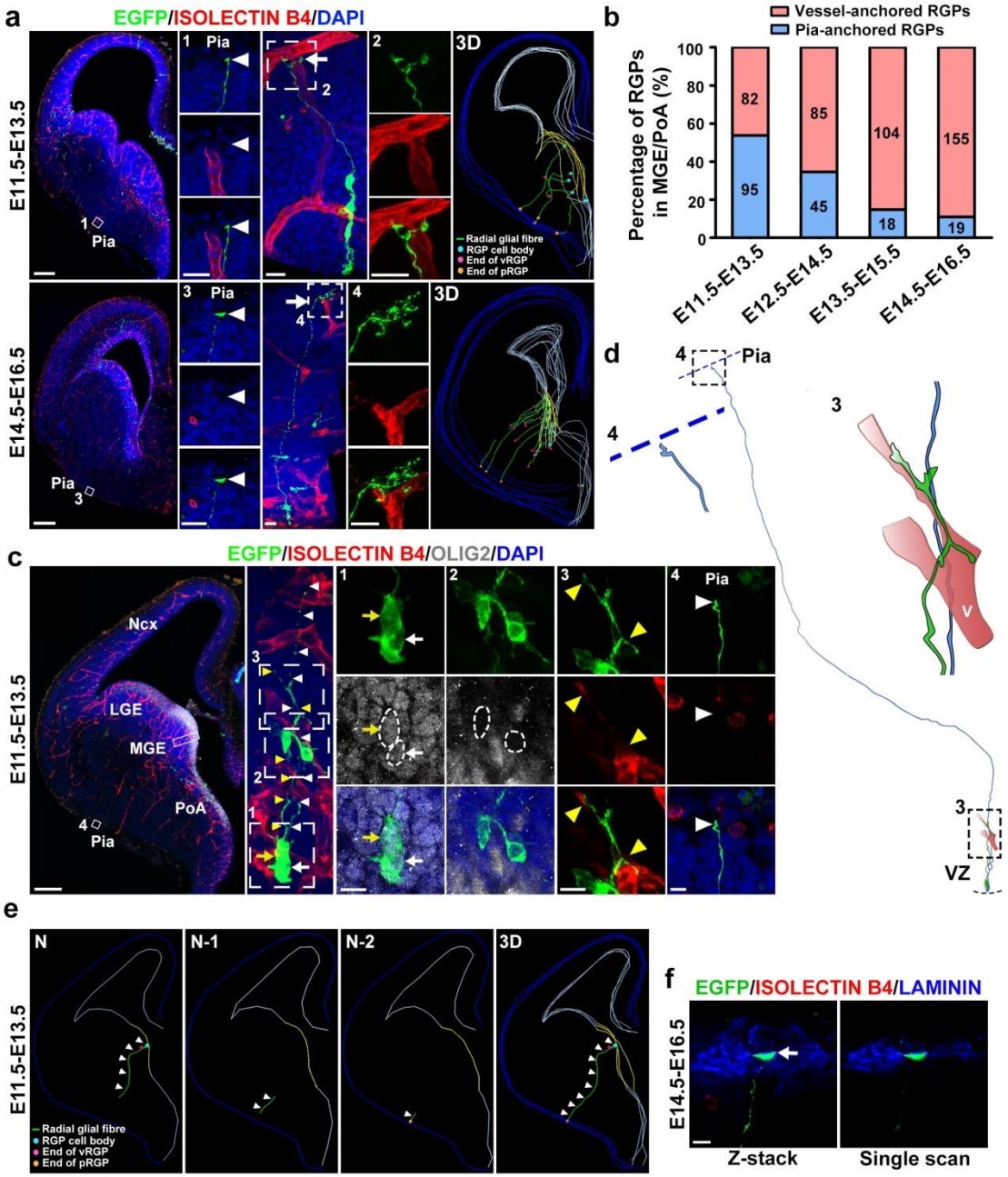


Figure 3.5: DiI labelling reveals the radial glial processes of RGPs in the ventral and dorsal telencephalon at a population level. (a) E11.5 and E14.5 brain sections labelled by DiI (red) at the VZ of the MGE and stained for ISOLECTIN B4 (green). At E14.5, DiI-labelled radial glial fibres aggregate at the periventricular vessels (areas 4 and 5, arrows) with few reaching the pia (area 6), while those at E11.5 do not obviously associate with vessels (areas 1 and 2) and mostly reach the pia (area 3). Scale bars: 200 μm (left), 20 μm (right) and 20 μm (bottom). **(b)** E10.5 and E14.5 neocortex labelled by DiI (red) at the VZ and stained for ISOLECTIN B4 (green). High magnification images of a region containing an extra-VZ vessel (broken lines) are shown to the right. Scale bars (from left to right): 20μm, 20μm, 40μm, 40 μm.

We next systematically analyzed the temporal development of RGP-vessel association in the MGE/PoA at the clonal level. We injected low-titre EGFP-expressing retrovirus into the lateral ventricle at E11.5, E12.5, E13.5 and E14.5 to label individual dividing RGPs in the MGE/PoA. Brains were harvested two days later and subjected to serial sectioning and 3D reconstruction analysis to recover all EGFP-labelled RGPs in the MGE/PoA and assess their radial glial fibre end location and configuration **(Figure 3.6a, b)**. As expected, we observed simple club-shaped

ends at the pial surface **(Figure 3.6a, areas 1 and 3, arrowheads)** that were clearly not associated with the vessel, as well as branched, claw-shaped ends associated with the periventricular vessel in the mantle region **(Figure 3.6a, areas 2 and 4, arrows)**. Notably, the pia-reaching radial glial fibre endings in the MGE/PoA shared a similar morphology with those in the dorsal telencephalon **(Figure 3.4)** and were also anchored to the pial basement membrane **(Figure 3.6f)**. At E13.5 (with retroviruses injected at E11.5), the fraction of pia-anchored radial glial fibre endings was similar to that of vessel-anchored radial glial fibre endings **(Figure 3.6a top, and b)**. However, as time proceeded, the fraction of pia-anchored endings progressively decreased, whereas the fraction of vessel-anchored endings concurrently increased. At E16.5 (with retroviruses injected at E14.5), the vast majority of proliferative RGPs (~90%) in the MGE/PoA possessed a short radial glial fibre that was anchored to the periventricular vessel in the mantle region **(Figure 3.6a bottom, and b)**.

Figure 3.6: Progressive generation of the periventricular vessel-anchored RGPs in the MGE/PoA. (a) E13.5 and E16.5 brain sections with retrovirus (green) injected two days earlier. High magnification images of a pia-anchored radial glial fibre (areas 1 and 3) and a periventricular vessel-anchored RGP (areas 2 and 4) are shown in the middle. 3D reconstruction images of the configuration of RGPs in the MGE/PoA are shown to the right. Blue and white lines indicate the contours of the pia and the VZ surface. Blue dots indicate RGP cell bodies, green lines indicate radial glial fibres, red dots indicate the radial glial fibre ends anchored to the vessel, and orange dots indicate the ends anchored to the pia. Scale bars: 200 μm, 20 μm, 20 μm, and 20 μm (from left to right). **(b)** Stereological quantification of the fraction of RGPs anchored to the vessel or the pia at different developmental stages. **(c)** An E13.5 brain section (left, retrovirus injected at E11.5) with a clone containing a pair of OLIG2-positive RGPs (right, yellow and white arrows, area 1). Note one RGP with a short fibre (yellow arrowheads) anchored to the periventricular vessel (yellow arrowheads, area 3), while the other with a long fibre reaching the pia (white arrowheads, area 4). Scale bars: 200 μm; 10 μm; 10 μm; 10 μm. **(d)** Schematic reconstruction of the RGP pair. The pia-reaching RGP is shown in blue and the vessel (v, red)-anchored RGP is shown in green. The highlighted areas (3 and 4) are corresponding to the same areas (3 and 4) in Figure 3.6c. **(e)** Schematic representation of consecutive sections and reconstruction of the clonal pair of RGPs shown in Figure 3.6c. Arrowheads indicate the pia-reaching radial glial fibre. **(f)** A pia-anchored radial glial fibre end (green, shown in Figure 3.6a, area 3) in the MGE stained for ISOLECTIN B4 (red) and LAMININ (blue). The projection image is shown to the left and the single-section image is shown to the right. Note that the pia-reaching radial glial fibre end attaches to the LAMININ-labelled pial basement membrane, but not the vessel. Scale bar: 10 μm.


a
EGFP/ISOLECTIN B4/DAPI
E11.5-E13.5
E14.5-E16.5
Pia
3D
Radial glial fibre
RGP cell body
End of vRGP
End of pRGP
b
Vessel-anchored RGPs
Pia-anchored RGPs
Percentage of RGPs in MGE/PoA (%)
82
95
85
45
104
18
155
19
E11.5-E13.5
E12.5-E14.5
E13.5-E15.5
E14.5-E16.5
c
EGFP/ISOLECTIN B4/OLIG2/DAPI
Ncx
LGE
MGE
PoA
d
V
VZ
e
N
N-1
N-2
f
EGFP/ISOLECTIN B4/LAMININ
Z-stack
Single scan

The progressive increase in vessel-anchored RGPs raised the possibility that they were generated by pia-anchored RGPs through proliferative divisions. Using the similar approach as above but with even lower titer of retroviruses, we aimed to label individual RGPs that could potentially go through proliferative division after the viral infection at E11.5. Two days later, isolated clusters of RGPs could be captured in the MGE/PoA, which most likely came from proliferative divisions of one infected RGP. These clusters could be divided into three types based on their cell composition. The first type of clusters contained one pia-anchored RGP and one cell that remained connected with the pia-anchored RGP at the VZ surface, likely representing the final stage of mitosis (**Figure 3.7a,** n=2). Since it was positive for progenitor cell marker OLIG2, a transcription factor selectively expressed in the VZ of the ventral telencephalon (Petryniak et al., 2007), we speculate that the later cell was a new-born RGP that did not inherit the long basal process of pia-anchored RGP and was in the beginning process of re-growing its own. The second type was composed of one pia-anchored RGP containing a long radial glial fibre reached the pia (white arrow and arrowheads) and the other possessing a short radial glial fibre attached to the periventricular vessel (yellow arrow and arrowheads) (**Figure 3.7c**, n=3). The third type included two RGPs that were both Olig2+ and possessed a short radial glial fibre anchored to the periventricular vessel **(Figure 3.7c, n=7)**, indicating a self-proliferation of vessel-anchored RGPs. Altogether, these results indicates that vessel-anchored RGPs are initially generated by pia-anchored RGPs, which comprise the major population of RGPs before the onset of neurogenesis in the MGE/PoA; after the proliferative division, new-born RGPs do not inherit any basal process and have to re-grow its own, which gradually anchor to nearby periventricular vessels and become vessel-anchored RGPs; these vessel-anchored RGPs can expand its own pool through proliferative divisions, which greatly contribute to the progressively increasing proportion of vessel-anchored RGPs through the embryonic development.

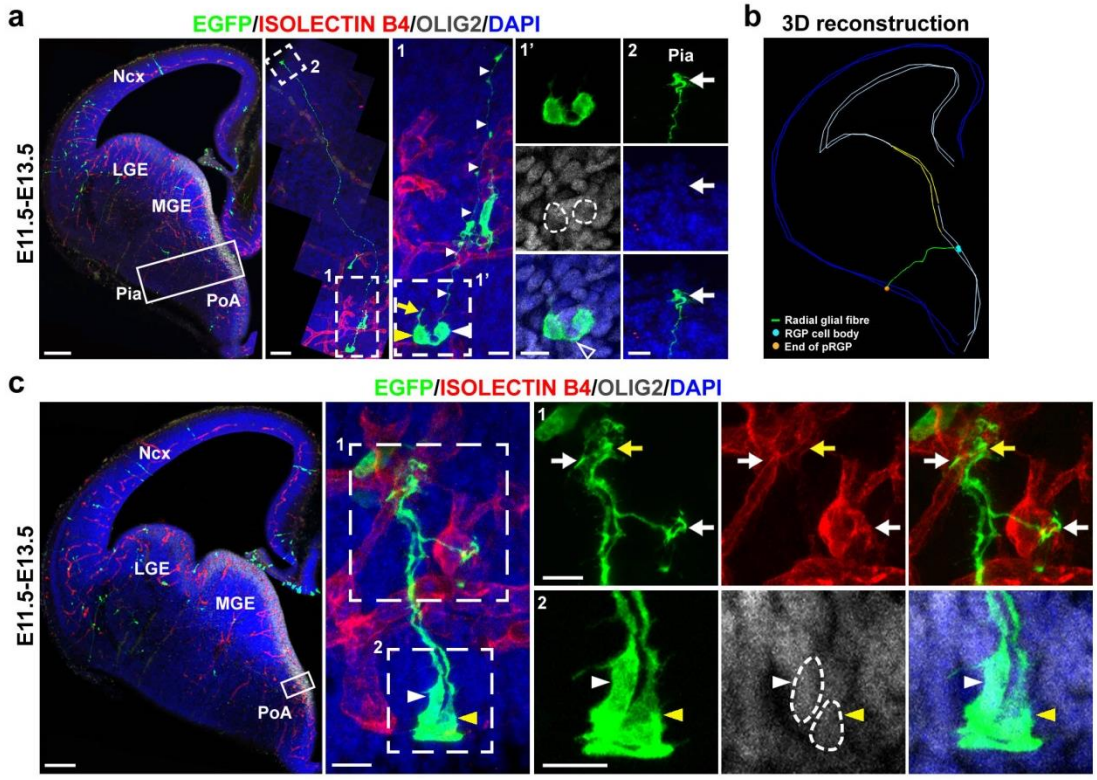


Figure 3.7: Generation of RGPs with short radial glial fibres anchored to the periventricular vessel. (a) An E13.5 brain section (with EGFP-expressing retrovirus injected at E11.5) stained for ISOLECTIN B4 (red) and OLIG2 (white), and with DAPI (blue). High magnification images of a clonal pair of RGPs (solid line) positive for OLIG2 (yellow and white arrowheads, area 1 and 1') are shown to the right. One RGP extends a radial glial fibre reaching the pia (area 2, white arrows), whereas the other RGP possesses a short radial glial fibre (yellow arrow). Note that the two RGPs remain connected at the VZ surface (open arrowhead). Scale bars: 200 μm, 40 μm, 20 μm, 10 μm and 10 μm (from left to right). **(b)** 3D reconstruction image of the clonal RGP pair in a. **(c)** An E13.5 brain section (with retrovirus injected at E11.5) stained for ISOLECTIN B4 (red) and OLIG2 (white), and with DAPI (blue). High magnification images of the clonal RGP pair positive for OLIG2 (area 2, arrowheads) are shown to the right. Note that the radial glial fibre ends of both RGPs interact with the periventricular vessel (area 1, arrows). Scale bars: 200 μm, 10 μm and 10 μm (from left to right).

3.5 Active interaction between radial glial fibre endings and periventricular vessels

The morphology of vessel-anchored radial glial fibre endings in the MGE/PoA was diverse and often complex, suggesting that this RGP-vessel interaction is dynamic. To test this, we performed live imaging experiments to monitor the

interaction. We took advantage of the *Tek-Cre* transgenic mouse line, in which Cre recombinase is selectively expressed in endothelial cells under the *Tek* (i.e. *Tie2*) promoter (Cao et al., 2004). By crossing it with *Ai14-tdTomato*, a Cre-dependent fluorescent reporter mouse line (Madisen et al., 2010), we labelled the vessels in red fluorescence **(Figure 3.8)**. We then performed *in utero* intraventricular injection of low titre EGFP-expressing retrovirus into the *Tek-Cre;Ai14-tdTomato* mouse embryos at E12.5 to label individual dividing RGPs in the MGE/PoA. Organotypic brain slice cultures were prepared two days later and subjected to time-lapse imaging analysis for up to 48 hours (Brown et al., 2011).

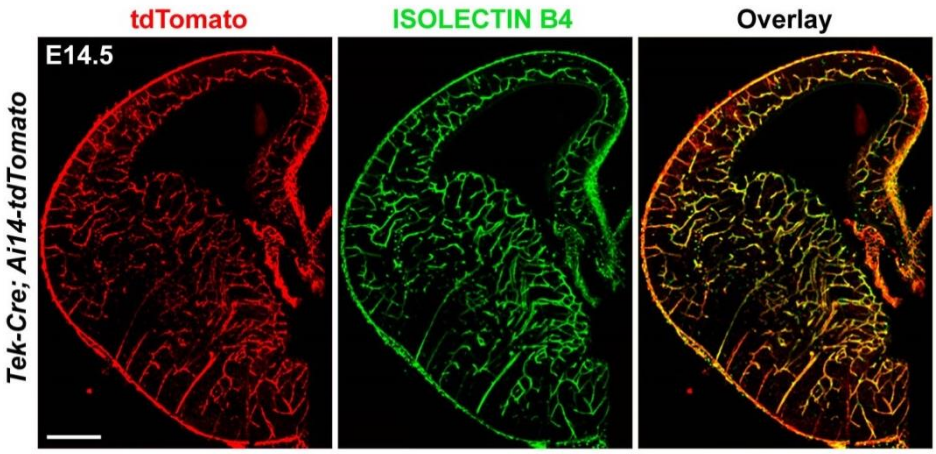
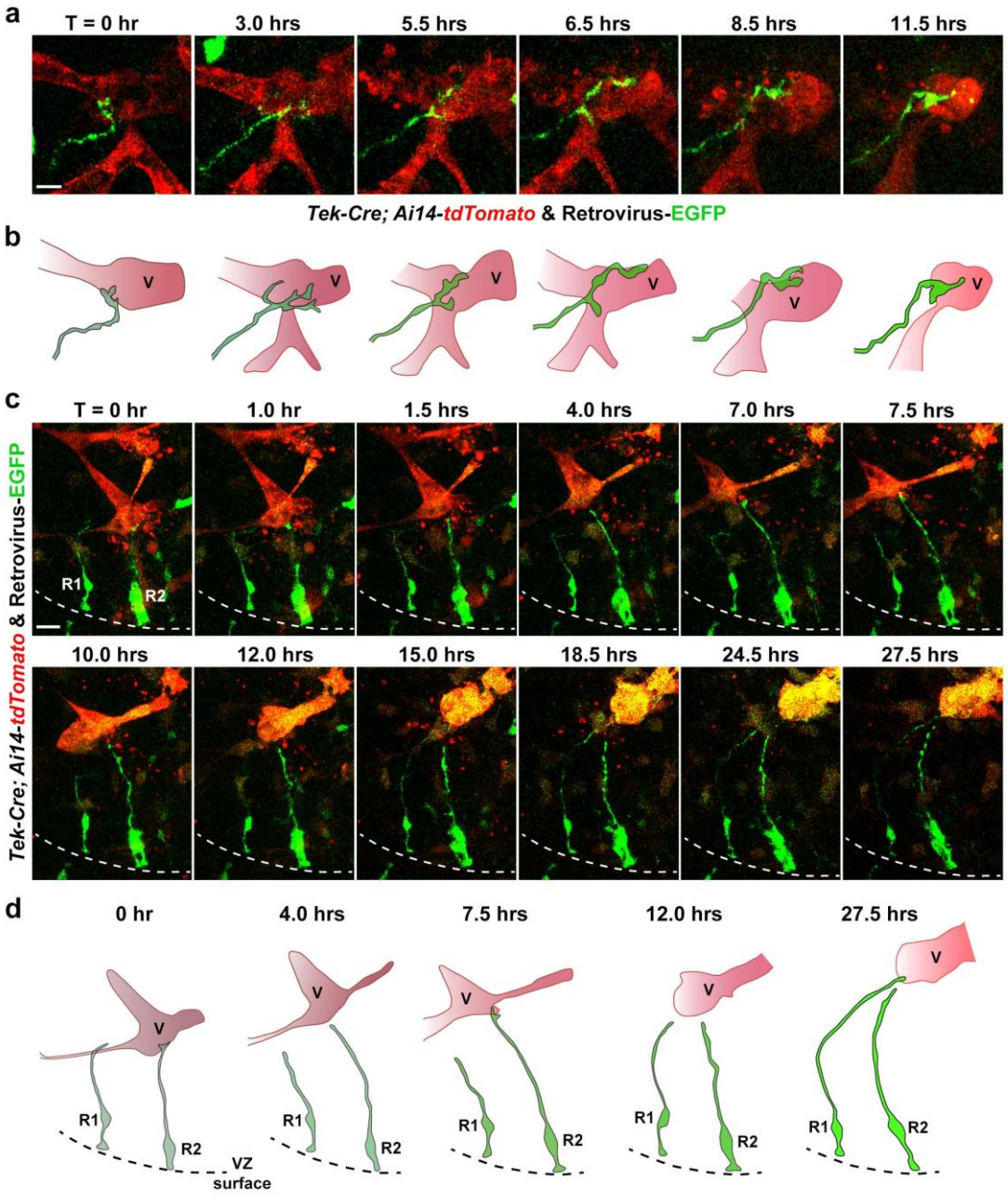


Figure 3.8: Labelling of vessels using *Tek-Cre;Ai14-tdTomato*. Representative images of an E14.5 brain section of *Tek-Cre;Ai14-tdTomato* mouse stained for ISOLECTIN B4 (green). Note the effective labelling of vessels by the expression of tdTomato (red). Scale bar: 200 μm.

Through the live imaging analysis, we observed that the radial glial basal endfoot continuously changed its morphology to probe the environment **(Figure 3.9a, b)**. Upon contacting with the vessel, the endfoot grew branches along the vessel and developed enlarged contact sites. Moreover, as the vessel remodelled its configuratio, the radial glial fibre actively adjusted its length and orientation to maintain or resume its interaction with the vessel **(Figure 3.9c, and d)**. Together, these results suggest

that RGPs actively search for the vessel in the mantle region of the MGE/PoA and that the association between the radial glial fibre end and the vessel is actively maintained. Additionally, these live imaging have further confirmed that the radial glial fibre endings of these RGPs are not static or stable structures, and instead they are highly dynamic in their ability to extend or retract branches, even when they are interacting with vasculature.

Figure 3.9: Dynamic and active interaction between the radial glial fibre and the periventricular vessel in the MGE/PoA. (a) Time-lapse images of a radial glial fibre end (green) and the periventricular vessel (red) in the MGE of an organotypic slice culture prepared from an E14.5 *Tek-Cre;Ai14-tdTomato* mouse brain that received *in utero* intraventricular injection of EGFP-expressing retrovirus (green) at E12.5. Time is indicated on the top. Note the active growth and enlargement of the radial glial fibre end upon contacting the vessel. Scale bar: 20 μm. **(b)** Schematic representation of the dynamic interaction between the radial glial fibre end and the vessel (V). **(c)** Time-lapse images of the interaction between two RGPs (green, R1 and R2) and a nearby periventricular vessel (red) in the MGE. Time is indicated on the top. Note that, as the vessel remodels, the radial glial fibres of the two RGPs actively change their orientation and grow towards the vessel. Scale bar: 20 μm. **(d)** Schematic representation of the active interaction between the radial glial fibre and the vessel (V). Broken lines indicate the VZ surface.


a
T = 0 hr
3.0 hrs
5.5 hrs
6.5 hrs
8.5 hrs
11.5 hrs
Tek-Cre; Ai14-tdTomato & Retrovirus-EGFP
b
V
c
T = 0 hr
1.0 hr
1.5 hrs
4.0 hrs
7.0 hrs
7.5 hrs
R1
R2
10.0 hrs
12.0 hrs
15.0 hrs
18.5 hrs
24.5 hrs
27.5 hrs
Tek-Cre; Ai14-tdTomato & Retrovirus-EGFP
d
0 hr
4.0 hrs
7.5 hrs
12.0 hrs
27.5 hrs
V
R1
R2
VZ
surface

3.6 Dividing RGPs maintain vessel anchorage

A defining feature of RGPs is their mitotic capability. As we previously showed, RGPs in the MGE/PoA displayed interkinetic nuclear migration and divided at the VZ surface to produce neocortical interneurons (Brown et al., 2011). Here, we found that throughout the process, RGPs maintained their association with the vessel, even though the morphology of the radial glial fibre endfeet changed dynamically **(Figure 3.10)**. Notably, a vast majority of dividing RGPs (25 out of 29) observed in our live imaging experiments had their radial glial endfeet associated with the vessel either through the mitosis or right before entering the anaphase, suggesting that the vessel anchorage may facilitate RGP division and neocortical interneuron neurogenesis.

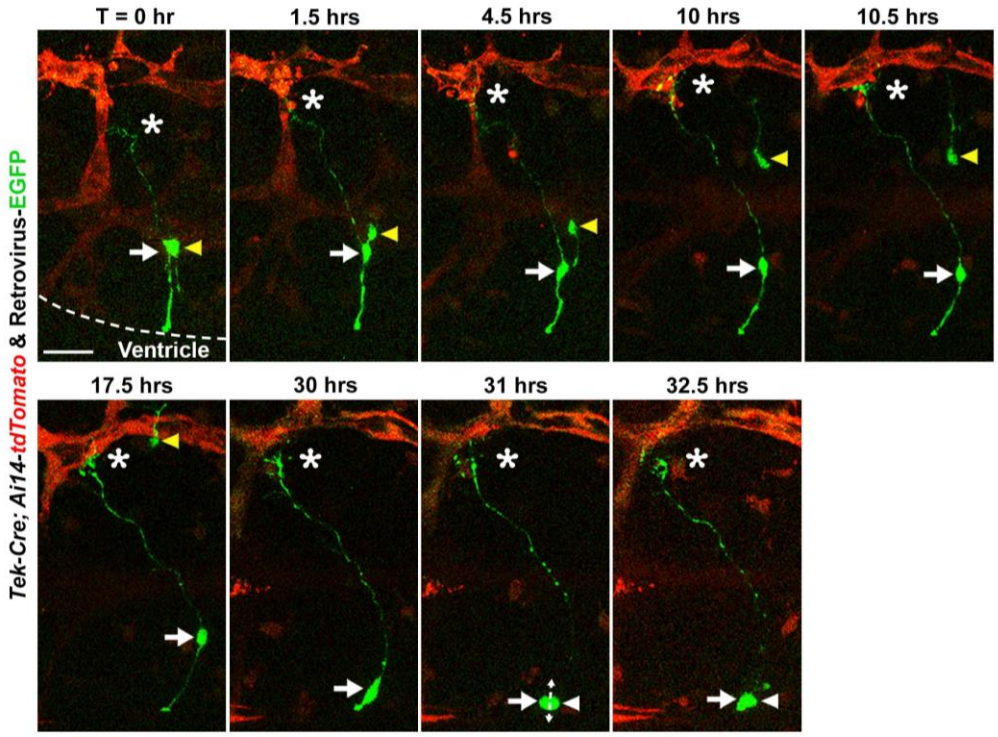


Figure 3.10: RGPs divide while maintaining anchorage to the periventricular vessel in the MGE/PoA. Time-lapse images of an RGP in the MGE that undergoes mitosis in an organotypic culture slice prepared from an E14.5 *Tek-Cre;Ai14-tdTomato* (red) mouse brain with EGFP-expressing retrovirus (green) injected at E12.5. Time is indicated on the top. Note that the RGP undergoes interkinetic nuclear migration (white arrows) and divides at the VZ surface (broken line), and throughout the process, the radial glial fibre end remains attached to the periventricular vessel (asterisks). The double-arrowed broken line indicates the cleavage plane. White arrowheads indicate the new born daughter cell and yellow arrowheads indicate a previously born daughter cell of the RGP that progressively migrates away. Scale bar: 30 μm.

3.7 Vascular anchorage of individual RGPs in the LGE

Located next to the MGE, the lateral ganglionic eminence (LGE) represents another major domain of the ventral telencephalon, which produces a distinct set of cell types including the projections neurons of the striatum (Kiel and Morrison, 2008; Wichterle et al., 2001; Yilmaz et al., 2006), and the interneurons that migrate rostrally to the olfactory bulb (Wichterle et al., 1999; Wichterle et al., 2001). In fact, the migration of new-born interneurons to the olfactory bulb is led by the LGE into

adulthood. The route that newly generated neurons take from the anterior subventricular zone to the olfactory bulb is called the Rostral Migratory Stream (RMS). Unlike the MGE, which gradually regresses through the development to a tiny domain in the adult brain, the LGE maintains and progressively transit to become the main domain of the subependymal zone (SEZ) where a relatively small proportion of neural stem cells persist in the adulthood (Ogawa et al., 1991).

Although there have been some studies suggesting that RGPs are the developmental source of adult neural stem cells (Lennartsson and Ronnstrand, 2006), very little is known about the RGP population in the LGE. In our studies, we found that periventricular vessel-anchored RGPs were readily observed not only in the MGE but also in the LGE **(Figure 3.11a)**. Although we did not quantitatively analyze the developmental change of the proportion of vessel-anchored RGPs, they could be easily found at different embryonic stages (E14.5, E15.5, and E16.5, **Figure 3.11b**). These results raise an intriguing possibility that these vessel-anchored RGPs are the more direct origin of adult neural stem cells that persist in the SEZ. It would be interesting to investigate further into the lineage relationship of these vessel-anchored RGPs and adult neural stem cells (Further discussed in Chapter 6).

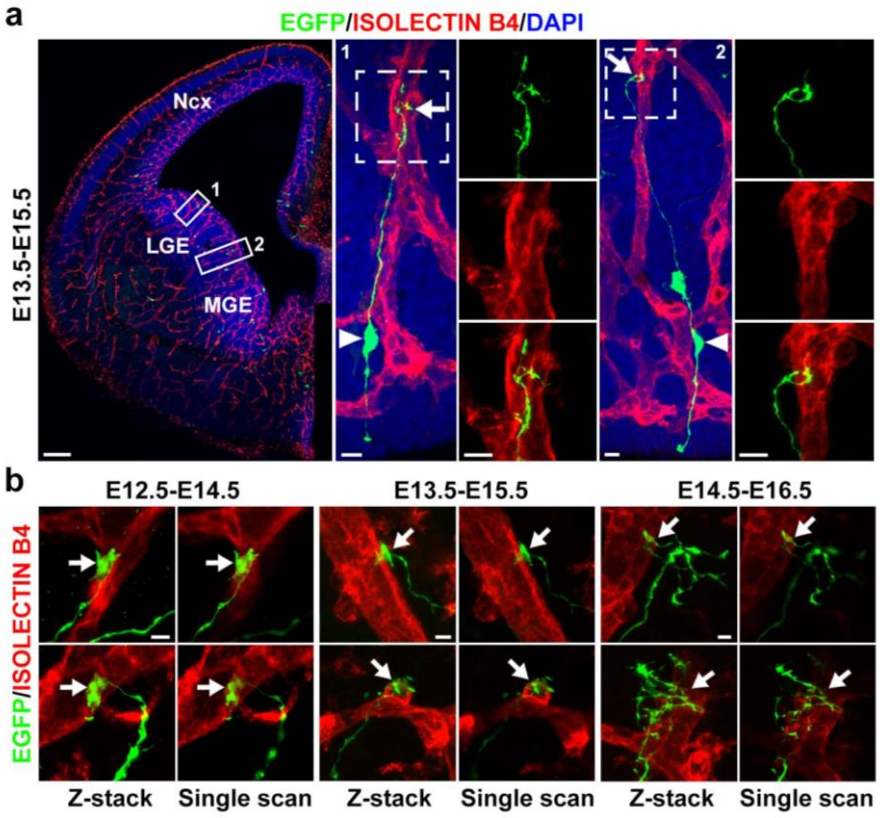


Figure 3.11: Anchorage of radial glial fibre ends to the periventricular vessels in the LGE. (a) An E15.5 brain section that received in utero intraventricular injection of EGFP-expressing retrovirus (green) at E13.5 and stained for ISOLECTIN B4 (red) and with DAPI (blue). High magnification images of two RGPs in the LGE (areas 1 and 2) with their radial glial fibre end (arrows and broken lines) attached to the vessel are shown to the right. Scale bars: 200 μm (left), 10 μm (others). **(b)** The dynamic interaction between radial glial fibre end and periventricular vessels (arrows) in the LGE at different embryonic stages. Projection images are shown to the left and single-section images are shown to the right. Scale bar: 10 μm.

3.8 Summary

As neural progenitors, RGPs throughout the CNS are thought to have similar properties and organization, i.e. expressing BLBP and GLAST, and in bipolar morphology with a short ventricular endfoot that reaches the ventricular zone surface and a long radial glial fibre that attaches to the pia surface (Anthony et al., 2004). Our study for the first time revealed the cellular organization heterogeneity of RGPs in the developing brain. While RGPs in the dorsal telencephalon responsible for producing

neocortical excitatory neurons are anchored to the pia surface, RGPs in the ventral telencephalon responsible for producing neocortical inhibitory interneurons are progressively anchored to the periventricular vessels. Given that the ventral telencephalon expands rapidly with the formation of the striatum between the ventricular zone and the pia, this unique organization of the ventral RGPs enables them to efficiently cope with the distinct developmental change in the ventral telencephalon. Using time lapse imaging analysis of the vessels and RGPs simultaneously, we demonstrated that the association between RGPs and the vessels is active and dynamic. Moreover, RGPs maintain the vessel association as they divide, suggesting the vascular niche anchorage is critical for progenitor division and maintenance. Our study not only for the first time revealed a robust association between the vessels and RGPs in the developing brain, but also demonstrated how this association progressively forms as development proceeds (i.e. proliferative division of RGPs and active interaction between RGPs and the vessels).

CHAPTER 4:

THE MECHANISM MEDIATING THE INTERACTION BETWEEN RADIAL GLIAL PROGENITORS AND THE VASCULATURE IN THE VENTRAL TELENCEPHALON

The blood vessels typically consist of endothelial cells that enclose blood cells and plasma, a basement membrane (BM) surrounding the endothelial cells and mural cells (pericytes or smooth muscle cells) (Nikolova et al., 2007). The BM is a thin sheet of highly specialized extracellular matrix (ECM) that is enriched with laminins, collagen IV, entactin-1/nidogen-1, and proteoglycans (Erickson and Couchman, 2000). Originally believed to serve as a selective barrier and scaffold to which cells adhere, it has become evident that the individual components of the BM are regulators of biological activities such as cell growth, differentiation, and migration, and that they influence tissue development and repair (Lennartsson et al., 1999; Thommes et al., 1999). Although BMs are widespread tissue components, their fine structure and composition varies from tissue to tissue, as well as within the same tissue at different developmental periods and during repair. All BMs contain laminins, entactin-1/nidogen-1, Type IV collagen, and heparan sulfate proteoglycans (HSPGs). It has been indicated that BM can not only provide the structural support to maintain the physical barriers, but also create an interactive interface between cells and surrounding environment that can mediate local and distant signals (Colognato and ffrench-Constant, 2004; Fuentealba et al., 2012; Wolf, 1978). *In vivo*, the endothelial-derived diffusible factors may bind to the vascular BM (Kerever et al., 2007; Mercier et al., 2002). As such, the rich vascular BM may be an important site for the integration of niche signals arising not only from the blood vessels, including pericytes, endothelial cells, and factors from the blood, but also from ependymal cells, mesenchymal cells, axon terminals, as well as the cerebrospinal fluid.

Among those ECM molecules, laminins have been considered as the structural components of BMs. The laminins are heterotrimeric proteins of the extracellular

matrix that are composed of α-, β- and γ-subunits. Currently, in mouse and human, genes encoding five α -, three β - and three γ-subunits have been identified. When known splice variants are included, these subunits assemble into at least 16 different laminin heterotrimers (Barker, 1994). Laminin heterotrimers are relatively large proteins (with molecular masses ranging from 400 to 900 kDa) and exist as cross-shaped molecules with two or three short arms and one long arm (Barker, 1994, 1997). A well-known family of receptors for LAMININs is transmembrane heterodimeric INTEGRIN receptors, in particular INTEGRIN α6β1 during embryonic stage, that mediate signaling initiated by ligand binding (Belkin and Stepp, 2000; Miranti and Brugge, 2002). Following ligand binding to ECM macromolecules, integrins undergo clustering that concentrates intracellular components involved in signaling. Integrins affect actin organization through modulation of small GTPase activities and can provide firm anchorage to the cell through linkages formed with recruited cytoplasmic proteins to F-actin (Wolf, 1978).

In this study, we set out to determine the molecular mechanism that mediates the interaction between RGPs and the vessels, in particular the role of INTEGRIN-mediated ECM adhesion in this interaction.

4.1 Expression of LAMININs and INTEGRIN receptors in the developing telencephalon

To explore the molecular basis of radial glial fibre ending-vessel interaction, we examined the adhesion molecules LAMININs and their receptor INTEGRINs that mediate cell-cell or cell-extracellular matrix (ECM) contacts (Buck and Horwitz, 1987; Troyanovsky, 1999). Our immunostaining results showed that through the embryonic development, LAMININ-enriched BMs are present surrounding the ISOLECTIN B4-labelled blood vessels (vascular BM) and also on the pial surface (pial BM) **(Figure 4.1a)**. It has been previously noted that the multipotent RGPs in the dorsal telencephalon span the entire neocortical wall and maintain contact both at the ventricular and pial surfaces. Even though the periventricular vessels start to

emerge in the cortical VZ from E11.5, the radial glial endfeet do not interact with them but are instead anchored to the pial BM (Avecilla et al., 2004; Erickson and Couchman, 2000) **(Figure 3.4)**. Of note, the radial glial processes of these RGPs also act as a guide for the migration of newly formed neurons in the ventricular zone to the pia, leading to the formation of cell layers (Kosodo and Huttner, 2009). Mutations affecting expression of laminins, the nidogen-binding site in the laminin γ1 subunit (Lmg1DLEb3), perlecan, α6 and β1-integrins, dystroglycan, focal adhesion kinase, and integrin-linked kinase have been found to result in separation of RGP basal endfeet from the pial BM because of apparent loss of ECM integrity, and defects of neuronal cell migration and cell layer formation leading to cobblestone-type lissencephaly (Beggs et al., 2003; Graus-Porta et al., 2001; Kohwi et al., 2005; Kwan et al., 2012; Marthiens et al., 2010; Niewmierzycka et al., 2005; Shibata et al., 2015; Sultan et al., 2013). Further analysis of the *Lmg1DLEb3* null, *integrin α6*-null and *perlecan*-null mice revealed that the pial anchorage was important for neuronal migration and cortical layer formation but not for proliferation or neurogenic capacity (Haubst et al., 2006). As the majority of RGPs in the ventral telencephalon is anchored to the vessels, would the vascular BM play a critical role in regulating the cellular behaviors and even proliferations of those RGPs?

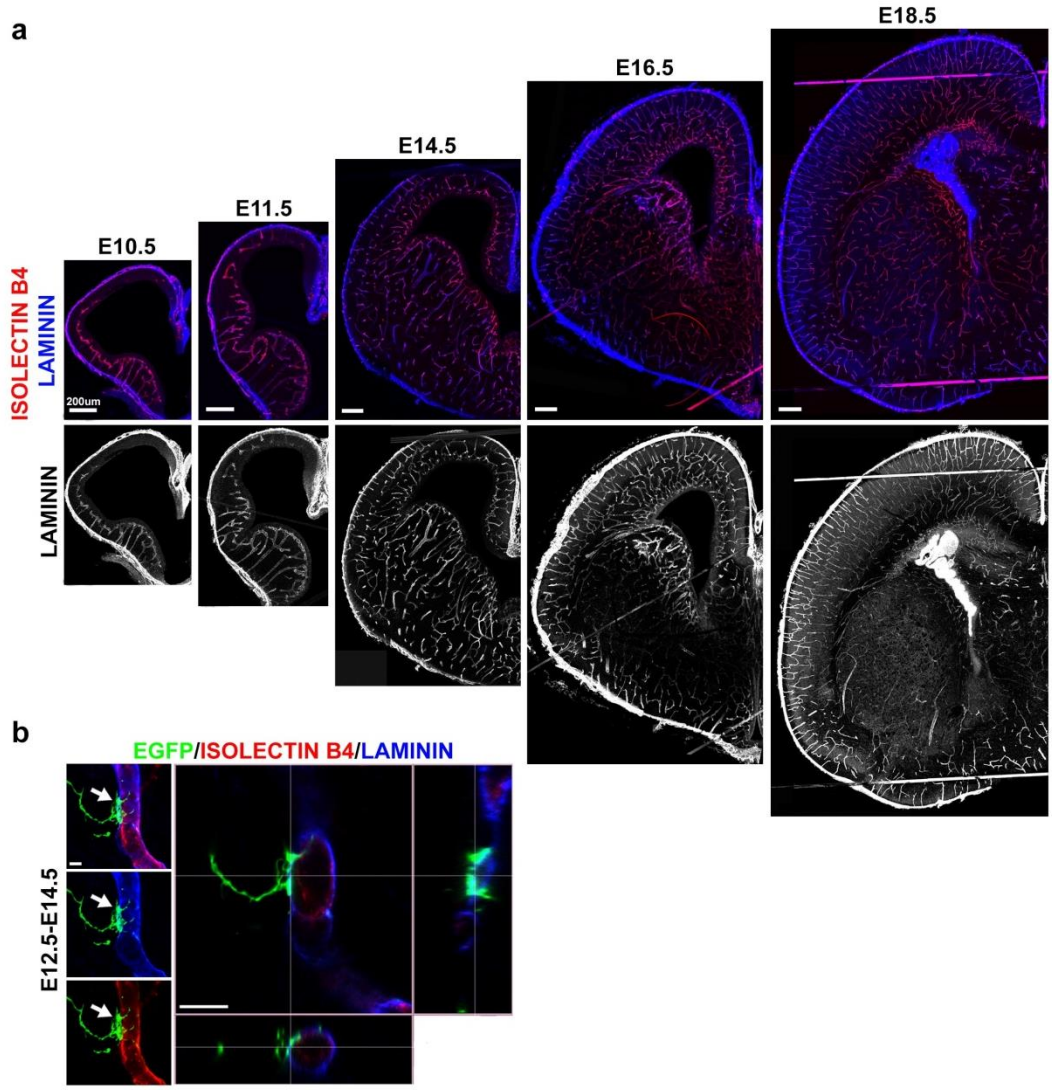


Figure 4.1: LAMININ expression in the vascular basement membrane through the embryonic development. (a) Coronal sections of mouse brains at different embryonic stages (E10.5, E11.5, E14.5, E16.5 and E18.5) stained for ISOLECTIN B4 (red) and LAMININ (red and white). Note that LAMININ is expressed in both vascular and pial basement membranes. Scale bars: 200 μm. **(b)** A radial glial fibre end (arrows) labelled by EGFP-expressing retrovirus (green) that anchored to the periventricular vessel labelled by ISOLECTIN B4 (red) and vascular basement membrane labelled by LAMININ (blue) staining. Cross-sectional images are shown to the right. Scale bars: 10 μm.

Interestingly, while the LAMININ expression level remains high in the pial BM through the brain development, its expression in the vascular BM is gradually down-regulated over time as neurogenesis diminishes and only retains on the vessels

within the periventricular zone in the ventral telencephalon during late embryogenesis (e.g. E16.5 and E18.5) **(Figure 4.1a)**.

The presence of the LAMININ-enriched BM surrounding the periventricular vessels leads us to wonder if the vascular BM is directly mediating the interaction between RGPs and the vessels. As revealed by the cross-sectional imaging, we found a LAMININ-enriched sheet outside of the ISOLECTIN B4-labelled vessel that was in direct contact with the radial glial fibre ending of RGPs in the MGE/PoA **(Figure 4.1b)**. Meanwhile, the LAMININ receptors, both INTEGRIN β1 and α6, were abundantly expressed in the MGE/PoA RGPs, especially in the radial glial fibres labelled by NESTIN and BLBP **(Figure 4.2, arrows)**. Notably, they were also expressed in the vessels **(Figure 4.2, arrowheads)**.

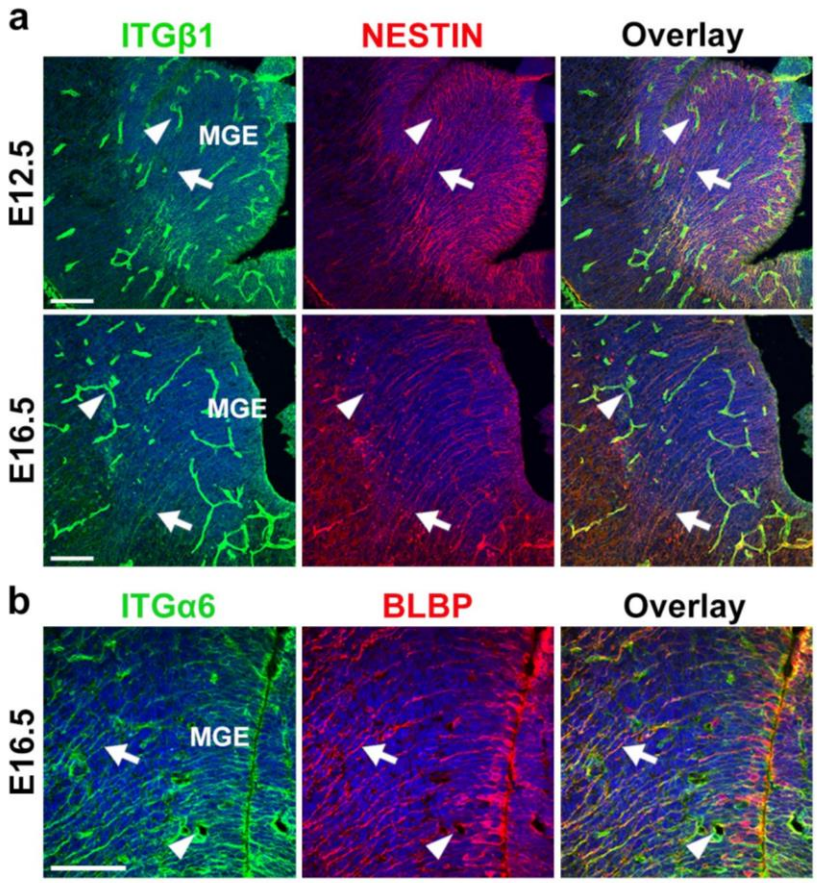


Figure 4.2: INTEGRINs expression in the ventral telencephalon. (a) An E12.5 (top) and E16.5 (bottom) MGE stained for ITGβ1 (green) and NESTIN (red), a RGP-specific marker. **(b)** An E16.5 MGE stained for ITGα6 (green) and BLBP (red), another RGP-specific marker. Note the high expression of ITGβ1 and ITGα6 in radial glial fibres labelled by NESTIN and BLBP (arrows), and the vessels (arrowheads). Scale bars: 100 μm.

4.2 Generation of mice with conditional knockout of INTEGRIN β1

The expression patterns of LAMININ on the surface of the vessels and INTEGRIN in the radial glial fibre of MGE/PoA RGPs suggest that they likely mediate the association between RGPs and vessels. To test this, we selectively removed INTEGRIN β1 (ITGβ1) in the MGE/PoA RGPs by crossing mice carrying the conditional allele of *Itgβ1* (*Itgβ1*$^{fl/fl}$) (Raghavan et al., 2000) with the *Nkx2.1-Cre* mice (Xu et al., 2008), in which Cre recombinase is selectively expressed in MGE/PoA progenitors under the *Nkx2.1* promoter. As confirmed by

immunohistochemistry, the expression of ITGβ1 was drastically reduced in RGPs labelled by NESTIN (arrows), but not in nearby vessels (arrowheads), in the MGE/PoA of *Itgβ1* conditional knockout (*Itgβ1* cKO) mice at E14.5 **(Figure 4.3)**.

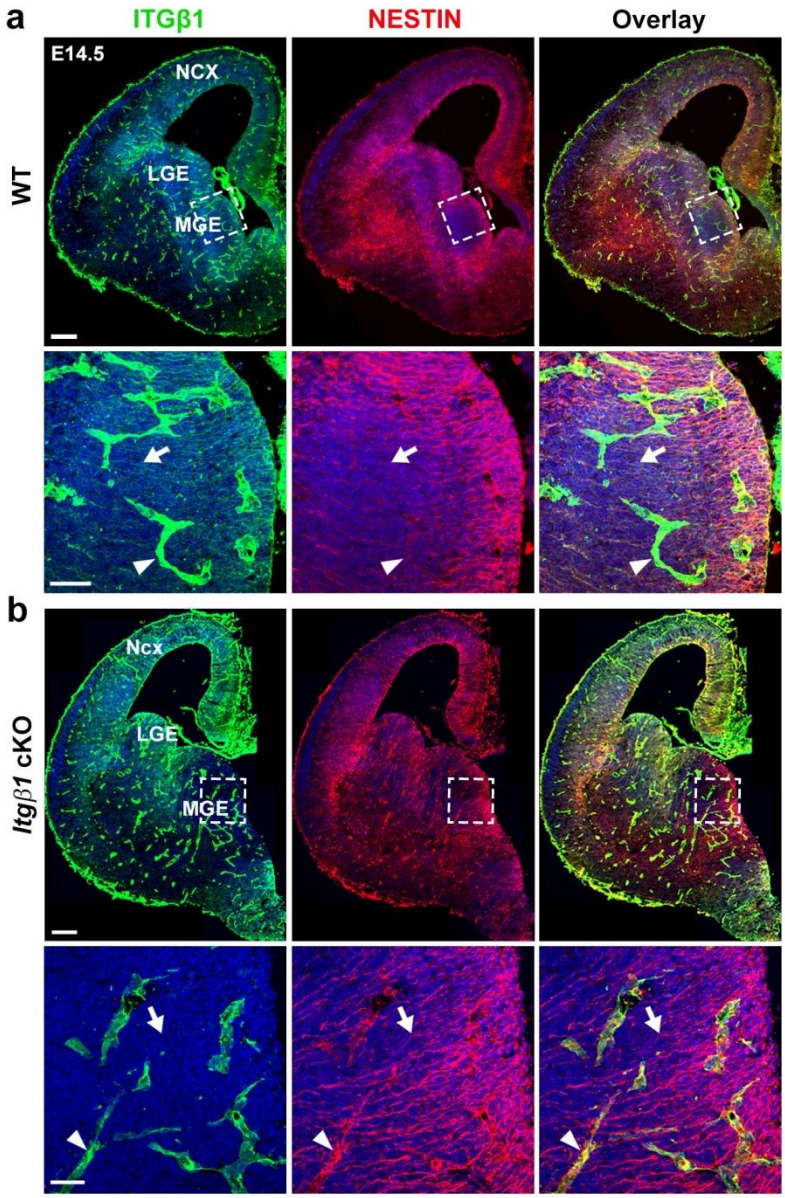


Figure 4.3: Selective removal of ITGβ1 in the MGE/PoA. Representative images of E14.5 brain sections of WT **(a)** and *Itgβ1* cKO **(b)** mice stained for ITGβ1 (green) and NESTIN (red), a RGP marker, and with DAPI (blue). High magnification images of the MGE (broken lines) are shown at the bottom. Note the loss of ITGβ1 in the RGPs including the radial glial fibres (arrows), but not in the vessels (arrowheads), in the MGE/PoA of the *Itgβ1* cKO mouse. Scale bars: 200 μm, 100 μm, 200 μm, and 40 μm (from top to bottom).

4.3 Loss of vascular anchorage after removing INTEGRIN β1 in RGPs

Interestingly, with retroviruses injected at E12.5, while radial glial fibres of MGE/PoA progenitors labelled by EGFP-expressing retrovirus were anchored to the periventricular vessel in the littermate wild type control mice **(Figure 4.4e left, c)**, more than half failed to anchor to the vessel in *Itgβ1* cKO mice at both E14.5 and E16.5 **(Figure 4.4e right, b and c)**, suggesting that ITGβ1 is indeed crucial for maintaining the association between the radial glial fibre ending and vessel in the MGE/PoA. Consistent with this, we found that DiI-labelled radial glial fibres did not obviously converge to the periventricular vessels in *Itgβ1* cKO mice **(Figure 4.5)**. Notably, we observed that the radial glial fibre ending of MGE/PoA progenitors grew more branches in *Itgβ1* cKO mice than those in the wild type control mice **(Figure 4.4d)**, suggesting that vessel anchorage stabilizes the radial glial fibre ending structure. Without the vessel association, the radial glial fibre ends appear to grow additional branches to probe the nearby environment for vessels. In addition, it has been implicated that long-term loss of one INTEGRIN could initiate the compensatory effect by other heterodimer combinations (Bovetti et al., 2007), therefore we expect that the phenotype in this cKO is underestimated. Altogether, these results indicate that INTEGRIN-mediated extracellualr matrix adhesion is a crucial part of the mechanism that mediates the interaction between RGPs and periventricular vessels in the MGE/PoA.

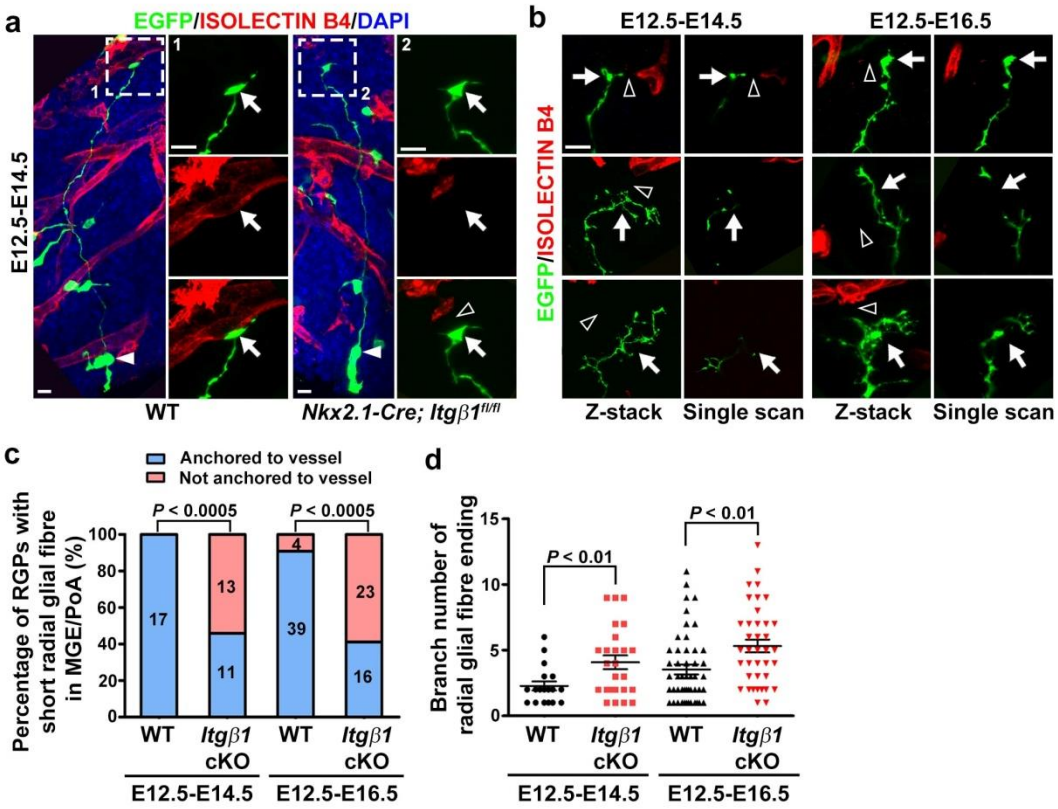


Figure 4.4: ITGβ1 mediates vessel anchorage of the radial glial fibre end in the MGE/PoA. (a) E14.5 MGE of the wild type (WT, left) and *Itgβ1* conditional knockout (*Itgβ1* cKO, right) mice with retrovirus (green) injected at E12.5 and stained for ISOLECTIN B4 (red) and with DAPI (blue). Scale bars: 10 μm. **(b)** Radial glial fibre ends in E14.5 (left) and E16.5 (right) MGE/PoA of *Itgβ1* cKO mice, labelled by EGFP-expressing retrovirus (green, arrows) at E12.5. Note the clear separation between radial glial fibre ends and the vessels (red, open arrowheads). Scale bar: 10 μm. **(c)** Percentage of non-pia reaching RGPs anchored to the periventricular vessel in the MGE/PoA of the WT and *Itgβ1* cKO mice. P values are shown at the top. **(d)** Number of branches of individual non-pia-reaching radial glial fibre ends in the MGE/PoA. Data are presented as mean ± s.e.m.

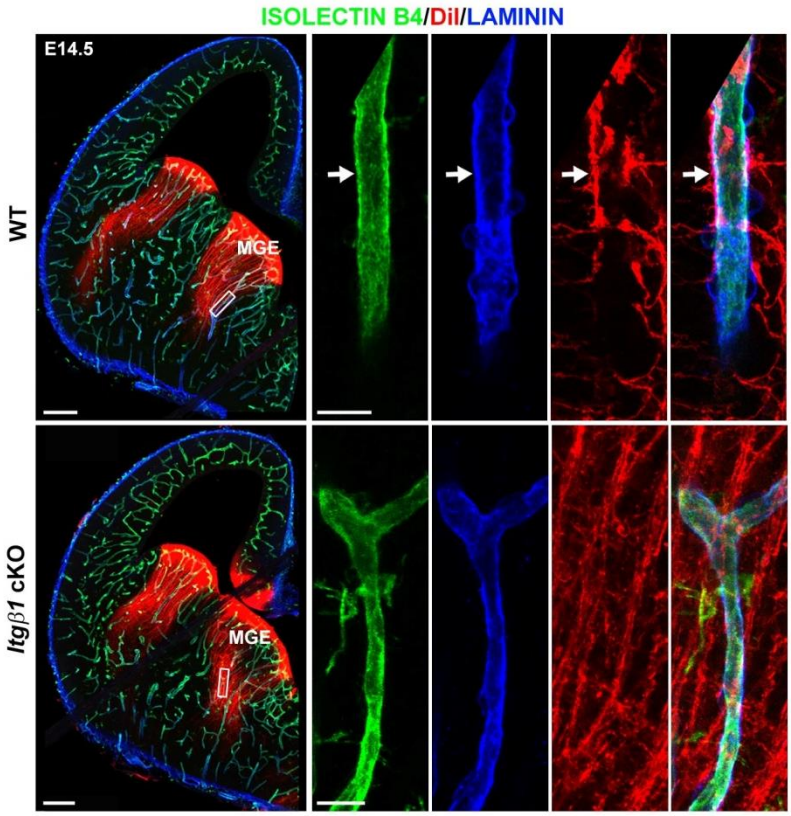


Figure 4.5: ITGβ1 removal disrupts the radial glial fibre and vessel association in the MGE at the population level. Representative images of an E14.5 brain section of WT (top) and *Itgβ1* cKO (bottom) mice labelled with DiI (red) at the VZ and stained for ISOLECTIN B4 (green) and LAMININ (blue). High magnification images of a region with the extra-VZ vessel in the MGE are shown to the right. Note that, while DiI-labelled radial glial fibres in the WT MGE aggregates at the vessel (arrows), those in the *Itgβ1* cKO MGE do not, suggesting a defect in radial glial fibre and vessel association. Scale bars: 200 μm and 20 μm (from left to right).

4.4 Loss of vascular anchorage impairs progenitor proliferation

Active anchorage of RGPs to the periventricular vessel in the MGE/PoA suggests that this progenitor-vessel interaction affects RGP behaviour and neocortical interneuron production. To test this, we examined progenitor proliferation in the MGE/PoA of the wild type control and *Itgβ1* cKO mice at E12.5, E14.5 and E16.5 **(Figure 4.6a, b)**. A crucial difference between RGPs and intermediate progenitors (IPs) is that only the former undergo interkinetic nuclear migration with the nucleus

migrating towards basal positions for S phase and then moving back apically to undergo M phase and cytokinesis at the ventricular surface (Gotz and Huttner, 2005). Similar to those in the dorsal telencephalon, RGPs in the MGE/PoA divide at the VZ surface to give rise to postmitotic interneurons or IPs that divide away from the VZ surface to produce neocortical interneurons (Brown et al., 2011). To identify the dividing progenitors, we stained the brain sections for phosphorylated histone H3 (PHH3), a specific marker for cells undergoing mitosis.

As expected, we observed $PHH3^+$ cells at the VZ surface as well as away from the VZ surface (Extra-VZ surface) **(Figure 4.6a)**. As shown in Figure 3.6b, the majority of proliferating RGPs (~90%) were shRGPs at E14.5, so we could assume here that the PHH3+ cells at the VZ surface at E14.5 and E16.5 were mostly shRGPs, whereas those away from the VZ surface were dividing IPs. Interestingly, while there was no significant difference in the number of $PHH3^+$ cells at the VZ or Extra-VZ surface in the MGE/PoA between the wild type control and *Itgβ1* cKO mice at E12.5 **(Figure 4.6a, b top)**, there was a significant decrease in the number of $PHH3^+$ cells at the VZ surface in the MGE/PoA of the *Itgβ1* cKO mice compared to that of the wild type control mice at E14.5 **(Figure 4.6a, b middle)**. As development proceeded, this decrease at the VZ surface became more pronounced at E16.5 (by 52.73% at E16.5, compared to 23.07% at E14.5) **(Figure 4.6a, b bottom)**. Moreover, there was a significant decrease at the Extra-VZ surface at E16.5 (by 20.55%, compared to WT). , Since IPs are initially produced by RGPs, the reduction of mitotic IPs may be the result of decreased RGP proliferation. Notably, the number of $PHH3^+$ cells in the LGE did not obviously change **(Figure 4.7)**, suggesting that the selective reduction in MGE/PoA progenitor division is due to ITGβ1 removal and a loss of vessel anchorage.

Moreover, injection of DNA-base analogue 5-bromo-2'-deoxyuridine (BrdU) 0.5 hour prior to sacrifice labels proliferating cells in S phase and resulted in a thick band of BrdU-labelled cells in the VZ and SVZ **(Figure 4.8a)**. The VZ and SVZ were defined according to DAPI staining. Similar to the $PHH3^+$ quantification, $BrdU^+$ cell

number per mm^2 in the VZ was significantly reduced in the *Itgβ1* cKO at both E14.5 and E16.5 compared to its littermate wild type control **(Figure 4.8b, top)**, while its cell density in the SVZ was significantly decreased only at E16.5 **(Figure 4.8b, bottom)**. Thus, these results further confirm that the proliferation of RGPs in the MGE is impeded after disrupting their vascular anchorage by removing ITGB1.

The proliferation deficiency of RGPs in *Itgβ1* cKO may be caused by: 1) loss of vascular interaction resulted by *Itgβ1* knockout, which further led to loss of signaling molecules (e.g. growth factors) from the vessels that promote RGP proliferation; 2) disruption of INTEGRIN β1 downstream signalling, which may also affect the progenitor proliferation; 3) both of the above two. From the time lapse imaging data (Chapter 3) that was collected in the wild type mice in which INTEGRIN β1 was normally expressed, the RGP proliferation rate dropped significantly after RGPs failed to interact with the vessel.

In addition, detachment of RGPs from the pial BM following INTEGRIN loss was found to be associated with apoptosis in the dorsal telencephalon (Blanpain et al., 2004). However, we did not observe any substantial increase in apoptosis in the MGE/PoA of *Itgβ1* cKO mice compared to the control, as revealed by immunostaining for apoptotic cell marker Cleaved Caspase 3 **(Figure 4.9)**. Together, these results suggest that disruption of radial glial fibre anchorage to the periventricular vessels leads to a decrease in RGP division at the VZ surface and a subsequent decrease in IP division away from the VZ surface in the MGE/PoA.

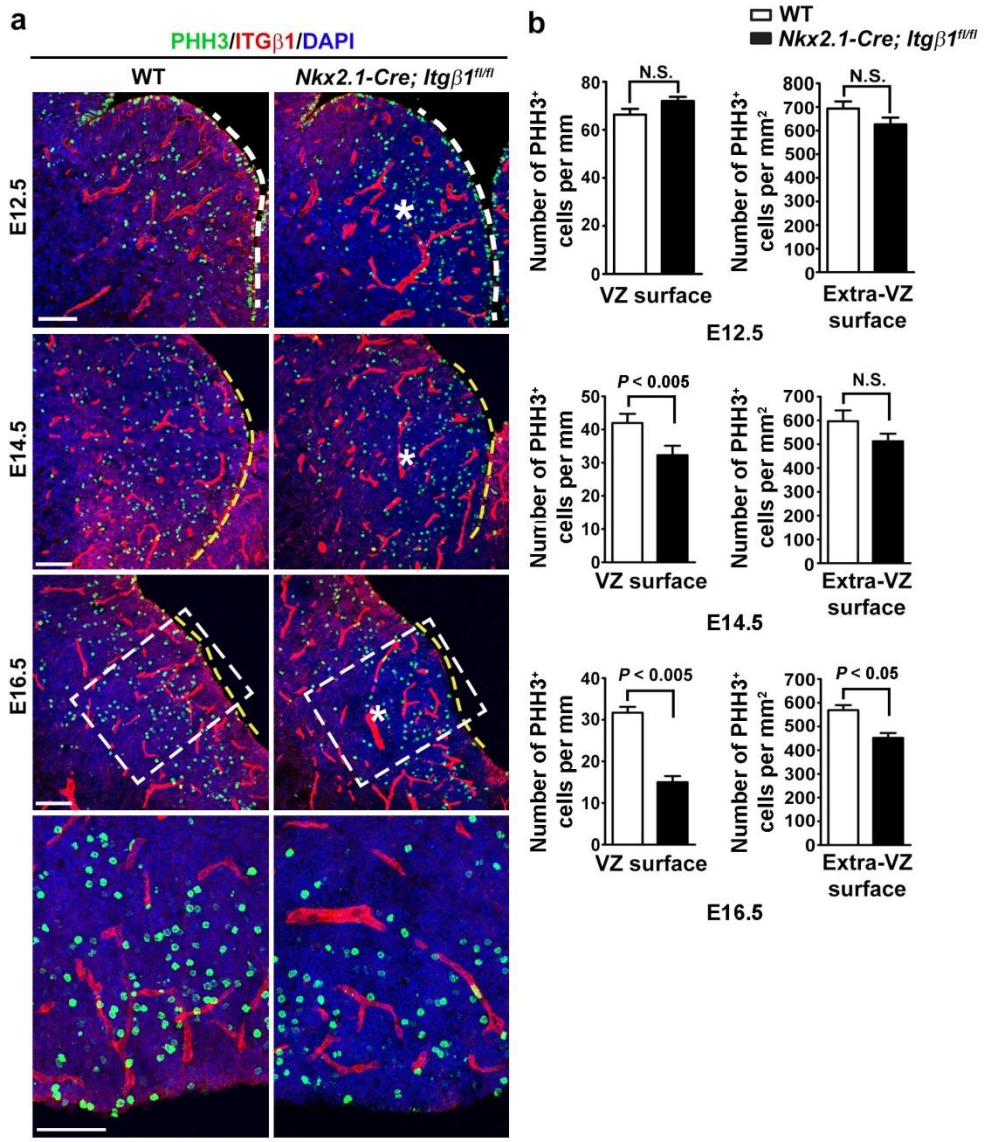


Figure 4.6: ITGβ1 removal in the MGE leads to a decrease in progenitor division at mid- and late-neurogenesis. (a) E12.5 (top), E14.5 (middle) and E16.5 (bottom) WT (left) or *Itgβ1* cKO (right) MGE/PoA stained for ITGβ1 (red) and phosphorylated Histone H3 (PHH3, green), and with DAPI (blue). Higher magnification images of the areas framed by white boxes are shown to the bottom of E16.5 MGE/PoA images. Yellow/white dash lines indicate the VZ surface of the targeted MGE/PoA. Note a selective loss of ITGβ1 in the MGE/PoA of *Itgβ1* cKO mice (asterisks). Scale bar: 100 μm. **(b)** Quantification of the number of $PHH3^+$ cells at the VZ surface and away from the VZ surface (Extra-VZ surface) at E12.5, E14.5 and E16.5. Data are all presented as mean ± s.e.m. P values are shown at the top. N.S., not significant.

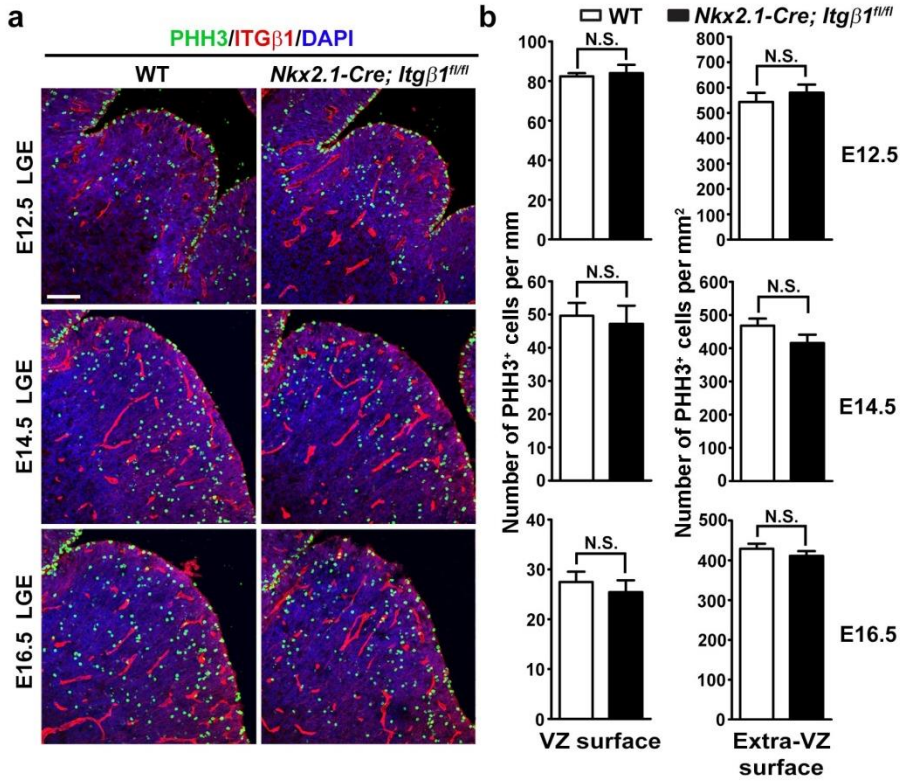


Figure 4.7: ITGβ1 removal in the MGE does not affect progenitor division in the LGE. (a) E12.5 (top), E14.5 (middle) and E16.5 (bottom) LGEs in WT (left) and *Itgβ1* cKO (right) mice stained for PHH3 (green) and ITGβ1 (red), and with DAPI (blue). Scale bar: 100 μm. **(b)** Quantification of the number of $PHH3^+$ cells at the VZ surface and away from the VZ surface in the LGE at different developmental stages. Data are presented as mean ± s.e.m. N.S., not significant.

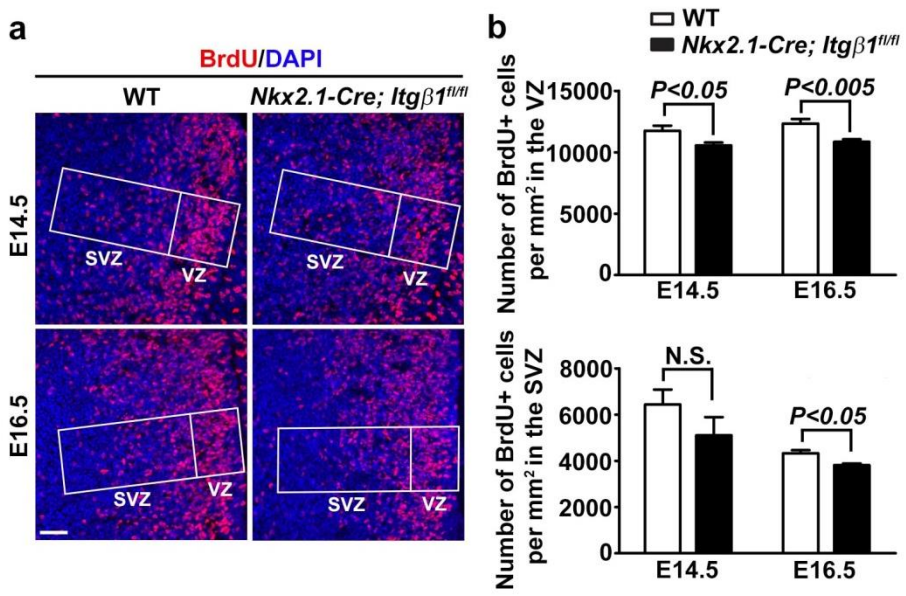


Figure 4.8: BrdU quantification confirms that ITGβ1 removal in the MGE leads to a decrease in progenitor division at mid- and late-neurogenesis. (a) E14.5 (top), and E16.5 (bottom) WT (left) or *Itgβ1* cKO (right) MGE/PoA stained for BrdU (red) and with DAPI (blue). White box indicate the VZ and SVZ regions that were measured for $BrdU^+$ cell numbers. Scale bar: 40 μm. **(b)** Quantification of the number of $BrdU^+$ cells at the VZ and SVZ at E14.5 and E16.5. Data are all presented as mean ± s.e.m. P values are shown at the top. N.S., not significant.

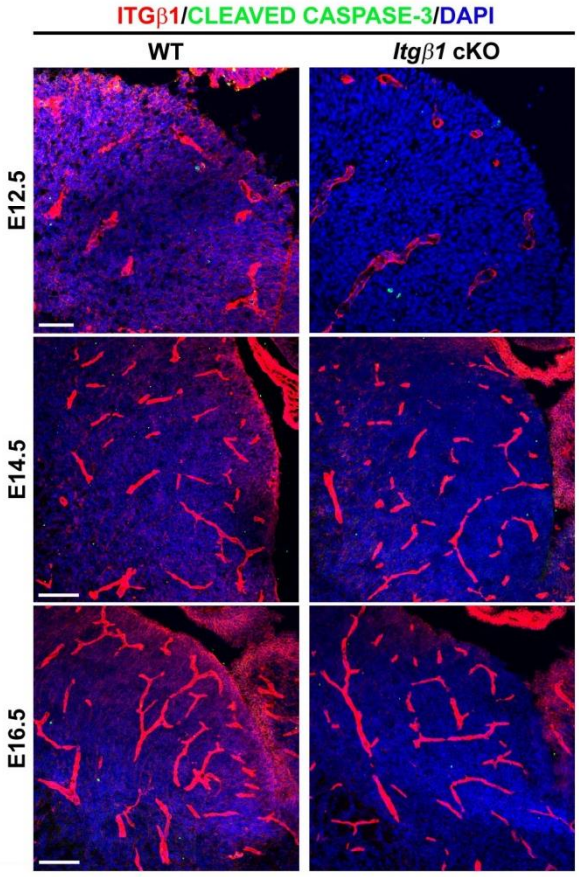


Figure 4.9: ITGβ1 removal does not cause apoptosis in the MGE/PoA. Representative images of E12.5 (top), E14.5 (middle) and E16.5 (bottom) MGEs in WT (left) and *Itgβ1* cKO (right) mice stained for CLEAVED CASPASE 3 (green) and ITGβ1 (red), and with DAPI (blue). Note no obvious apoptosis in the *Itgβ1* cKO MGEs compared to WT. Scale bar: 20 μm, 100 μm and 100 μm (from top to bottom).

4.5 Vascular anchorage may be important for RGP maintenance

Using time lapse imaging as described in Chapter 3, we observed an example in which the RGP was interacting with the vessel and went through one cell division at the ventricular surface **(Figure 4.10a, from t= 22 hrs to 23.5 hrs)**. The radial glial fiber was not visible at anaphase (t=23 hrs), but quickly reappeared after the division (t=23.5 hrs) and the endfoot was still interacting with the initial vessel, as the new-born cell lowly migrated along the fiber away from the VZ. However, as the RGP started to lose its interaction with the vessel at t=35.5 hrs, the new-born cell stopped migrating from that point, and then the RGP detached from the ventricular

surface (t=37 hrs) and moved out of the VZ, which never divided again (until t=48 hrs). In total, we have observed 18 RGPs that lost their initial vascular anchorage and did not be able to resume the interaction with alternative vessels. Among these RGPs, 44.44% (8/18) remained their position in the VZ but never divided again within the time window of imaging, while the other 55.56% (10/18) lost their apical anchorage at the ventricular surface, moved out of the VZ and never divided after failing to resume their vascular interaction. These results suggest that the vascular anchorage may be important for not only the proliferation but also the maintenance of RGPs in the VZ. However, using retroviral labeling in the fixed tissue of *Itgβ1* cKO, although we did capture some RGPs detached from the ventricular surface, we did not find any significant increase of detached RGP number compared to wild type control. It is possible that the effect on disrupting the vascular anchorage in *Itgβ1* cKO is not as severe as that in the slice culture, or that since the retroviruses only label dividing cells on the ventricular surface, it may not infect those that were strongly affected – not proliferating or not dividing at the ventricular surface.

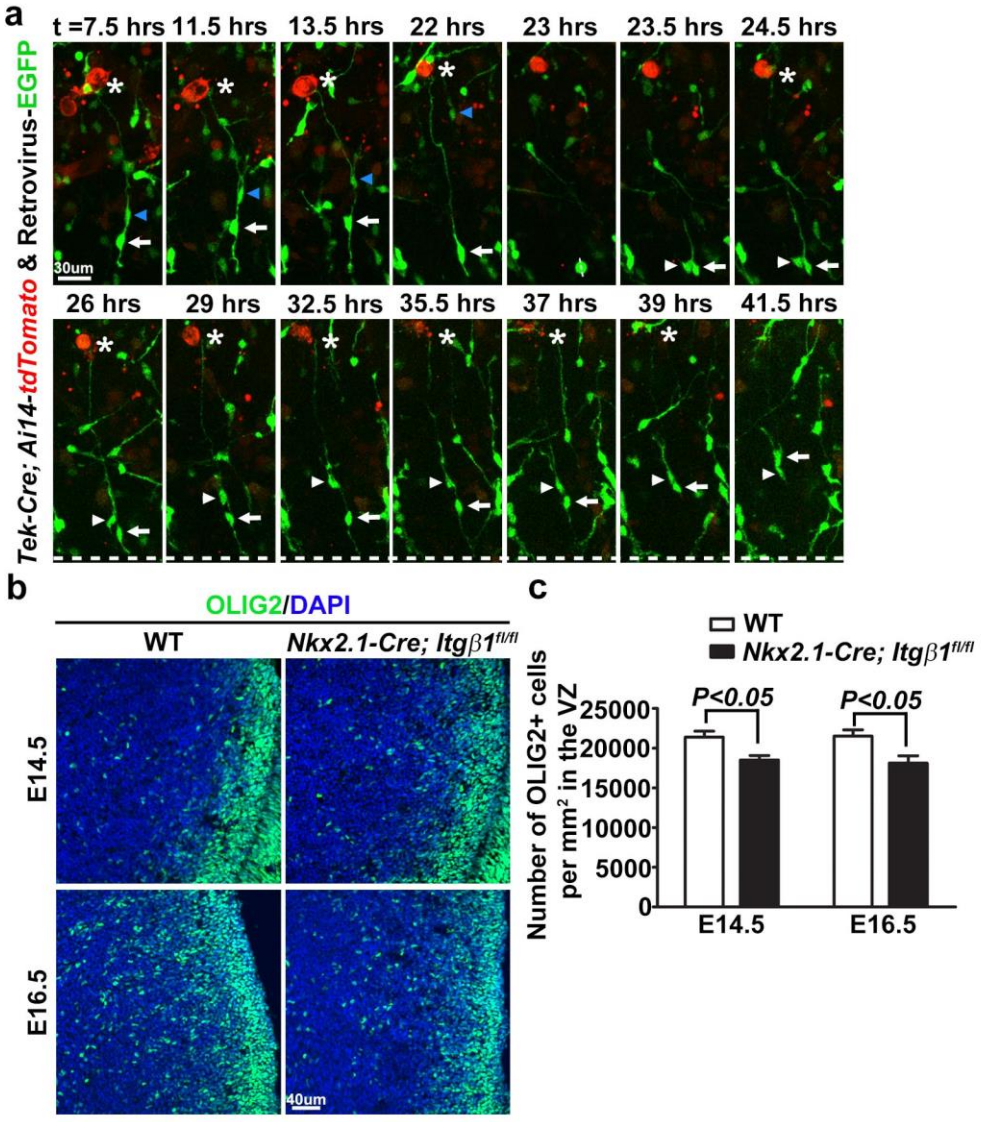


Figure 4.10: Vascular anchorage may be important for RGP maintenance. (a) Time-lapse images of a RGP (green, arrows) and the periventricular vessel (red, asterisk) in the MGE of an organotypic slice culture prepared from an E14.5 *Tek-Cre;Ai14-tdTomato* mouse brain that received *in utero* intraventricular injection of EGFP-expressing retrovirus (green) at E12.5. Time is indicated on the top. Dash lines indicate the VZ surface. Note the new-born cell (arrowheads) that migrates along the radial glial fibre. Scale bar: 30 μm. **(b)** E14.5 (top), and E16.5 (bottom) WT (left) or *Itgβ1* cKO (right) MGE/PoA stained for OLIG2 (green) and with DAPI (blue). Scale bar: 40 μm. **(b)** Quantification of the number of $OLIG2^+$ cells at the VZ at E14.5 and E16.5. Data are all presented as mean ± s.e.m. P values are shown at the top.

Furthermore, we compared the progenitor pool size in the VZ of wild type control and *Itgβ1* cKO. In the MGE, the greatest number of $OLIG2^+$ cells is found in

the VZ, where they comprise more than 90% of cells, which are mainly multipotent progenitor RGPs and new-born cells generated by RGPs (Carmeliet, 2003). The proportion of $OLIG2^+$ cells declined sharply in the SVZ, which are most likely IPs and differentiating oligodendrocyte precursor cells. We found that $OLIG2^+$ cell number in the VZ was significantly reduced in the cKO at both E14.5 and E16.5 **(Figure 4.10b, c)**, while its cell density in the SVZ was significantly decreased only at E16.5 **(Figure 4.10b, c)**. These results indicate that the VZ progenitor pool size in the MGE is significantly reduced after removing ITGB1, which may be the result of both decreased proliferative division and compromised progenitor maintenance.

4.6 Loss of vascular anchorage leads to decreased interneuron cell numbers in the somatosensory cortex

A variety of genetic fate-mapping and transplantation studies have suggested that MGE/PoA progenitors are responsible for producing a majority of neocortical interneurons, mostly PARVALBUMIN (PV)- and SOMATOSTATIN (SST)-expressing interneurons (Butt et al., 2008; Fogarty et al., 2007; Gelman et al., 2011; Xu et al., 2008). To test if the decrease in MGE/PoA progenitor division in *Itgβ1* cKO mice indeed leads to a loss of neocortical interneurons, we crossed *Nkx2.1-Cre;Itgβ1*$^{fl/+}$ or *Nkx2.1-Cre;Itgβ1*$^{fl/fl}$ mice with *Ai14-tdTomato* reporter mice, which allowed selective labelling of all progeny arising from the NKX2.1-expressing MGE/PoA progenitors in the presence or absence of ITGβ1 **(Figure 4.11a)**. We systematically analyzed the number of tdTomato-expressing cells in the somatosensory neocortex at P21-30 **(Figure 4.11a, b)**. Compared to the control, while the overall thickness of the cortex did not change, the numbers of tdTomato-expressing cells in both the superficial (1-4) and deep (5-6) layers were significantly decreased in *Itgβ1* cKO mice, suggesting a loss of neuronal progeny in the neocortex.

To confirm the neuron loss and reveal the identity of lost neurons, we performed immunohistochemistry experiments using antibodies against PV and SST **(Figure**

4.11a). Interestingly, we found that compared to the control the number of PV-expressing interneurons was significantly decreased in *Itgβ1* cKO mice, whereas the number of SST-expressing interneurons did not change substantially **(Figure 4.11b)**. In contrast, the interneuron cell number was not affected in the dorsal striatum **(Figure 4.12a, b)** in the *Itgβ1* cKO at P21, excluding the possibility that the interneuron loss in the dorsal cortex is caused by migratory defects of MGE/PoA-originated interneurons and missing neurons remain in the ventral region.

Together, these results demonstrate that vessel anchorage of RGPs in the MGE/PoA is crucial for proper production of neocortical interneurons, especially the subtype expressing PV. It has previously been suggested that, while SST-expressing interneurons are predominantly generated at the early developmental stages, PV-expressing interneurons are produced persistently throughout the MGE/PoA neurogenesis (Miyoshi et al., 2007). Therefore, the selective loss of PV-expressing interneurons is consistent with the decrease in progenitor division in the late developmental stage (E14.6-E16.5) and likely reflects the progressive appearance of vessel-anchored RGPs and the temporal difference in the production of PV- and SST-expressing neocortical interneurons. Consistent with this possibility, the interneuron population in the hippocampus, which is also generate at the early developmental stage in the MGE (E9.5 - E12.5) (Holmes and Zachary, 2005), is also not affected **(Figure 4.12c, d).**

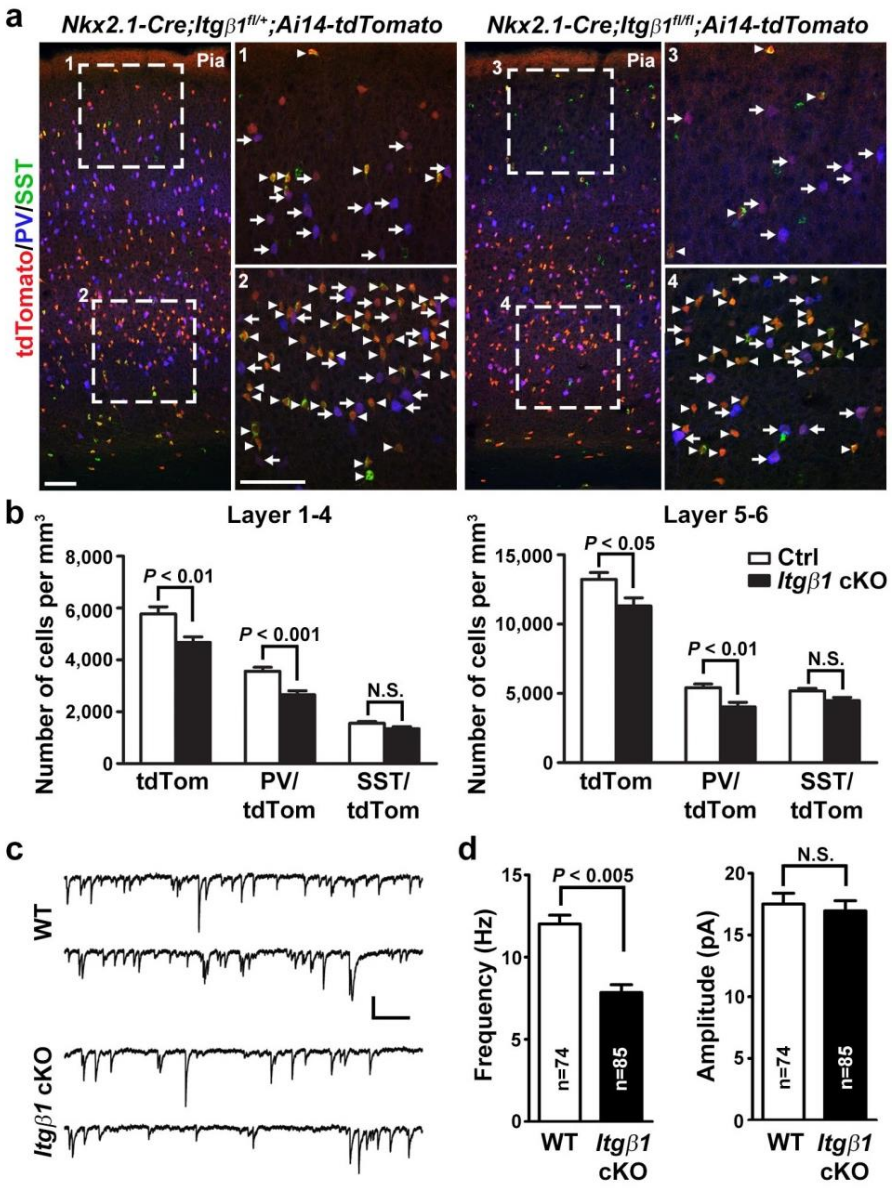


Figure 4.11: Loss of vessel anchorage results in a PV$^+$ interneuron loss and a reduced synaptic inhibition in the somatosensory cortex. (a) The somatosensory neocortex of P30 *Nkx2.1-Cre;Itgβ1$^{fl/+}$;Ai14-tdTomato* (control, left) and *Nkx2.1-Cre;Itgβ1$^{fl/fl}$;Ai14-tdTomato* (*Itgβ1* cKO, right) mice stained for PV (blue) and SST (green). High magnification images of the superficial (Layer 1-4; area 1) and deep (Layer 5-6; area 2) layers are shown to the right. Arrows indicate PV/tdTomato double positive neurons; arrowheads indicate SST/ tdTomato double positive neurons. Scale bars: 100 μm. **(b)** Stereological quantification of the number of cells positive for tdTomato, PV/tdTomato or SST/tdTomato in the somatosensory neocortex. **(c)** Representative sample traces of mIPSCs recorded from excitatory neurons in the somatosensory neocortex of P21 WT or *Itgβ1* cKO mice. Scale bars: 25 pA and 200 msec. **(d)** Quantification of the frequency and amplitude of mIPSCs. Data are all presented as mean ± s.e.m. P values are shown at the top. N.S., not significant.

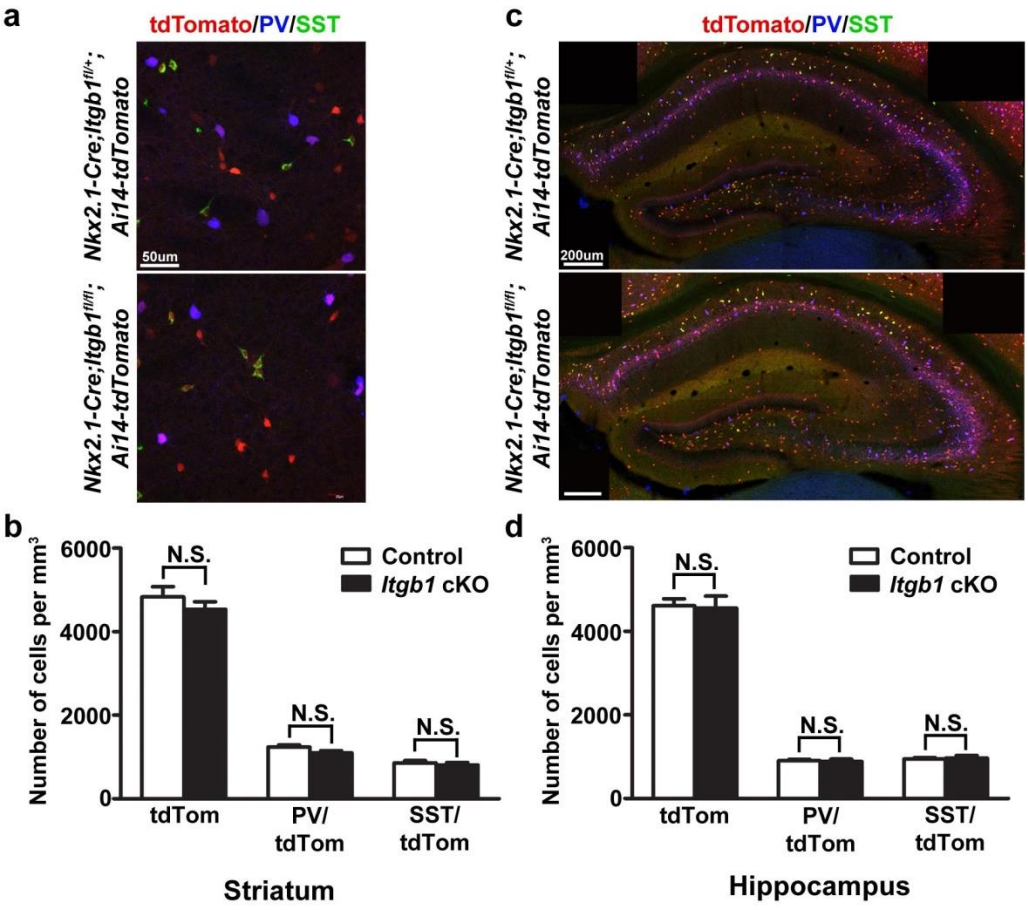


Figure 4.12: Interneuron cell number is not affected in the striatum or the hippocampus at P21. (a) High magnification images of the striatum of P21 *Nkx2.1-Cre;Itgβ1*$^{fl/+}$*;Ai14-tdTomato* (control, top) and *Nkx2.1-Cre;Itgβ1*$^{fl/fl}$*;Ai14-tdTomato* (*Itgβ1* cKO, bottom) mouse brain sections stained for PV (blue) and SST (green). Scale bar: 50 μm. **(b)** Stereological quantification of the number of cells positive for tdTomato, PV/tdTomato or SST/tdTomato in the striatum. **(c)** The hippocampus of P21 *Nkx2.1-Cre;Itgβ1*$^{fl/+}$*;Ai14-tdTomato* (control, top) and *Nkx2.1-Cre;Itgβ1*$^{fl/fl}$*;Ai14-tdTomato* (*Itgβ1* cKO, bottom) mice stained for PV (blue) and SST (green). Scale bar: 200 μm. **(d)** Stereological quantification of the number of cells positive for tdTomato, PV/tdTomato or SST/tdTomato in the hippocampus. N.S., not significant.

4.7 Synaptic inhibition is reduced in the somatosensory cortex of integrin β1 conditional knockout

PV-expressing interneurons provide essential synaptic inhibition to excitatory neurons in the neocortex (Isaacson and Scanziani, 2011; Markram et al., 2004; Pfeffer et al., 2013). To test whether the loss of PV-expressing interneurons causes any

functional deficit in the neocortex, we performed whole-cell patch clamp recording on excitatory neurons in the somatosensory neocortex and examined the miniature inhibitory synaptic currents (mIPSCs) **(Figure 4.9c and d)**. We found that, while the amplitude of mIPSCs did not significantly change between the wild type control and *Itgβ1* cKO neurons, the frequency of mIPSCs was substantially reduced in *Itgβ1* cKOneurons. These results showed that loss of RGP anchorage to the periventricular vessels in the MGE/PoA leads to a reduction in synaptic inhibition onto excitatory neurons in the neocortex.

4.8 Summary

Here, we identified cell adhesion molecule ITGβ1 as a key component in maintaining the progenitor-vessel interaction in the MGE/PoA. Selective deletion of ITGβ1 in the MGE/PoA RGPs results in a dissociation between the radial glial fibres and the periventricular vessels, and a progressive decrease in progenitor division. Notably, RGPs in the MGE/PoA divide while maintaining the association with the periventricular vessels, indicating that the vascular anchorage may regulate RGP maintenance and/or division. The decrease in progenitor division initially at the VZ surface then in the SVZ in *Itgβ1* cKO mice likely reflects that a decrease in RGP division at the VZ surface progressively leads to a reduction in intermediate progenitors that divide in the SVZ. ITGβ1 has previously been shown to be expressed in RGPs of the dorsal telencephalon and mediate the attachment of radial glial fibres to the pial basement membrane (Graus-Porta et al., 2001). However, its removal or disruption of the pial basement membrane attachment does not affect RGP proliferation in the dorsal telencephalon (Graus-Porta et al., 2001; Haubst et al., 2006). This difference is likely related to the distinct cellular organization of RGPs in the ventral versus dorsal telencephalon, and also suggests that certain signalling sourced from the periventricular vessels other than ITGβ1 may regulate RGP division.

The decrease in progenitor division in the MGE/PoA in *Itgβ1* cKO mice leads to a substantial (~20-30%) loss of PV-, but not SST-, expressing interneurons in the

neocortex, and a clear reduction of synaptic inhibition in neocortical excitatory neurons. The selective loss of PV-expressing interneurons is consistent with the notion that the vessel-anchored RGPs progressively form towards the late embryonic stage, and that PV-, but not SST-, expressing interneurons are persistently generated in the MGE/PoA (Miyoshi et al., 2007).

CHAPTER 5:
VESSEL-DERIVED FACTOR THAT MAY REGULATE THE PROLIFERATION OF NEURAL PROGENITOR CELLS IN THE VENTRAL TELENCEPHALON

With the introduction of the 'stem cell niche' concept in 1978, Shofield described the potential for the existence of an extracellular environment that would be able to maintain a stem cell in its undifferentiated, proliferating state (Schofield, 1978). Through the years, this concept has been supported by many other literatures reporting the specialized niche microenvironments supporting lifelong self-renewal and production of differentiated cells in various organs (Fuchs et al., 2004; Morrison and Spradling, 2008). For example, within the adult mammalian brain, stem cells harvested from non-neurogenic regions can generate neurons and astrocytes when cultured in vitro, but only make glia *in vivo* (Gage, 2000). Moreover, primary cells from neurogenic areas transplanted into non-neurogenic regions exhibit very limited neurogenesis (Temple, 2001). In contrast, upon transplantation into the SVZ, RMS or SGZ, cultured neural stem cells derived from non-neurogenic regions can generate neurons appropriate to the region (Temple, 2001). All these previous observations have indicated the existence of neurogenic niches, which have an instructive role in directing neuronal production and stem cell maintenance as well as shield ongoing neurogenesis from possible external inhibitory influences. In other tissues, it has also been found that stem cells are maintained in specialized microenvironments - termed niches - in which supporting cells secrete factors that promote stem cell maintenance (Morrison and Spradling, 2008). For example, the haematopoietic stem cell (HSC) niche is revealed to be perivascular, where endothelial and/or perivascular cells secrete various factors that regulate HSC proliferation and maintenance (Ding et al., 2012; Kiel and Morrison, 2008; Kiel et al., 2005).

Among those growth factors, stem cell factor (SCF, also called Steel factor or Kit

ligand), originated from endothelial and perivascular cells, has been shown to be a key niche component and essential for the maintenance of HSCs *in vivo* (Ding et al., 2012; Ogawa et al., 1991). Differential splicing and proteolytic cleavage lead to the expression of a membrane-bound form and a soluble form of SCF (Lennartsson and Ronnstrand, 2012). HSCs are depleted in *Sl/Sl*^{d} mutant mice (Barker, 1994), which express soluble SCF but lack the membrane-bound form, indicating that membrane-bound SCF is particularly important for HSC maintenance (Barker, 1997). Mice with a mixture of wild-type and *Sl/Sl*^{d} stromal cells only exhibit normal haematopoiesis in the immediate vicinity of the wild-type cells, demonstrating that SCF acts locally in creating the niche (Wolf, 1978). Although the critical role of SCF for HSC maintenance has been extensively studied, its function in the neural system is still unclear.

In this study, we set out to explore the vessel-derived factors that may regulate the proliferation of RGPs in the MGE/PoA, in particular the potential role of SCF in interneuron neurogenesis.

5.1 Expression of SCF in the periventricular vessels in the developing telencephalon

To explore the signaling mechanism between the periventricular vessels and RGPs, we examined the expression and function of SCF. Taking advantage of the *Scf*^{gfp} knock-in mice with the insertion of enhanced green fluorescence protein (*EGFP)* into the endogenous *Scf* locus (Ding et al., 2012), we found that SCF was abundantly expressed in the thin flattened endothelial cells that constitute the periventricular vessels in the ventral telencephalon, including the MGE/PoA **(Figure 5.1)**, co-labelled with blood vessel marker ISOLECTIN B4.

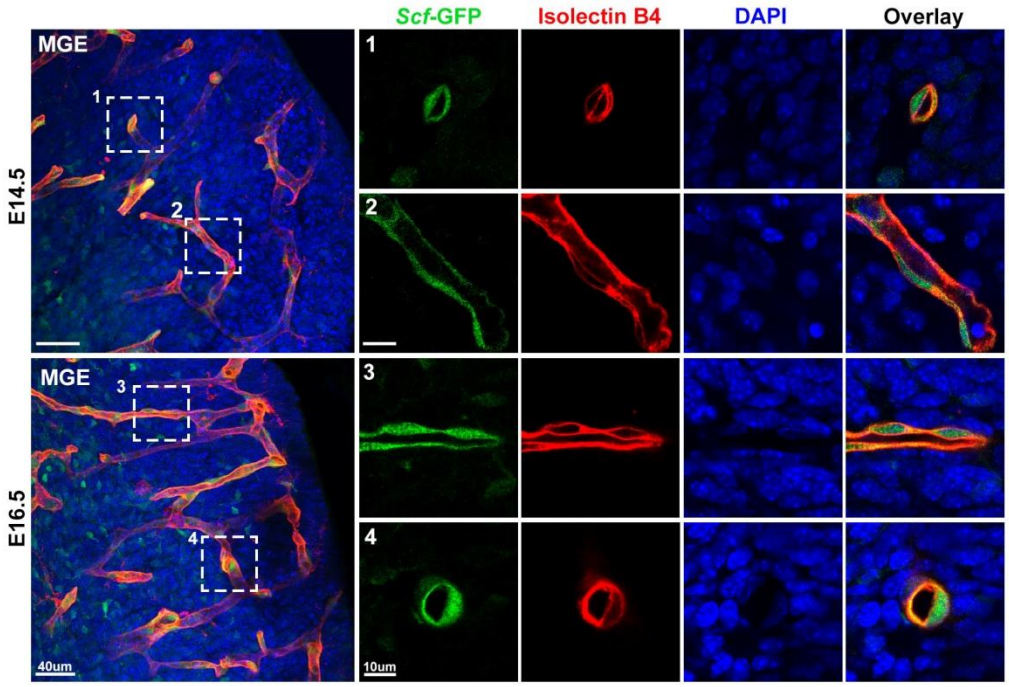


Figure 5.1: Stem cell factor (SCF) is highly expressed in the endothelial cells in the ventral telencephalon. Projection image of an E14.5 (top) and E16.5 (bottom) $Scf^{gfp/+}$ MGE stained for ISOLECTIN B4 (red) and with DAPI (blue). High magnification single-section images of the marked areas (1 - 4) are shown to the right. Scale bar: 40 μm (left) and 10 μm (right).

5.2 Loss of SCF in endothelial cells impairs progenitor proliferation in the MGE/PoA

Previous study showed that $Scf^{gfp/gfp}$ mice have a severe loss of SCF function (Ding et al., 2012). To determine if SCF is important for regulating the neural progenitor proliferation, we examined the progenitor proliferation in the MGE of the wild type control and $Scf^{gfp/gfp}$ mice by staining brain sections with the phosphorylated histone H3 (PHH3), a specific marker for cells undergoing mitosis. Interestingly, we found that the numbers of $PHH3^{+}$ cells at the VZ surface and away from the VZ surface in the MGE were significantly reduced in the $Scf^{gfp/gfp}$ mice compared to the wild type littermate control mice at E14.5 **(Figure 5.2a, b)**.

As SCF is expressed by more than one cell types and there is a complete loss of function of SCF in all cell types in $Scf^{gfp/gfp}$ mice, we used a floxed allele of *Scf* (Scf^{fl}),

which allowed deleting *Scf* conditionally (Ding et al., 2012). To selectively remove SCF in endothelial cells, we crossed the Scf^{fl} mice with the *Tek-Cre* mice, in which the *Cre* is specifically expressed in endothelial and haematopoietic cells (Koni et al., 2001). Because haematopoietic cells do not express *Scf* (Ding et al., 2012), the use of *Tek-Cre* allowed us to test whether SCF expression by endothelial cells regulates neural progenitor proliferation. Interestingly, we found that the numbers of $PHH3^{+}$ cells at the VZ surface and away from the VZ surface in the MGE were also significantly reduced in the $Tek\text{-}Cre^{+/-};Scf^{gfp/fl}$ mice compared to the control $Tek\text{-}Cre^{-/-};Scf^{gfp/+}$ mice at E14.5 **(Figure 5.2c, d)**. Although slightly milder, the decrease is comparable to that observed in $Scf^{gfp/gfp}$ mice. These results suggest that, similar to ITGβ1 removal in RGPs, SCF deletion in the endothelial cells causes a reduction in progenitor cell division in the MGE.

In addition, we did not observe any substantial increase in apoptosis in the MGE/PoA of $Scf^{gfp/gfp}$ mice compared to the control, as revealed by immunostaining for apoptotic cell marker Cleaved Caspase 3 **(Figure 5.3)**. Notably, the density and organization of the vascular structure in the developing brain was not disrupted in the *Scf* cKO animals **(Figure 5.4)**, indicating that the vascular development was not affected.

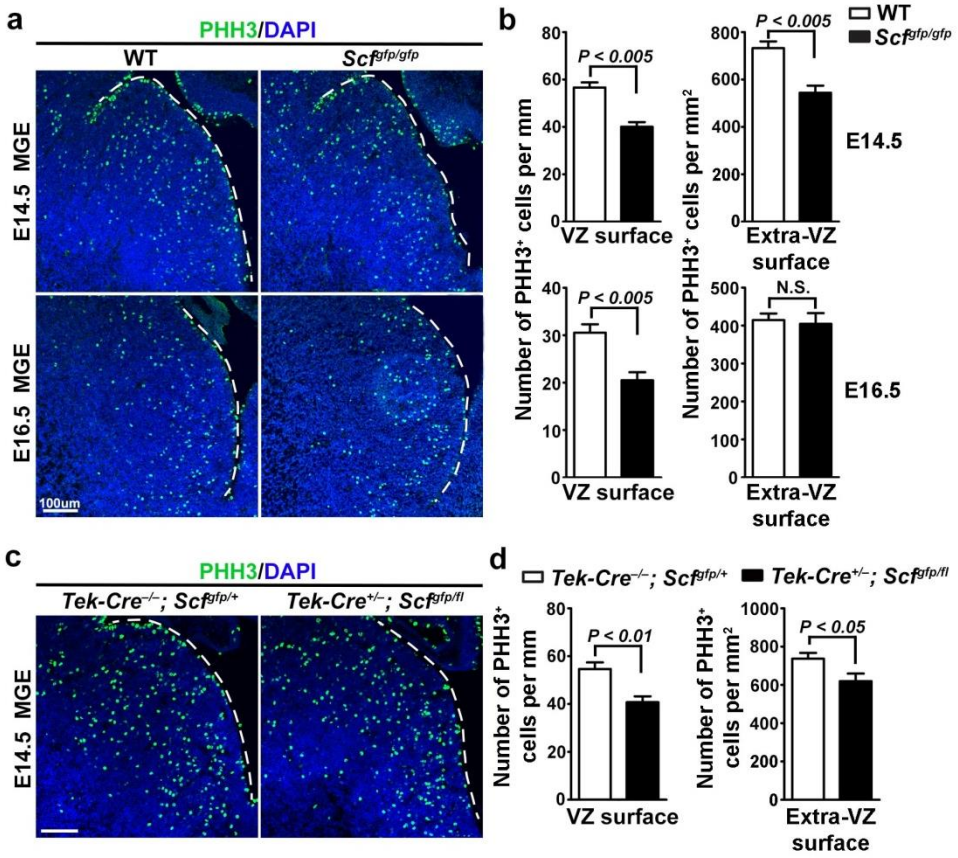


Figure 5.2: SCF removal from the endothelial cells in the MGE leads to a decrease in progenitor division at mid- and late-neurogenesis. (a) E14.5 (top) and E16.5 (bottom) of wild type control (left) or *Scf*$^{gfp/gfp}$ (right) MGE stained for PHH3 (green) and with DAPI (blue). Broken lines indicate the VZ surface of the MGE. Scale bar: 100 μm. **(b)** Quantificatin of the number of PHH3^{+} cells at the VZ surface and away from the VZ surface at E14.5 (top) and E16.5 (bottom). **(c)** E14.5 *Tek-Cre*$^{-/-}$*;Scf*$^{gfp/+}$ (left) or *Tek-Cre*$^{+/-}$*;Scf*$^{gfp/fl}$(right) MGE stained for PHH3 (green) and with DAPI (blue). Broken lines indicate the VZ surface of the MGE. Scale bar: 100 μm. **(d)** Quantification of the number of PHH3^{+} cells at the VZ surface and away from the VZ surface at E14.5. Data are all presented as mean ± s.e.m. P values are shown at the top. N.S., not significant.

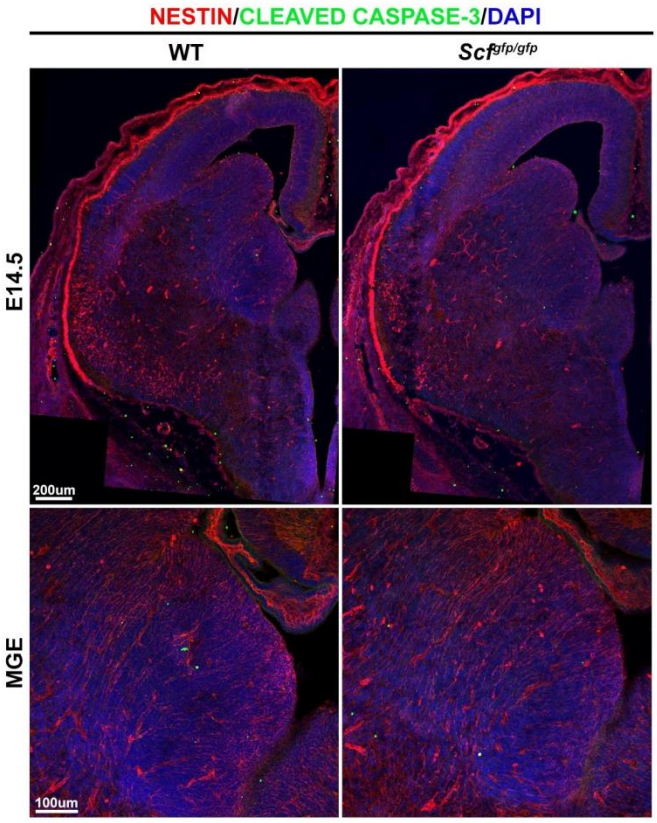


Figure 5.3: SCF removal does not cause apoptosis in the MGE/PoA. Representative images of E14.5 coronal brain section of WT (left) and *Scf*$^{gfp/gfp}$ (right) mice stained for CLEAVED CASPASE 3 (green) and NESTIN (red), and with DAPI (blue). High magnification images of corresponding MGEs are shown to the bottom. Note no obvious apoptosis in the *Scf*$^{gfp/gfp}$ MGEs compared to WT. Scale bar: 200 μm and 100 μm (from top to bottom).

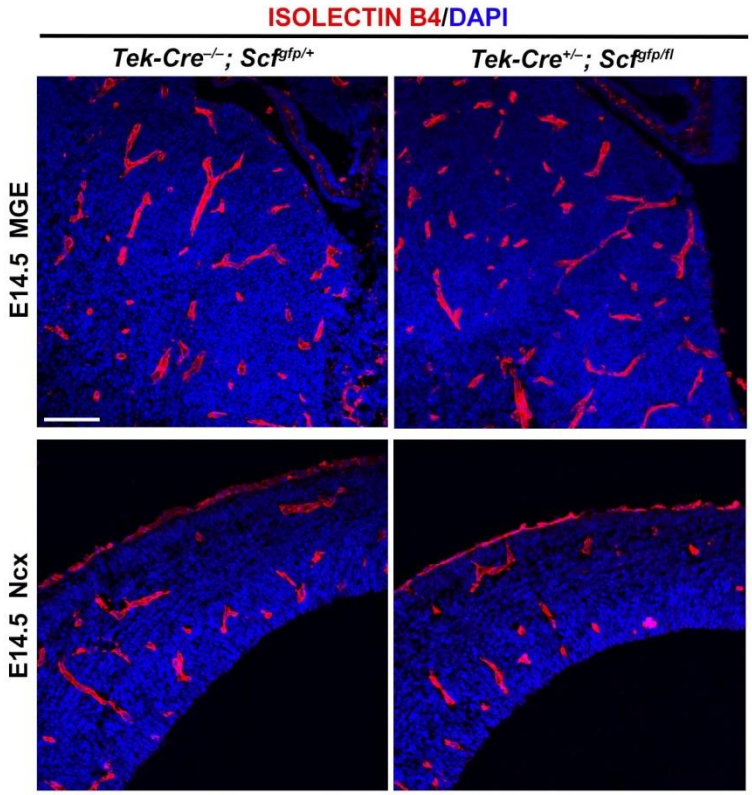


Figure 5.4: SCF removal does not affect the vascular organization in the brain. Representative images of E14.5 *Tek-Cre*$^{-/-}$;*Scf*$^{gfp/+}$ (left) or *Tek-Cre*$^{+/-}$;*Scf*$^{gfp/fl}$ (right) MGE (top) and neocortex (bottom) stained for ISOLECTIN B4 (red) and with DAPI (blue). Note the density and organization of the vessels are not disrupted in the *Tek-Cre*$^{+/-}$;*Scf*$^{gfp/fl}$. Scale bar: 100 μm.

5.3 Loss of SCF in endothelial cells does not affect progenitor proliferation in the dorsal neocortex

As expected, SCF is expressed widely in the vessels over all the developing telencephalon, including those in the dorsal telencephalon **(Figure 5.5a)**. However, despite its presence, we observed no change in the number of PHH3^{+} cells in the dorsal neocortex of *Tek-Cre*$^{+/-}$;*Scf*$^{gfp/fl}$ (*Scf* cKO) mice at E14.5 **(Figure 5.5)**, suggesting the vessel-derived SCF is not important for the neural progenitor proliferation in the dorsal neocortex, in contrast to the results we have observed in the MGE. The difference in the phenotypes after SCF removal is consistent with the

distinct organization of RGPs and the vessels between the dorsal and ventral telencephalon, since the vascular interaction is much more prominent and more critical for the RGPs in the ventral telencephalon.

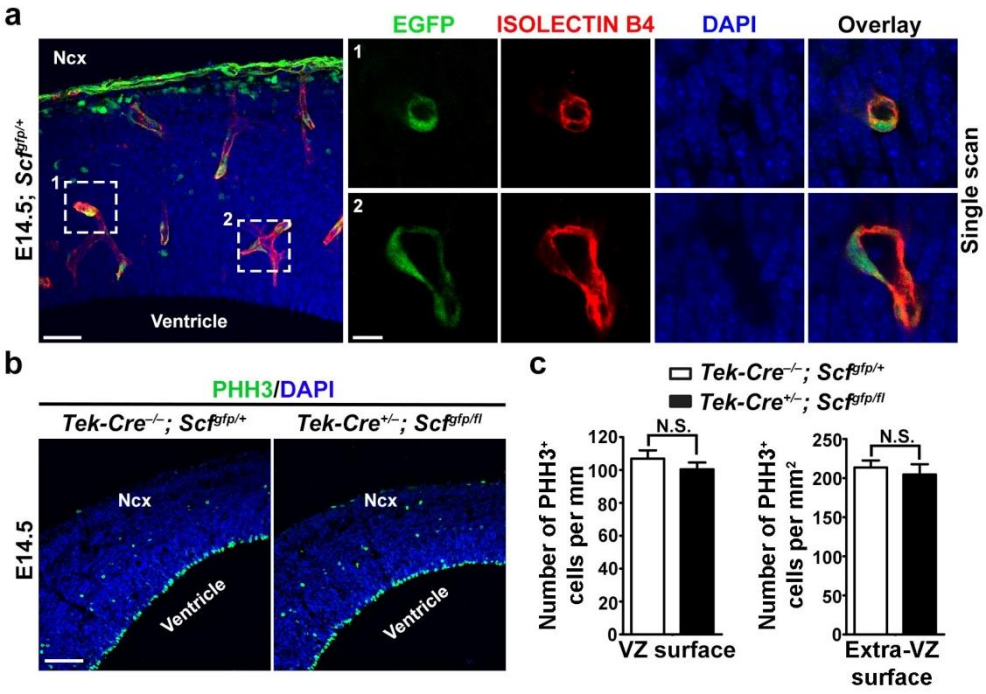


Figure 5.5: SCF removal does not impair progenitor division in the dorsal telencephalon. (a) Representative image of an E14.5 *Scf*$^{gfp/+}$ neocortex stained for ISOLECTIN B4 (red) and with DAPI (blue). High magnification single-section images of the marked areas (1 and 2) are shown to the right. Note the abundant expression of EGFP in ISOLECTIN B4-labelled endothelial cells that constitute the vessels. Scale bars: 40 μm (left) and 10 μm (right). **(b)** Representative images of E14.5 *Tek-Cre*$^{-/-}$*; Scf*$^{gfp/+}$ (left) or *Tek-Cre*$^{+/-}$*; Scf*$^{gfp/fl}$ (right) neocortex stained for PHH3 (green) and with DAPI (blue). Scale bar: 100 μm. **(c)** Quantification of the number of PHH3^{+} dividing cells at the VZ surface and away from the VZ surface at E14.5 neocortex. Data are presented as mean ± s.e.m. N.S., not significant.

5.4 Loss of SCF in endothelial cells leads to decreased interneuron cell number in somatosensory cortex

Scf$^{gfp/gfp}$ mice died perinatally (Ding et al., 2012), thus preventing the examination of the production of neocortical interneurons. *Tek-Cre*$^{+/-}$*; Scf*$^{gfp/fl}$ mice do

not appear to survive postnatal either. Therefore, to test if the decrease in MGE/PoA progenitor division in *Scf* cKO mice indeed leads to a loss of neocortical interneurons, we crossed the *Tek-Cre* mice with the Scf^{fl} mice to generate $Tek\text{-}Cre^{+/-}$; $Scf^{fl/fl}$ and examined the numbers of PV- and SST-expressing interneurons in the somatosensory cortex at P21-30. We found that compared to the wild type control, the number of PV-, but not SST-, expressing interneurons was significantly decreased in both the superficial and deep layers **(Figure 5.6)**. This reduction was in line with the decrease in progenitor cell division at the embryonic stage and similar to the effect of ITGβ1 removal in RGPs (~20% reduction). We observed no change in neocortical lamination and thickness, indicating that neocortical RGPs and excitatory neuron production are not affected. Together, these results strongly suggest that SCF in the endothelial cells could be a key signalling component in regulating the progenitor behaviour in the MGE/PoA and the production of neocortical interneurons.

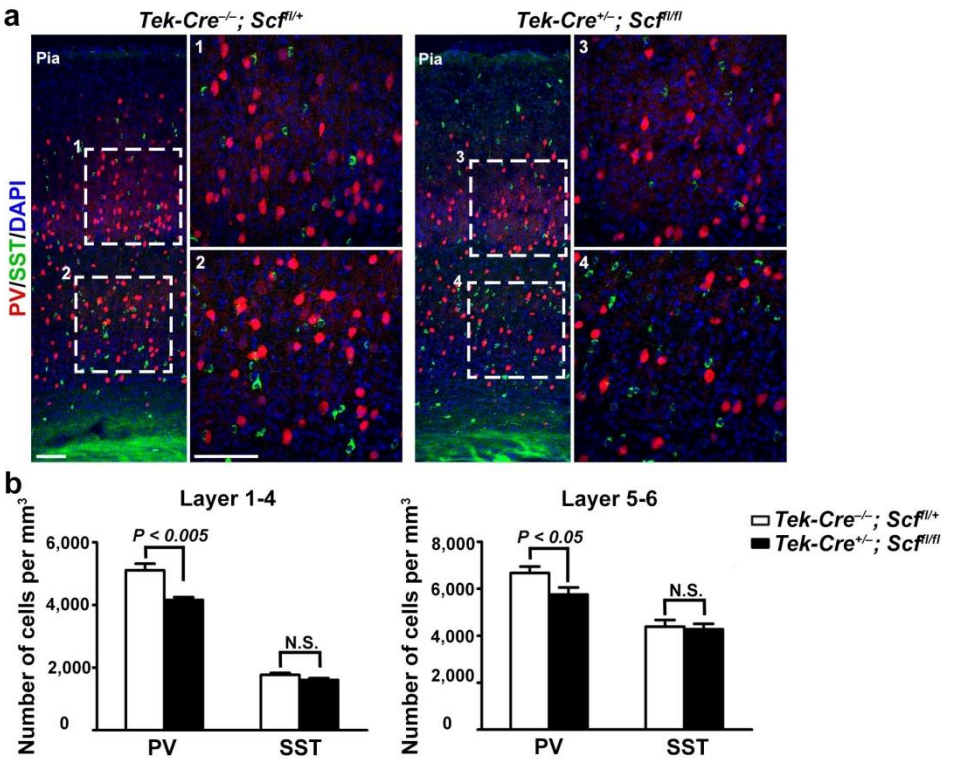


Figure 5.6: SCF from endothelial cells may regulate neocortical interneuron production. (a) The somatosensory neocortex of P27 *Tek-Cre*$^{-/-}$;*Scf*$^{fl/+}$ (WT, left) and *Tek-Cre*$^{+/-}$;*Scf*$^{fl/fl}$ (*Scf* cKO, right) mice stained for PV (red) and SST (green). High magnification images of the superficial (areas 1 and 3) and deep (areas 2 and 4) layers are shown to the right. Scale bars: 100 μm. **(b)** Stereological quantification of the number of cells positive for PV or SST in the somatosensory neocortex. Data are all presented as mean ± s.e.m. P values are shown at the top. N.S., not significant.

5.5 Summary

While endothelial cells have been shown to stimulate neural progenitor division in culture, the nature of the signalling between the vessel and neuronal progenitor remain unknown. In our study, we showed that Stem cell factor (Scf), a growth factor, is expressed in endothelial cells in the developing brain. Moreover, consistent with a region-specific vascular regulation, selective removal of *Scf* in endothelial cells leads to a decrease in progenitor division in the ventral, but not dorsal, telencephalon, and a loss of PV-expressing neocortical interneurons. These results suggest that Scf from the vessels could be a crucial signalling component in regulating RGP division and neocortical interneuron production specifically in the ventral telencephalon.

CHAPTER 6:

DISCUSSION AND FUTURE DIRECTIONS

Neocortical interneurons are an extraordinarily diverse group of neurons that play essential roles in controlling circuit dynamics and operation (Blatow et al., 2005; Fishell and Rudy, 2011; Huang et al., 2007; Kepecs and Fishell, 2014; Markram et al., 2004; Petilla Interneuron Nomenclature et al., 2008). While it is well-established that the vast majority of neocortical interneurons are produced by RGPs in the ventral telencephalon (Batista-Brito and Fishell, 2009; Flames and Marin, 2005; Wonders and Anderson, 2006), the cellular properties of these progenitors remain largely unknown. Here, we adapted a method for selectively labeling individual mitotic progenitor cells in the MGE/PoA that produce a majority of neocortical interneurons. By utilizing these techniques, we revealed a unique organization of RGPs in the MGE/PoA: 1) At early embryonic stage (before E13.5), the majority of RGPs are anchored to the pia surface, which we call pia-anchored RGPs (pRGPs), similar to those in the dorsal neocortex; 2) After E13.5, the dividing progenitor population progressively shifts towards vessel-anchored RGPs (vRGPs) that directly and actively interact with the periventricular vessels, but not the pial basement membrane **(Figure 6.1a)**.

Our experiments suggest that the interaction between the radial glial fibre of RGPs and the periventricular vessels is active and robust. We also identified cell adhesion molecule ITGβ1 as a key component in maintaining this progenitor-vessel interaction. Selective deletion of ITGβ1 in the MGE/PoA RGPs results in a dissociation between the radial glial fibres and the periventricular vessels, and a progressive decrease in progenitor division. Notably, RGPs in the MGE/PoA divide while maintaining the association with the periventricular vessels, indicating that the vascular anchorage may regulate RGP maintenance and/or division. The decrease in progenitor division initially at the VZ surface then in the SVZ in *Itgβ1* cKO mice likely reflects that a decrease in RGP division at the VZ surface progressively leads to a reduction in intermediate progenitors that divide in the SVZ. ITGβ1 has previously

been shown to be expressed in RGPs of the dorsal telencephalon and mediate the attachment of radial glial fibres to the pial basement membrane (Graus-Porta et al., 2001). However, its removal or disruption of the pial basement membrane attachment does not affect RGP proliferation in the dorsal telencephalon (Graus-Porta et al., 2001; Haubst et al., 2006). This difference is likely related to the distinct cellular organization of RGPs in the ventral versus dorsal telencephalon, and also suggests that certain signalling sourced from the periventricular vessels other than ITGβ1 may regulate RGP division. Consistent with a region-specific vascular regulation, we found that selective removal of SCF in endothelial cells causes a similar reduction in progenitor division in the ventral, but not dorsal, telencephalon. These results suggest that SCF may be particularly important as a niche component for RGPs in the MGE/PoA and act locally, as previously shown in the regulation of haematopoietic stem cells (Ding et al., 2012).

The decrease in progenitor division in the MGE/PoA in *Itgβ1* and *Scf* cKO mice leads to a substantial (~20-30%) loss of PV-, but not SST-, expressing interneurons in the neocortex, and a clear reduction of synaptic inhibition in neocortical excitatory neurons. The selective loss of PV-expressing interneurons is consistent with the notion that the vessel-anchored RGPs progressively form towards the late embryonic stage, and that PV-, but not SST-, expressing interneurons are persistently generated in the MGE/PoA (Miyoshi et al., 2007).

Given their diversity, their early appearance and critical role during embryonic development, and their ubiquitous presence within all tissues, it is not surprising that blood vessels can function far beyond their basic roles as suppliers of oxygen and nutrients. This unique ability of vascular endothelium has been proposed for some time, and now with the rapid advancement of research methods, their diverse roles in stem cell maintenance and niche regulation as well as the inter-relationship between the vascular and nervous systems are beginning to be revealed.

The new insights from this study will have a fundamental influence on our understanding of the vascular niche in the developing brain, and the unique regulation

of RGP behavior and neocortical interneuron production in the ventral telencephalon. They also shed lights on the developmental change in the migratory behavior of interneurons, and the embryonic origin and niche regulation of adult stem cells.

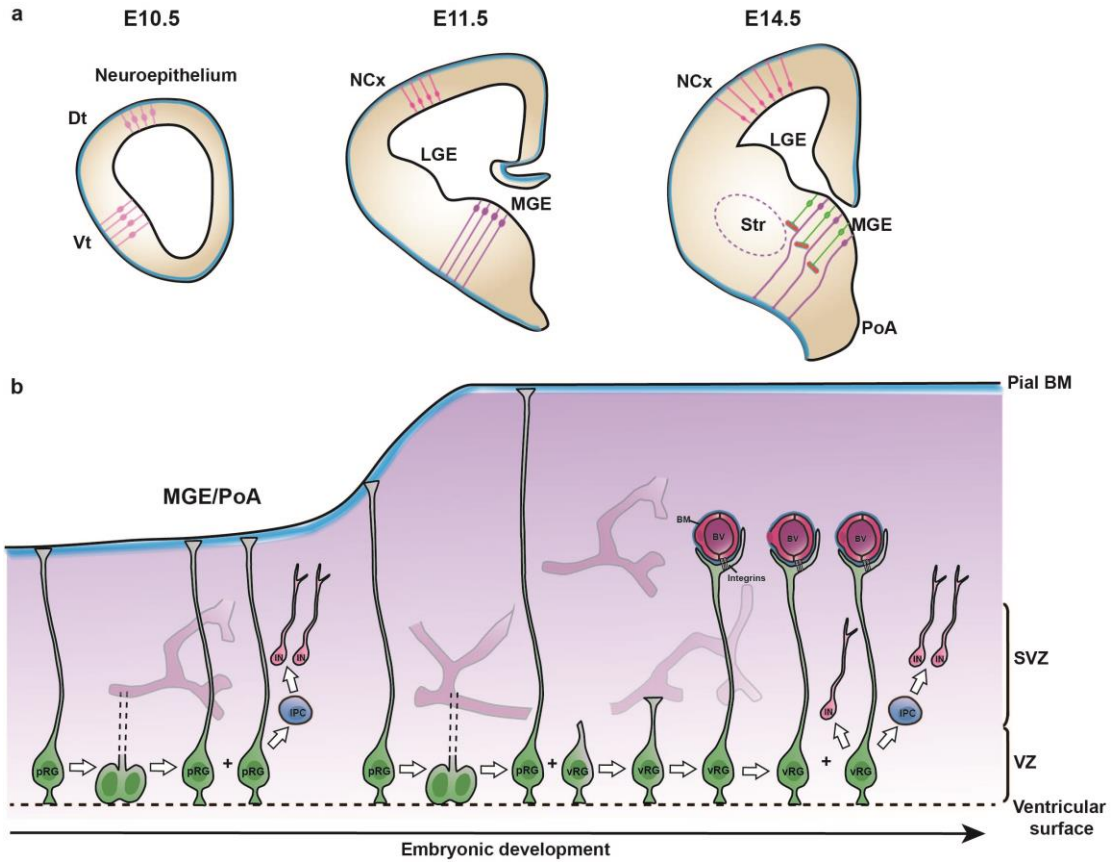


Figure 6.1: Proposed model illustrating the progressive generation of vessel-anchored RGPs in the ventral telencephalon. (a) At early embryonic stage (before E13.5), the majority of RGPs in the MGE/PoA are pia-anchored RGPs (pRGPs) with a long radial glial fibre reaching the pial surface, similar to those in the dorsal neocortex. After E13.5, the dividing progenitor population progressively shifts towards vessel-anchored RGPs (vRGPs) that directly and actively interact with the periventricular vessels. Dt, dorsal telencephalon; Vt, ventral telencephalon; Ncx, neocortex; LGE, lateral ganglionic eminence; MGE, medial ganglionic eminence; PoA, preoptic area; Str, striatum. **(b)** At the clonal level, vessel-anchored RGPs (vRGs) are generated by pia-anchored RGPs (pRGs) through proliferative division, in which the vRGs do not inherit the long radial glial fibre and have to grow their own that eventually attach to the periventricular blood vessels (BV). The INTEGRIN-mediated ECM adhesion is partly responsible for this vascular interaction. The vRG is capable of proliferative division that generates two vRGs as well as asymmetric division that produce interneurons (INs) either directly or indirectly through the intermediate progenitor cells (IPCs). VZ, ventricular zone; SVZ, subventricular zone.

6.1 Pia-anchored and vessel-anchored RGPs: molecularly distinct populations?

Neocortical interneurons are remarkably diverse, and are often classified by distinctive morphology, expression of neurochemical markers, firing pattern, and synaptic connectivity (Ascoli et al., 2008; Markram et al., 2004; Qiao et al., 2007). Proper production of a correct number of each of these different subgroups of neocortical interneurons is essential for constructing a functional neocortex. The diversity and precision in generating neocortical interneurons most certainly requires intricate regulation of progenitor cell division pattern and dynamics. In the dorsal telencephalon, single multipotential progenitor cells exist that are intrinsically capable of generating a diversity of neuronal and glial cell types; fate-restricted progenitor cells are also present (Luskin, 1998; Shen et al., 2006). Results from our study suggest that the MGE and PoA contain a heterogeneous population of RGPs with an array of proliferative behaviors, at least morphologically.

We found that the generation and propagation of the vessel-anchored RGPs temporally coincides with the rapid expansion of the ventral telencephalon and the emergence of the striatum between the VZ and the pia, which would demand the development of exceedingly long radial glial fibres for RGPs to maintain the characteristic bipolar morphology, should they be anchored to the pial surface. Thus, the shift from the pial basement membrane anchorage to the periventricular vessel anchorage can be an efficient way for RGPs to cope with the distinct developmental change in structural organization of the ventral telencephalon. On the other hand, vessel-anchored RGPs may also receive distinct signalling molecules from the periventricular vessels that lead to the different proliferative behaviours between RGPs in the dorsal and ventral telencephalon. Both periventricular and pial vessels are a source of factors that serve as instructive cues for neuronal proliferation and differentiation, and have been suggested to directly signal via the radial glial fibre to regulate RGP activities (Kiel and Morrison, 2008; Shen et al., 2004; Sirko et al., 2007). Recent studies have suggested that endothelial cells of the periventricular vessels have molecular identities distinct from those of the pial vessels (Won et al.,

2013). Could the different sets of signaling molecules/growth factors from the periventricular and pial vessels result in distinct behaviours and even different progenies of vessel- versus pia-anchored RGPs? Identifying the molecules uniquely expressed by periventricular vessels and their functions in regulating RGP activities would be needed in order to address this question.

Given their different morphologies and extrinsic niche environments, it is likely that pia-anchored and vessel-anchored RGPs are molecularly distinct groups of neural progenitors that could contribute to the heterogeneity of the interneuron population. In this study, we characterized these two RGP groups morphologically through the embryonic development. Future work will aim to more thoroughly identify the key difference of their expression profiles that make them molecularly and functionally distinct. A few molecular markers that could distinguish these two RGP groups would be necessary to make further characterizations happen.

Some evidence supports the notion that progenitor potential is cell autonomous and generation of different neuronal subtypes is due to maturation. As an example, single cells isolated from E10.5 cortex and grown in culture are multipotent and sequentially generate neuronal then glial restricted progenitor, resembling the in vivo developmental sequence (Qiang et al., 2000). However, environmental cues may also contribute to progenitor production of distinct interneuron subtypes. Sonic hedgehog signaling has already been shown to influence MGE progenitor transcription factor expression and is vital for production of MGE-derived interneuron fate. It is also possible that cell progeny could provide fate-modulating signals that influence clone composition (Kriegstein and Alvarez-Buylla, 2009). Considering the different niche environment for pia- and vessel-anchored RGPs, could these two types of RGPs produce different interneuron subtypes? We found that disruption of the vascular anchorage by conditionally removal of ITGB1 in RGPs leads to a significant reduction of PV- but not SST-expressing interneurons **(Figure 4.11)**. It has previously been suggested that, while SST-expressing interneurons are predominantly generated at the early developmental stages, PV-expressing interneurons are produced

persistently throughout the MGE/PoA neurogenesis (Miyoshi et al., 2007). Therefore, it is likely that the pia-anchored RGPs generate most of the SST-expressing interneurons, whereas the vessel-anchored RGPs predominately produce PV-expressing interneurons. Recent studies suggest that Chandelier cells, a morphologically well-defined interneuron subtype that synapses exclusively to the axon initial segment of excitatory neurons, are produced predominantly at the late embryonic stage (Inan et al., 2012; Taniguchi et al., 2013). It will be interesting to explore whether a selective population of PV-expressing interneurons are lost in the mutant neocortex with an impaired vascular regulation, resulting in a deficit in inhibitory synaptic transmission.

6.2 Coordinated migration of neocortical interneurons

It has been known that RGPs in the dorsal neocortex are not only neural progenitors that generate neurons and glia, but also serve as a scaffold for new-born neurons to migrate along into the cortical plate (Campbell and Gotz, 2002). Originated from the ventral telencephalon, neocortical interneurons have to take a lengthy tangential migration to reach the dorsal telencephalon before migrating radially to their ultimate layer destination in the neocortex. Similar to excitatory neurons, inhibitory interneurons generated in the MGE and the PoA display birth date-dependent laminar distribution in the neocortex (Ang et al., 2003; Batista-Brito and Fishell, 2009; Miyoshi and Fishell, 2011), thereby arguing for a regulated process of interneuron migration.

The tangential migration of neocortical interneurons can be segregated to two spatially distinct streams: a superficial route along the marginal zone (MZ) close to the pia surface, and a deeper stream along the SVZ/IZ (Anderson et al., 2001). Early during development (E11.5-E12.5), neocortical interneurons arise primarily from the MGE and PoA, and follow a superficial route (Anderson et al., 2001). At mid-embryonic stages (E12.5-E14.5), the MGE appears to be the principal source of neocortical interneurons, some of which start to take the deeper stream. At later stages

(E15.5), the majority of immature interneurons follow a deep route (Morrison and Spradling, 2008). The most widely accepted model for GABAergic interneuron migration suggests that it is dictated by the simultaneous activity of chemorepulsive and chemoattractive gradients (Kiel et al., 2005; Yilmaz et al., 2006). Several families of ligands/receptors (Slit/Robo, semaphorin/neuropilin, Neuregulin-1/ErbB4 (Lennartsson and Ronnstrand, 2012; Marin et al., 2001; Ogawa et al., 1991)), motogenic factors (HGF, SDF1(CXCL12), GDNF and BDNF (Polleux et al., 2002; Powell et al., 2001; Stumm et al., 2003; Zhu et al., 1999)), as well as projection neuron precursors in the IZ-SVZ via CXCL12/CXCR4 signalling (Tiveron et al., 2006), have been proposed as candidates for guiding trajectories of GABA neurons. Recent studies suggest that the embryonic vascular networks could also provide support and critical guidance cues that regulate the divided migratory routes of GABAergic interneurons (Won et al., 2013). Nevertheless, key aspects into how exactly these factors influence formation and segregation of GABAergic neurons into a dual stream remain undetermined, and in this regard the enigma of GABAergic neuron tangential journey has remained elusive.

Interestingly, our results revealed that the increasing population of vessel-anchored RGPs coincides with the emergence of the deeper migratory stream, which starts from E12.5 and becomes the main route after E14.5. This raises the possibility that due to the relatively shorter radial glial fibre, vessel-anchored RGPs do not provide the scaffold for new-born daughter cells to reach the pia surface, which therefore take the deeper route after detaching from the radial glial fibre. Cell-cell contact plays important roles throughout neuronal development via transmembrane receptor molecules and local accumulation of secreted signals. Consistent with our earlier studies (Brown et al., 2011), our time lapse imaging showed that MGE/PoA-derived new-born daughter cells are frequently in direct contact with the mother radial glial fibre. This may allow daughter cells to begin neuronal differentiation prior to tangential migration. It has been suggested that direct contact with radial glial fibre promotes GABAergic interneuron differentiation (Wu et al.,

2008). Moreover, *in vitro* assays have shown newly generated GABAergic neurons acquire excitability more rapidly when co-cultured with RGPs than when grown alone (Li et al., 2008). Our earlier work has also indicated that cells within individual clones with the most pronounced neuronal and physiological characteristics are located furthest away from the ventricular zone (Brown et al., 2011). Early electrical activity is characteristic of interneurons shortly after birth and is necessary to promote migration via calcium driven cell motility (Cuzon et al., 2006; Lopez-Bendito et al., 2003; Morante-Oria et al., 2003). Consistent with this, we observed that the new-born daughter cell stopped migrating after the interaction with the mother radial glial fibre was disrupted as the RGP detached from the ventricular surface **(Figure 4.10a)**. It is therefore possible that the direct interaction observed between daughter cells and mother radial glial fibers promotes functional development until the cells are mature enough to initiate tangential migration. However, different from the RGPs in the dorsal neocortex where the radial glial fibres are anchored to the pial surface and all possess a similar length, the radial glial fibres of the RGPs in the MGE/PoA appear to have quite variable lengths and therefore could potentially lead to variable maturity of daughter interneurons detached from the fibre. To consolidate the role of radial glial fibre contact in the interneuron maturation process, further investigation will be needed to determine if there is a correlation between the length of radial glial fibre and the differentiation/maturity of daughter interneurons in the MGE/PoA.

6.3 Vessel-anchored RGPs and adult neural stem cells in the V-SVZ

Endothelial cells have been shown to stimulate self-renewal and promote neurogenesis of neural stem cells in culture (Tavazoie et al., 2008). However, the context of this vascular regulation remains largely unclear. Recent studies have revealed a vascular niche of adult neurogenesis (Kokovay et al., 2012; Leventhal et al., 1999; Tavazoie et al., 2008; Temple, 2001). Two germinal regions that function as unique neural stem cell (NSC) niches have been found persist in the adult mammalian brain: the ventricular-subventricular zone (V-SVZ), which generates neurons destined

for the olfactory bulb, and the subgranular zone (SGZ) of the hippocampal dentate gyrus (Fuentealba et al., 2012). Previous studies have identified the major neural cell types and their lineal relationship in the adult V-SVZ: SVZ astrocytes (Type B cells) give rise to immature precursors (Type C transit-amplifying cells), which in turn generate the neuroblasts (Type A cells) **(Figure 6.2b)**. These neurogenic tubes lie on the striatal wall of the lateral ventricle, directly beneath the ependymal layer. Occasionally, an SVZ NSC extends a process between ependymal cells to contact the lateral ventricle and exhibits a short, single cilium characteristic of RGPs at embryonic stages. They also extend a radial glial fibre (also called basal process) which contacts nearby blood vessels **(Figure 6.2b)**. Blood vessels in the V-SVZ shows specialized features including an altered BBB and easier access to blood-derived signals that functionally distinguish blood vessels in the V-SVZ niche from vessels in nonneurogenic brain regions (Tavazoie et al., 2008). Vascular niches for adult NSCs are complex and encompass diverse aspects of the vascular system, which include local signals derived from endothelial cells and direct contact with endothelial and perivascular cells as well as the vascular BM (Kokovay et al., 2010; Louissaint et al., 2002; Ramirez-Castillejo et al., 2006).

For the first time, our studies revealed and characterized the vessel-anchored RGPs in the ventral telencephalon (LGE, MGE and PoA) that emerge along the embryonic development. Vessel-anchored RGPs possess a short apical process anchored to the ventricular surface and a longer radial glial fibre attached to the periventricular vessels **(Figure 6.2a)**. This similar organization between stem/progenitor cells and vessels in the adult and embryonic stages suggests that adult NSCs in the SVZ may originate from the periventricular vessel-anchored RGPs in the ventral telencephalon. It has been previously suggested that adult NSCs in the SVZ are mostly descendants of the LGE (Young et al., 2007). For example, transcription factors that are expressed in the LGE of the embryo, such as Dlx1/2, Er81, Gsh2, and Pax6, are also present within the adult SVZ and RMS (Kohwi et al., 2005; Parmar et al., 2003; Stenman et al., 2003). In addition, viral lineage tracing of radial glia in the

perinatal LGE has demonstrated that some of the progeny of these cells come to reside in the postnatal SVZ and contribute significantly to the adult neurosphere-forming activity (Merkle et al., 2004). However, it is still unknown how the adult NSCs are generated by radial glia and maintained through the prenatal and postnatal development. With a better understanding of the heterogeneity of the RGPs in the LGE, we also wonder if there is a specific group of RGPs that are responsible for producing or transitioning to adult NSCs. A recent study suggests that a substantial fraction of adult V-SVZ NSCs is derived from a slowly dividing subpopulation of embryonic neural progenitors that are produced between E13.5 and E15.5 (Furutachi et al., 2015). Interestingly, this observation is consistent with the time window we have found when the vessel-anchored RGPs are generated and populated in the ventral telencephalon. Further studies will aim to investigate the lineage relationship between embryonic vessel-anchored RGPs and adult NSCs in the V-SVZ, and if the vascular anchorage of RGPs at the embryonic stage is critical for maintaining the NSCs to the adulthood.

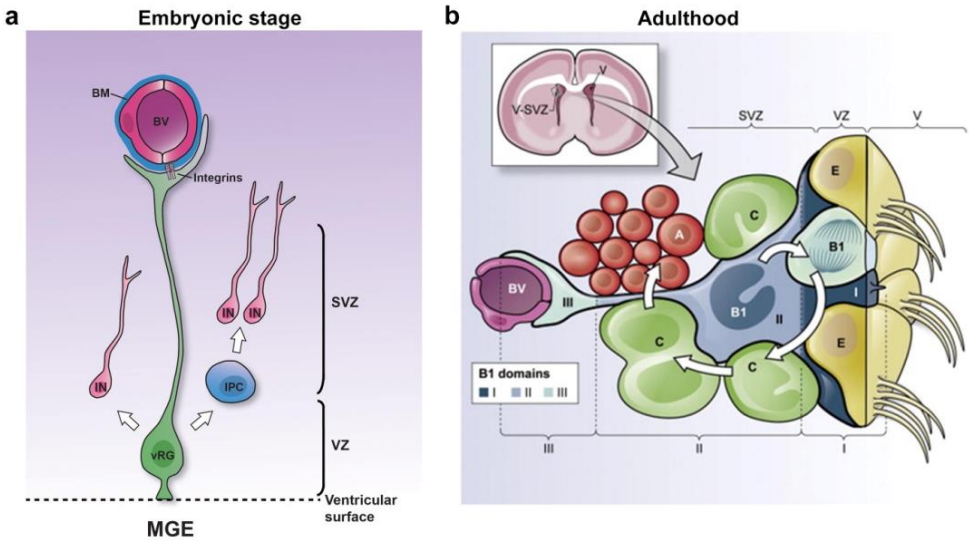


Figure 6.2: Vessel-anchored RGPs in the embryonic ventral telencephalon and adult neural stem cells in the V-SVZ. (a) Schematic of the vessel-anchored RGP (vRG) in the ventral telencephalon at the embryonic stage. vRGs possess a short apical process anchored to the ventricular surface and a longer radial glial fibre attached to the periventricular blood vessels (BV). They can produce daughter interneurons either directly or indirectly through the intermediate progenitor cells (IPCs). **(b)** Schematic of the different domains of adult neural stem cells (NSCs, also called B1 cells) within the adult ventricular-subventricular zone (V-SVZ). The upper left panel shows a frontal cross-section of the adult mouse brain showing the location of the V-SVZ, where neurogenesis in walls of the lateral ventricles (V) continues throughout life. The lower panel shows cellular composition of the adult V-SVZ niche and domains of NSCs (B1 cells, blue). B1 cells are surrounded by multiciliated ependymal cells (E) forming pinwheel-like structures on the ventricular surface. B1 cells give rise to IPCs (or C cells, green), which correspond to transit-amplifying cells that divide to generate neuroblasts (type A cells, red). B1 cells retain epithelial properties, with a thin apical process (containing a primary cilium) that contacts the lateral ventricle (V) and a long basal process ending on blood vessels (BV, purple). Adapted from (Fuentealba et al., 2012).

REFERENCES:

Aaku-Saraste, E., Hellwig, A., Huttner, W.B., 1996. Loss of occludin and functional tight junctions, but not ZO-1, during neural tube closure--remodeling of the neuroepithelium prior to neurogenesis. Dev Biol 180, 664-679.

Abbott, N.J., Ronnback, L., Hansson, E., 2006. Astrocyte-endothelial interactions at the blood-brain barrier. Nat Rev Neurosci 7, 41-53.

Alifragis, P., Liapi, A., Parnavelas, J.G., 2004. Lhx6 regulates the migration of cortical interneurons from the ventral telencephalon but does not specify their GABA phenotype. The Journal of neuroscience : the official journal of the Society for Neuroscience 24, 5643-5648.

Alvarez-Buylla, A., Kohwi, M., Nguyen, T.M., Merkle, F.T., 2008. The heterogeneity of adult neural stem cells and the emerging complexity of their niche. Cold Spring Harbor symposia on quantitative biology 73, 357-365.

Anderson, S., Mione, M., Yun, K., Rubenstein, J.L., 1999. Differential origins of neocortical projection and local circuit neurons: role of Dlx genes in neocortical interneuronogenesis. Cereb Cortex 9, 646-654.

Anderson, S.A., Eisenstat, D.D., Shi, L., Rubenstein, J.L., 1997. Interneuron migration from basal forebrain to neocortex: dependence on Dlx genes. Science 278, 474-476.

Anderson, S.A., Kaznowski, C.E., Horn, C., Rubenstein, J.L., McConnell, S.K., 2002. Distinct origins of neocortical projection neurons and interneurons in vivo. Cereb Cortex 12, 702-709.

Anderson, S.A., Marin, O., Horn, C., Jennings, K., Rubenstein, J.L., 2001. Distinct cortical migrations from the medial and lateral ganglionic eminences. Development 128, 353-363.

Ang, E.S., Jr., Haydar, T.F., Gluncic, V., Rakic, P., 2003. Four-dimensional migratory coordinates of GABAergic interneurons in the developing mouse cortex. J Neurosci 23, 5805-5815.

Angevine, J.B., Jr., Sidman, R.L., 1961. Autoradiographic study of cell migration during histogenesis of cerebral cortex in the mouse. Nature 192, 766-768.

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, 881-890.

Anton, E.S., Kreidberg, J.A., Rakic, P., 1999. Distinct functions of alpha3 and alpha(v) integrin receptors in neuronal migration and laminar organization of the cerebral cortex. Neuron 22, 277-289.

Ascoli, G.A., Alonso-Nanclares, L., Anderson, S.A., Barrionuevo, G., Benavides-Piccione, R., Burkhalter, A., Buzsaki, G., Cauli, B., Defelipe, J., Fairen, A., Feldmeyer, D., Fishell, G., Fregnac, Y., Freund, T.F., Gardner, D., Gardner, E.P., Goldberg, J.H., Helmstaedter, M., Hestrin, S., Karube, F., Kisvarday, Z.F., Lambolez, B., Lewis, D.A., Marin, O., Markram, H., Munoz, A., Packer, A., Petersen, C.C., Rockland, K.S., Rossier, J., Rudy, B., Somogyi, P., Staiger, J.F., Tamas, G., Thomson, A.M., Toledo-Rodriguez, M., Wang, Y., West, D.C., Yuste, R., 2008. Petilla terminology: nomenclature of features of GABAergic interneurons of the cerebral cortex. Nature reviews. Neuroscience 9, 557-568.

Avecilla, S.T., Hattori, K., Heissig, B., Tejada, R., Liao, F., Shido, K., Jin, D.K., Dias, S., Zhang, F., Hartman, T.E., Hackett, N.R., Crystal, R.G., Witte, L., Hicklin, D.J., Bohlen, P., Eaton, D., Lyden, D., de Sauvage, F., Rafii, S., 2004. Chemokine-mediated interaction of hematopoietic progenitors with the bone marrow vascular niche is required for thrombopoiesis. Nat Med 10, 64-71.

Barker, J.E., 1994. Sl/Sld hematopoietic progenitors are deficient in situ. Experimental hematology 22, 174-177.

Barker, J.E., 1997. Early transplantation to a normal microenvironment prevents the development of Steel hematopoietic stem cell defects. Experimental hematology 25, 542-547.

Batista-Brito, R., Fishell, G., 2009. The developmental integration of cortical interneurons into a functional network. Curr Top Dev Biol 87, 81-118.

Bayer, S.A., Altman, J., 1991. Development of the endopiriform nucleus and the claustrum in the rat brain. Neuroscience 45, 391-412.

Beggs, H.E., Schahin-Reed, D., Zang, K., Goebbels, S., Nave, K.A., Gorski, J., Jones, K.R., Sretavan, D., Reichardt, L.F., 2003. FAK deficiency in cells contributing to the basal lamina results in cortical abnormalities resembling congenital muscular dystrophies. Neuron 40, 501-514.

Belkin, A.M., Stepp, M.A., 2000. Integrins as receptors for laminins. Microscopy research and technique 51, 280-301.

Bentivoglio, M., Mazzarello, P., 1999. The history of radial glia. Brain Res Bull 49, 305-315.

Berry, M., Rogers, A.W., 1965. The migration of neuroblasts in the developing cerebral cortex. J Anat 99, 691-709.

Bertrand, N., Castro, D.S., Guillemot, F., 2002. Proneural genes and the specification of neural cell types. Nature reviews. Neuroscience 3, 517-530.

Blanpain, C., Lowry, W.E., Geoghegan, A., Polak, L., Fuchs, E., 2004. Self-renewal, multipotency, and the existence of two cell populations within an epithelial stem cell niche. Cell 118, 635-648.

Bovetti, S., Hsieh, Y.C., Bovolin, P., Perroteau, I., Kazunori, T., Puche, A.C., 2007. Blood vessels form a scaffold for neuroblast migration in the adult olfactory bulb. J Neurosci 27, 5976-5980.

Breier, G., Albrecht, U., Sterrer, S., Risau, W., 1992. Expression of vascular endothelial growth factor during embryonic angiogenesis and endothelial cell differentiation. Development 114, 521-532.

Briscoe, J., Pierani, A., Jessell, T.M., Ericson, J., 2000. A homeodomain protein code specifies progenitor cell identity and neuronal fate in the ventral neural tube. Cell 101, 435-445.

Britanova, O., Akopov, S., Lukyanov, S., Gruss, P., Tarabykin, V., 2005. Novel transcription factor Satb2 interacts with matrix attachment region DNA elements in a tissue-specific manner and demonstrates cell-type-dependent expression in the developing mouse CNS. Eur J Neurosci 21, 658-668.

Brown, K.N., Chen, S., Han, Z., Lu, C.H., Tan, X., Zhang, X.J., Ding, L., Lopez-Cruz, A., Saur, D., Anderson, S.A., Huang, K., Shi, S.H., 2011. Clonal production and organization of inhibitory interneurons in the neocortex. Science 334, 480-486.

Brunstrom, J.E., Gray-Swain, M.R., Osborne, P.A., Pearlman, A.L., 1997. Neuronal heterotopias in the developing cerebral cortex produced by neurotrophin-4. Neuron 18, 505-517.

Buck, C.A., Horwitz, A.F., 1987. Cell surface receptors for extracellular matrix molecules. Annual review of cell biology 3, 179-205.

Bulfone, A., Puelles, L., Porteus, M.H., Frohman, M.A., Martin, G.R., Rubenstein, J.L., 1993. Spatially restricted expression of Dlx-1, Dlx-2 (Tes-1), Gbx-2, and Wnt-3 in the embryonic day 12.5 mouse forebrain defines potential transverse and longitudinal segmental boundaries. The Journal of neuroscience : the official journal of the Society for Neuroscience 13, 3155-3172.

Bultje, R.S., Castaneda-Castellanos, D.R., Jan, L.Y., Jan, Y.N., Kriegstein, A.R., Shi, S.H., 2009. Mammalian Par3 regulates progenitor cell asymmetric division via notch signaling in the developing neocortex. Neuron 63, 189-202.

Butt, S.J., Fuccillo, M., Nery, S., Noctor, S., Kriegstein, A., Corbin, J.G., Fishell, G., 2005. The temporal and spatial origins of cortical interneurons predict their physiological subtype. Neuron 48, 591-604.

Butt, S.J., Sousa, V.H., Fuccillo, M.V., Hjerling-Leffler, J., Miyoshi, G., Kimura, S., Fishell, G., 2008. The requirement of Nkx2-1 in the temporal specification of cortical interneuron subtypes. Neuron 59, 722-732.

Cameron, R.S., Rakic, P., 1991. Glial cell lineage in the cerebral cortex: a review and synthesis. Glia 4, 124-137.

Campbell, K., Gotz, M., 2002. Radial glia: multi-purpose cells for vertebrate brain development. Trends Neurosci 25, 235-238.

Campos, L.S., Duarte, A.J., Branco, T., Henrique, D., 2001. mDll1 and mDll3 expression in the developing mouse brain: role in the establishment of the early cortex. Journal of neuroscience research 64, 590-598.

Cao, L., Jiao, X., Zuzga, D.S., Liu, Y., Fong, D.M., Young, D., During, M.J., 2004. VEGF links hippocampal activity with neurogenesis, learning and memory. Nat Genet 36, 827-835.

Caric, D., Gooday, D., Hill, R.E., McConnell, S.K., Price, D.J., 1997. Determination of the migratory capacity of embryonic cortical cells lacking the transcription factor Pax-6. Development 124, 5087-5096.

Carmeliet, P., 2003. Blood vessels and nerves: common signals, pathways and diseases. Nat Rev Genet 4, 710-720.

Cauli, B., Audinat, E., Lambolez, B., Angulo, M.C., Ropert, N., Tsuzuki, K., Hestrin, S., Rossier, J., 1997. Molecular and physiological diversity of cortical nonpyramidal cells. The Journal of neuroscience : the official journal of the Society for Neuroscience 17, 3894-3906.

Cavanagh, M.E., Parnavelas, J.G., 1989. Development of vasoactive-intestinal-polypeptide-immunoreactive neurons in the rat occipital cortex: a combined immunohistochemical-autoradiographic study. The Journal of comparative neurology 284, 637-645.

Caviness, V.S., Jr., Rakic, P., 1978. Mechanisms of cortical development: a view from mutations in mice. Annu Rev Neurosci 1, 297-326.

Chae, T., Kwon, Y.T., Bronson, R., Dikkes, P., Li, E., Tsai, L.H., 1997. Mice lacking p35, a neuronal specific activator of Cdk5, display cortical lamination defects, seizures, and adult lethality. Neuron 18, 29-42.

Chenn, A., McConnell, S.K., 1995. Cleavage orientation and the asymmetric inheritance of Notch1 immunoreactivity in mammalian neurogenesis. Cell 82, 631-641.

Chow, A., Erisir, A., Farb, C., Nadal, M.S., Ozaita, A., Lau, D., Welker, E., Rudy, B., 1999. K(+) channel expression distinguishes subpopulations of parvalbumin- and somatostatin-containing neocortical interneurons. The Journal of neuroscience : the official journal of the Society for Neuroscience 19, 9332-9345.

Cina, C., Maass, K., Theis, M., Willecke, K., Bechberger, J.F., Naus, C.C., 2009. Involvement of the cytoplasmic C-terminal domain of connexin43 in neuronal migration. J Neurosci 29, 2009-2021.

Cobos, I., Borello, U., Rubenstein, J.L., 2007. Dlx transcription factors promote migration through repression of axon and dendrite growth. Neuron 54, 873-888.

Colognato, H., ffrench-Constant, C., 2004. Mechanisms of glial development. Curr Opin Neurobiol 14, 37-44.

D'Arcangelo, G., 2001. The role of the Reelin pathway in cortical development. Symp Soc Exp Biol, 59-73.

D'Arcangelo, G., Miao, G.G., Chen, S.C., Soares, H.D., Morgan, J.I., Curran, T., 1995. A protein related to extracellular matrix proteins deleted in the mouse mutant reeler. Nature 374, 719-723.

de Carlos, J.A., Lopez-Mascaraque, L., Valverde, F., 1996. Dynamics of cell migration from the lateral ganglionic eminence in the rat. J Neurosci 16, 6146-6156.
de Rooij, D.G., Repping, S., van Pelt, A.M., 2008. Role for adhesion molecules in the spermatogonial stem cell niche. Cell Stem Cell 3, 467-468.

DeDiego, I., Smith-Fernandez, A., Fairen, A., 1994. Cortical cells that migrate beyond area boundaries: characterization of an early neuronal population in the lower intermediate zone of prenatal rats. The European journal of neuroscience 6, 983-997.
DeFelipe, J., Garcia Sola, R., Marco, P., del Rio, M.R., Pulido, P., Ramon y Cajal, S., 1993. Selective changes in the microorganization of the human epileptogenic neocortex revealed by parvalbumin immunoreactivity. Cereb Cortex 3, 39-48.

DeFelipe, J., Jones, E.G., 1988. A light and electron microscopic study of serotonin-immunoreactive fibers and terminals in the monkey sensory-motor cortex. Experimental brain research. Experimentelle Hirnforschung. Experimentation cerebrale 71, 171-182.

Denaxa, M., Chan, C.H., Schachner, M., Parnavelas, J.G., Karagogeos, D., 2001. The adhesion molecule TAG-1 mediates the migration of cortical interneurons from the ganglionic eminence along the corticofugal fiber system. Development 128, 4635-4644.

Devenport, D., Brown, N.H., 2004. Morphogenesis in the absence of integrins: mutation of both Drosophila beta subunits prevents midgut migration. Development 131, 5405-5415.

Ding, L., Saunders, T.L., Enikolopov, G., Morrison, S.J., 2012. Endothelial and perivascular cells maintain haematopoietic stem cells. Nature 481, 457-462.

Doetsch, F., 2003. A niche for adult neural stem cells. Curr Opin Genet Dev 13, 543-550.

Drakew, A., Frotscher, M., Deller, T., Ogawa, M., Heimrich, B., 1998. Developmental distribution of a reeler gene-related antigen in the rat hippocampal formation visualized by CR-50 immunocytochemistry. Neuroscience 82, 1079-1086.

Elias, L.A., Wang, D.D., Kriegstein, A.R., 2007. Gap junction adhesion is necessary for radial migration in the neocortex. Nature 448, 901-907.

Emsley, J.G., Hagg, T., 2003. alpha6beta1 integrin directs migration of neuronal precursors in adult mouse forebrain. Exp Neurol 183, 273-285.

Englund, C., Fink, A., Lau, C., Pham, D., Daza, R.A., Bulfone, A., Kowalczyk, T., Hevner, R.F., 2005. Pax6, Tbr2, and Tbr1 are expressed sequentially by radial glia, intermediate progenitor cells, and postmitotic neurons in developing neocortex. J Neurosci 25, 247-251.

Erickson, A.C., Couchman, J.R., 2000. Still more complexity in mammalian basement membranes. J Histochem Cytochem 48, 1291-1306.

Fietz, S.A., Kelava, I., Vogt, J., Wilsch-Brauninger, M., Stenzel, D., Fish, J.L., Corbeil, D., Riehn, A., Distler, W., Nitsch, R., Huttner, W.B., 2010. OSVZ progenitors of human and ferret neocortex are epithelial-like and expand by integrin signaling. Nat Neurosci 13, 690-699.

Flames, N., Pla, R., Gelman, D.M., Rubenstein, J.L., Puelles, L., Marin, O., 2007. Delineation of multiple subpallial progenitor domains by the combinatorial expression of transcriptional codes. The Journal of neuroscience : the official journal of the Society for Neuroscience 27, 9682-9695.

Fogarty, M., Grist, M., Gelman, D., Marin, O., Pachnis, V., Kessaris, N., 2007. Spatial genetic patterning of the embryonic neuroepithelium generates GABAergic interneuron diversity in the adult cortex. J Neurosci 27, 10935-10946.

Forster, E., Tielsch, A., Saum, B., Weiss, K.H., Johanssen, C., Graus-Porta, D., Muller, U., Frotscher, M., 2002. Reelin, Disabled 1, and beta 1 integrins are required for the formation of the radial glial scaffold in the hippocampus. Proc Natl Acad Sci U S A 99, 13178-13183.

Fuccillo, M., Rallu, M., McMahon, A.P., Fishell, G., 2004. Temporal requirement for hedgehog signaling in ventral telencephalic patterning. Development 131, 5031-5040.
Fuchs, E., Tumbar, T., Guasch, G., 2004. Socializing with the neighbors: stem cells and their niche. Cell 116, 769-778.

Fuchs, E.J., Whartenby, K.A., 2004. Hematopoietic stem cell transplant as a platform for tumor immunotherapy. Current opinion in molecular therapeutics 6, 48-53.

Fuentealba, L.C., Obernier, K., Alvarez-Buylla, A., 2012. Adult neural stem cells bridge their niche. Cell Stem Cell 10, 698-708.

Gage, F.H., 2000. Mammalian neural stem cells. Science 287, 1433-1438.

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. J Neurosci 26, 1045-1056.

Garaschuk, O., Linn, J., Eilers, J., Konnerth, A., 2000. Large-scale oscillatory calcium waves in the immature cortex. Nature neuroscience 3, 452-459.

Gelman, D., Griveau, A., Dehorter, N., Teissier, A., Varela, C., Pla, R., Pierani, A., Marin, O., 2011. A wide diversity of cortical GABAergic interneurons derives from the embryonic preoptic area. The Journal of neuroscience : the official journal of the Society for Neuroscience 31, 16570-16580.

Gelman, D.M., Marin, O., 2011. Generation of interneuron diversity in the mouse cerebral cortex. The European journal of neuroscience 31, 2136-2141.

Gelman, D.M., Martini, F.J., Nobrega-Pereira, S., Pierani, A., Kessaris, N., Marin, O., 2009. The embryonic preoptic area is a novel source of cortical GABAergic

interneurons. J Neurosci 29, 9380-9389.

Georges-Labouesse, E., Mark, M., Messaddeq, N., Gansmuller, A., 1998. Essential role of alpha 6 integrins in cortical and retinal lamination. Curr Biol 8, 983-986.

Gerhardt, H., Golding, M., Fruttiger, M., Ruhrberg, C., Lundkvist, A., Abramsson, A., Jeltsch, M., Mitchell, C., Alitalo, K., Shima, D., Betsholtz, C., 2003. VEGF guides angiogenic sprouting utilizing endothelial tip cell filopodia. J Cell Biol 161, 1163-1177.

Gerhardt, H., Ruhrberg, C., Abramsson, A., Fujisawa, H., Shima, D., Betsholtz, C., 2004. Neuropilin-1 is required for endothelial tip cell guidance in the developing central nervous system. Dev Dyn 231, 503-509.

Gleeson, J.G., Allen, K.M., Fox, J.W., Lamperti, E.D., Berkovic, S., Scheffer, I., Cooper, E.C., Dobyns, W.B., Minnerath, S.R., Ross, M.E., Walsh, C.A., 1998. Doublecortin, a brain-specific gene mutated in human X-linked lissencephaly and double cortex syndrome, encodes a putative signaling protein. Cell 92, 63-72.

Gonchar, Y., Burkhalter, A., 1997. Three distinct families of GABAergic neurons in rat visual cortex. Cereb Cortex 7, 347-358.

Gotz, M., Huttner, W.B., 2005. The cell biology of neurogenesis. Nat Rev Mol Cell Biol 6, 777-788.

Graus-Porta, D., Blaess, S., Senften, M., Littlewood-Evans, A., Damsky, C., Huang, Z., Orban, P., Klein, R., Schittny, J.C., Muller, U., 2001. Beta1-class integrins regulate the development of laminae and folia in the cerebral and cerebellar cortex. Neuron 31, 367-379.

Gritti, A., Parati, E.A., Cova, L., Frolichsthal, P., Galli, R., Wanke, E., Faravelli, L., Morassutti, D.J., Roisen, F., Nickel, D.D., Vescovi, A.L., 1996. Multipotential stem cells from the adult mouse brain proliferate and self-renew in response to basic fibroblast growth factor. J Neurosci 16, 1091-1100.

Guillemot, F., 2005. Cellular and molecular control of neurogenesis in the mammalian telencephalon. Current opinion in cell biology 17, 639-647.

Gulacsi, A., Anderson, S.A., 2006. Shh maintains Nkx2.1 in the MGE by a Gli3-independent mechanism. Cereb Cortex 16 Suppl 1, i89-95.

Gupta, A., Wang, Y., Markram, H., 2000. Organizing principles for a diversity of GABAergic interneurons and synapses in the neocortex. Science 287, 273-278.

Hansen, D.V., Lui, J.H., Parker, P.R., Kriegstein, A.R., 2010. Neurogenic radial glia in the outer subventricular zone of human neocortex. Nature 464, 554-561.

Hartfuss, E., Galli, R., Heins, N., Gotz, M., 2001. Characterization of CNS precursor subtypes and radial glia. Dev Biol 229, 15-30.

Hatten, M.E., 1990. Riding the glial monorail: a common mechanism for glial-guided neuronal migration in different regions of the developing mammalian brain. Trends in neurosciences 13, 179-184.

Hatten, M.E., 1999. Central nervous system neuronal migration. Annual review of neuroscience 22, 511-539.

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. Proc Natl Acad Sci U S A 101, 3196-3201.

Haubst, N., Georges-Labouesse, E., De Arcangelis, A., Mayer, U., Gotz, M., 2006. Basement membrane attachment is dispensable for radial glial cell fate and for proliferation, but affects positioning of neuronal subtypes. Development 133, 3245-3254.

Hensch, T.K., 2005. Critical period plasticity in local cortical circuits. Nature reviews. Neuroscience 6, 877-888.

Hogan, B.L., Kolodziej, P.A., 2002. Organogenesis: molecular mechanisms of tubulogenesis. Nat Rev Genet 3, 513-523.

Hogan, K.A., Ambler, C.A., Chapman, D.L., Bautch, V.L., 2004. The neural tube patterns vessels developmentally using the VEGF signaling pathway. Development 131, 1503-1513.

Holmes, D.I., Zachary, I., 2005. The vascular endothelial growth factor (VEGF) family: angiogenic factors in health and disease. Genome biology 6, 209.

Huang, Z.J., Di Cristo, G., Ango, F., 2007. Development of GABA innervation in the cerebral and cerebellar cortices. Nature reviews. Neuroscience 8, 673-686.

Isaacson, J.S., Scanziani, M., 2011. How inhibition shapes cortical activity. Neuron 72, 231-243.

Jimenez, D., Lopez-Mascaraque, L.M., Valverde, F., De Carlos, J.A., 2002. Tangential migration in neocortical development. Dev Biol 244, 155-169.

Kageyama, R., Ohtsuka, T., Shimojo, H., Imayoshi, I., 2008. Dynamic Notch signaling in neural progenitor cells and a revised view of lateral inhibition. Nature neuroscience 11, 1247-1251.

Kamme, F., Salunga, R., Yu, J., Tran, D.T., Zhu, J., Luo, L., Bittner, A., Guo, H.Q., Miller, N., Wan, J., Erlander, M., 2003. Single-cell microarray analysis in hippocampus CA1: demonstration and validation of cellular heterogeneity. The Journal of neuroscience : the official journal of the Society for Neuroscience 23, 3607-3615.

Kearney, J.B., Kappas, N.C., Ellerstrom, C., DiPaola, F.W., Bautch, V.L., 2004. The VEGF receptor flt-1 (VEGFR-1) is a positive modulator of vascular sprout formation and branching morphogenesis. Blood 103, 4527-4535.

Kerever, A., Schnack, J., Vellinga, D., Ichikawa, N., Moon, C., Arikawa-Hirasawa, E., Efird, J.T., Mercier, F., 2007. Novel extracellular matrix structures in the neural stem cell niche capture the neurogenic factor fibroblast growth factor 2 from the extracellular milieu. Stem Cells 25, 2146-2157.

Kiel, M.J., Morrison, S.J., 2008. Uncertainty in the niches that maintain haematopoietic stem cells. Nature reviews. Immunology 8, 290-301.

Kohwi, M., Osumi, N., Rubenstein, J.L., Alvarez-Buylla, A., 2005. Pax6 is required for making specific subpopulations of granule and periglomerular neurons in the olfactory bulb. J Neurosci 25, 6997-7003.

Kohwi, M., Petryniak, M.A., Long, J.E., Ekker, M., Obata, K., Yanagawa, Y., Rubenstein, J.L., Alvarez-Buylla, A., 2007. A subpopulation of olfactory bulb GABAergic interneurons is derived from Emx1- and Dlx5/6-expressing progenitors. The Journal of neuroscience : the official journal of the Society for Neuroscience 27, 6878-6891.

Kopan, R., Ilagan, M.X., 2009. The canonical Notch signaling pathway: unfolding the activation mechanism. Cell 137, 216-233.

Kornack, D.R., Rakic, P., 1995. Radial and horizontal deployment of clonally related cells in the primate neocortex: relationship to distinct mitotic lineages. Neuron 15, 311-321.

Kosodo, Y., Huttner, W.B., 2009. Basal process and cell divisions of neural progenitors in the developing brain. Dev Growth Differ 51, 251-261.

Kosodo, Y., Roper, K., Haubensak, W., Marzesco, A.M., Corbeil, D., Huttner, W.B., 2004. Asymmetric distribution of the apical plasma membrane during neurogenic

divisions of mammalian neuroepithelial cells. EMBO J 23, 2314-2324.

Kubota, Y., Kawaguchi, Y., 1994. Three classes of GABAergic interneurons in neocortex and neostriatum. The Japanese journal of physiology 44 Suppl 2, S145-148.

Kubota, Y., Kawaguchi, Y., 1997. Two distinct subgroups of cholecystokinin-immunoreactive cortical interneurons. Brain research 752, 175-183.

Kwan, K.Y., Sestan, N., Anton, E.S., 2012. Transcriptional co-regulation of neuronal migration and laminar identity in the neocortex. Development 139, 1535-1546.

Lavdas, A.A., Grigoriou, M., Pachnis, V., Parnavelas, J.G., 1999. The medial ganglionic eminence gives rise to a population of early neurons in the developing cerebral cortex. J Neurosci 19, 7881-7888.

Lawson, N.D., Scheer, N., Pham, V.N., Kim, C.H., Chitnis, A.B., Campos-Ortega, J.A., Weinstein, B.M., 2001. Notch signaling is required for arterial-venous differentiation during embryonic vascular development. Development 128, 3675-3683.

Lee, J.C., Cho, Y.J., Kim, J., Kim, N., Kang, B.G., Cha, C.I., Joo, K.M., 2010. Region-specific changes in the immunoreactivity of vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide receptors (VPAC2, and PAC1 receptor) in the aged rat brains. Brain research 1351, 32-40.

Lennartsson, J., Blume-Jensen, P., Hermanson, M., Ponten, E., Carlberg, M., Ronnstrand, L., 1999. Phosphorylation of Shc by Src family kinases is necessary for stem cell factor receptor/c-kit mediated activation of the Ras/MAP kinase pathway and c-fos induction. Oncogene 18, 5546-5553.

Lennartsson, J., Ronnstrand, L., 2006. The stem cell factor receptor/c-Kit as a drug target in cancer. Current cancer drug targets 6, 65-75.

Lewis, J., 1998. Notch signalling and the control of cell fate choices in vertebrates. Seminars in cell & developmental biology 9, 583-589.

Liodis, P., Denaxa, M., Grigoriou, M., Akufo-Addo, C., Yanagawa, Y., Pachnis, V., 2007. Lhx6 activity is required for the normal migration and specification of cortical interneuron subtypes. J Neurosci 27, 3078-3089.

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, 763-779.

Long, J.E., Cobos, I., Potter, G.B., Rubenstein, J.L., 2009. Dlx1&2 and Mash1 transcription factors control MGE and CGE patterning and differentiation through parallel and overlapping pathways. Cereb Cortex 19 Suppl 1, i96-106.

Louissaint, A., Jr., Rao, S., Leventhal, C., Goldman, S.A., 2002. Coordinated interaction of neurogenesis and angiogenesis in the adult songbird brain. Neuron 34, 945-960.

Lu, S.J., Quan, C., Li, F., Vida, L., Honig, G.R., 2002. Hematopoietic progenitor cells derived from embryonic stem cells: analysis of gene expression. Stem Cells 20, 428-437.

Madisen, L., Zwingman, T.A., Sunkin, S.M., Oh, S.W., Zariwala, H.A., Gu, H., Ng, L.L., Palmiter, R.D., Hawrylycz, M.J., Jones, A.R., Lein, E.S., Zeng, H., 2010. A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. Nat Neurosci 13, 133-140.

Magdaleno, S., Keshvara, L., Curran, T., 2002. Rescue of ataxia and preplate splitting by ectopic expression of Reelin in reeler mice. Neuron 33, 573-586.

Malatesta, P., Hack, M.A., Hartfuss, E., Kettenmann, H., Klinkert, W., Kirchhoff, F., Gotz, M., 2003. Neuronal or glial progeny: regional differences in radial glia fate. Neuron 37, 751-764.

Malatesta, P., Hartfuss, E., Gotz, M., 2000. Isolation of radial glial cells by fluorescent-activated cell sorting reveals a neuronal lineage. Development 127, 5253-5263.

Marin, O., Anderson, S.A., Rubenstein, J.L., 2000. Origin and molecular specification of striatal interneurons. J Neurosci 20, 6063-6076.

Marin, O., Plump, A.S., Flames, N., Sanchez-Camacho, C., Tessier-Lavigne, M., Rubenstein, J.L., 2003. Directional guidance of interneuron migration to the cerebral cortex relies on subcortical Slit1/2-independent repulsion and cortical attraction. Development 130, 1889-1901.

Marin, O., Rubenstein, J.L., 2001. A long, remarkable journey: tangential migration in the telencephalon. Nat Rev Neurosci 2, 780-790.

Marin, O., Rubenstein, J.L., 2003. Cell migration in the forebrain. Annu Rev Neurosci 26, 441-483.

Marin, O., Yaron, A., Bagri, A., Tessier-Lavigne, M., Rubenstein, J.L., 2001. Sorting of striatal and cortical interneurons regulated by semaphorin-neuropilin interactions.

Science 293, 872-875.

Marinkovich, M.P., 2007. Tumour microenvironment: laminin 332 in squamous-cell carcinoma. Nature reviews. Cancer 7, 370-380.

Markram, H., Toledo-Rodriguez, M., Wang, Y., Gupta, A., Silberberg, G., Wu, C., 2004. Interneurons of the neocortical inhibitory system. Nat Rev Neurosci 5, 793-807.
Marthiens, V., Kazanis, I., Moss, L., Long, K., Ffrench-Constant, C., 2010. Adhesion molecules in the stem cell niche--more than just staying in shape? J Cell Sci 123, 1613-1622.

Martinez-Cerdeno, V., Noctor, S.C., Kriegstein, A.R., 2006. The role of intermediate progenitor cells in the evolutionary expansion of the cerebral cortex. Cereb Cortex 16 Suppl 1, i152-161.

Martynoga, B., Morrison, H., Price, D.J., Mason, J.O., 2005. Foxg1 is required for specification of ventral telencephalon and region-specific regulation of dorsal telencephalic precursor proliferation and apoptosis. Dev Biol 283, 113-127.

Mercier, F., Kitasako, J.T., Hatton, G.I., 2002. Anatomy of the brain neurogenic zones revisited: fractones and the fibroblast/macrophage network. J Comp Neurol 451, 170-188.

Mione, M.C., Danevic, C., Boardman, P., Harris, B., Parnavelas, J.G., 1994. Lineage analysis reveals neurotransmitter (GABA or glutamate) but not calcium-binding protein homogeneity in clonally related cortical neurons. The Journal of neuroscience : the official journal of the Society for Neuroscience 14, 107-123.

Miranti, C.K., Brugge, J.S., 2002. Sensing the environment: a historical perspective on integrin signal transduction. Nature cell biology 4, E83-90.

Miyata, T., Kawaguchi, A., Okano, H., Ogawa, M., 2001. Asymmetric inheritance of radial glial fibers by cortical neurons. Neuron 31, 727-741.

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, 3133-3145.

Miyoshi, G., Butt, S.J., Takebayashi, H., Fishell, G., 2007. Physiologically distinct temporal cohorts of cortical interneurons arise from telencephalic Olig2-expressing precursors. J Neurosci 27, 7786-7798.

Miyoshi, G., Hjerling-Leffler, J., Karayannis, T., Sousa, V.H., Butt, S.J., Battiste, J., Johnson, J.E., Machold, R.P., 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, 1582-1594.

Mollgoard, K., Saunders, N.R., 1975. Complex tight junctions of epithelial and of endothelial cells in early foetal brain. J Neurocytol 4, 453-468.

Molyneaux, B.J., Arlotta, P., Menezes, J.R., Macklis, J.D., 2007. Neuronal subtype specification in the cerebral cortex. Nature reviews. Neuroscience 8, 427-437.

Monje, M.L., Mizumatsu, S., Fike, J.R., Palmer, T.D., 2002. Irradiation induces neural precursor-cell dysfunction. Nat Med 8, 955-962.

Monyer, H., Markram, H., 2004. Interneuron Diversity series: Molecular and genetic tools to study GABAergic interneuron diversity and function. Trends Neurosci 27, 90-97.

Mori, T., Buffo, A., Gotz, M., 2005. The novel roles of glial cells revisited: the contribution of radial glia and astrocytes to neurogenesis. Curr Top Dev Biol 69, 67-99.

Morrison, S.J., Spradling, A.C., 2008. Stem cells and niches: mechanisms that promote stem cell maintenance throughout life. Cell 132, 598-611.

Mountcastle, V.B., 1997. The columnar organization of the neocortex. Brain : a journal of neurology 120 (Pt 4), 701-722.

Muzio, L., DiBenedetto, B., Stoykova, A., Boncinelli, E., Gruss, P., Mallamaci, A., 2002. Conversion of cerebral cortex into basal ganglia in Emx2(-/-) Pax6(Sey/Sey) double-mutant mice. Nat Neurosci 5, 737-745.

Nery, S., Fishell, G., Corbin, J.G., 2002. The caudal ganglionic eminence is a source of distinct cortical and subcortical cell populations. Nature neuroscience 5, 1279-1287.

Nieto, M., Monuki, E.S., Tang, H., Imitola, J., Haubst, N., Khoury, S.J., Cunningham, J., Gotz, M., Walsh, C.A., 2004. Expression of Cux-1 and Cux-2 in the subventricular zone and upper layers II-IV of the cerebral cortex. J Comp Neurol 479, 168-180.

Niewmierzycka, A., Mills, J., St-Arnaud, R., Dedhar, S., Reichardt, L.F., 2005. Integrin-linked kinase deletion from mouse cortex results in cortical lamination defects resembling cobblestone lissencephaly. J Neurosci 25, 7022-7031.

Nikolova, G., Strilic, B., Lammert, E., 2007. The vascular niche and its basement

membrane. Trends in cell biology 17, 19-25.

Noctor, S.C., Flint, A.C., Weissman, T.A., Dammerman, R.S., Kriegstein, A.R., 2001. Neurons derived from radial glial cells establish radial units in neocortex. Nature 409, 714-720.

Noctor, S.C., Flint, A.C., Weissman, T.A., Wong, W.S., Clinton, B.K., Kriegstein, A.R., 2002. Dividing precursor cells of the embryonic cortical ventricular zone have morphological and molecular characteristics of radial glia. The Journal of neuroscience : the official journal of the Society for Neuroscience 22, 3161-3173.

Noctor, S.C., 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, 136-144.

Noctor, S.C., Martinez-Cerdeno, V., Kriegstein, A.R., 2008. Distinct behaviors of neural stem and progenitor cells underlie cortical neurogenesis. The Journal of comparative neurology 508, 28-44.

Ogawa, M., Matsuzaki, Y., Nishikawa, S., Hayashi, S., Kunisada, T., Sudo, T., Kina, T., Nakauchi, H., 1991. Expression and function of c-kit in hemopoietic progenitor cells. The Journal of experimental medicine 174, 63-71.

Ogawa, M., Miyata, T., Nakajima, K., Yagyu, K., Seike, M., Ikenaka, K., Yamamoto, H., Mikoshiba, K., 1995. The reeler gene-associated antigen on Cajal-Retzius neurons is a crucial molecule for laminar organization of cortical neurons. Neuron 14, 899-912.

Ohshima, T., Ward, J.M., Huh, C.G., Longenecker, G., Veeranna, Pant, H.C., Brady, R.O., Martin, L.J., Kulkarni, A.B., 1996. Targeted disruption of the cyclin-dependent kinase 5 gene results in abnormal corticogenesis, neuronal pathology and perinatal death. Proc Natl Acad Sci U S A 93, 11173-11178.

Palmer, T.D., Willhoite, A.R., Gage, F.H., 2000. Vascular niche for adult hippocampal neurogenesis. J Comp Neurol 425, 479-494.

Parnavelas, J.G., 2002. The origin of cortical neurons. Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas / Sociedade Brasileira de Biofisica ... [et al.] 35, 1423-1429.

Parnavelas, J.G., Alifragis, P., Nadarajah, B., 2002. The origin and migration of cortical neurons. Progress in brain research 136, 73-80.

Parnavelas, J.G., Barfield, J.A., Franke, E., Luskin, M.B., 1991. Separate progenitor

cells give rise to pyramidal and nonpyramidal neurons in the rat telencephalon. Cereb Cortex 1, 463-468.

Petryniak, M.A., Potter, G.B., Rowitch, D.H., Rubenstein, J.L., 2007. Dlx1 and Dlx2 control neuronal versus oligodendroglial cell fate acquisition in the developing forebrain. Neuron 55, 417-433.

Pfeffer, C.K., Xue, M., He, M., Huang, Z.J., Scanziani, M., 2013. Inhibition of inhibition in visual cortex: the logic of connections between molecularly distinct interneurons. Nat Neurosci 16, 1068-1076.

Pleasure, S.J., Anderson, S., Hevner, R., Bagri, A., Marin, O., Lowenstein, D.H., Rubenstein, J.L., 2000. Cell migration from the ganglionic eminences is required for the development of hippocampal GABAergic interneurons. Neuron 28, 727-740.

Polleux, F., Whitford, K.L., Dijkhuizen, P.A., Vitalis, T., Ghosh, A., 2002. Control of cortical interneuron migration by neurotrophins and PI3-kinase signaling. Development 129, 3147-3160.

Porteus, M.H., Bulfone, A., Liu, J.K., Puelles, L., Lo, L.C., Rubenstein, J.L., 1994. DLX-2, MASH-1, and MAP-2 expression and bromodeoxyuridine incorporation define molecularly distinct cell populations in the embryonic mouse forebrain. The Journal of neuroscience : the official journal of the Society for Neuroscience 14, 6370-6383.

Price, J., Williams, B., Grove, E., 1991. Cell lineage in the cerebral cortex. Development Suppl 2, 23-28.

Puelles, E., Rubenstein, J.L., Puelles, L., 2001. Chicken Nkx6.1 expression at advanced stages of development identifies distinct brain nuclei derived from the basal plate. Mechanisms of development 102, 279-282.

Puelles, L., Kuwana, E., Puelles, E., Bulfone, A., Shimamura, K., Keleher, J., Smiga, S., Rubenstein, J.L., 2000. Pallial and subpallial derivatives in the embryonic chick and mouse telencephalon, traced by the expression of the genes Dlx-2, Emx-1, Nkx-2.1, Pax-6, and Tbr-1. The Journal of comparative neurology 424, 409-438.

Raab, S., Beck, H., Gaumann, A., Yuce, A., Gerber, H.P., Plate, K., Hammes, H.P., Ferrara, N., Breier, G., 2004. Impaired brain angiogenesis and neuronal apoptosis induced by conditional homozygous inactivation of vascular endothelial growth factor. Thrombosis and haemostasis 91, 595-605.

Raghavan, S., Bauer, C., Mundschau, G., Li, Q., Fuchs, E., 2000. Conditional ablation of beta1 integrin in skin. Severe defects in epidermal proliferation, basement

membrane formation, and hair follicle invagination. The Journal of cell biology 150, 1149-1160.

Rakic, P., 1978. Neuronal migration and contact guidance in the primate telencephalon. Postgraduate medical journal 54 Suppl 1, 25-40.

Rakic, P., 1988a. Defects of neuronal migration and the pathogenesis of cortical malformations. Prog Brain Res 73, 15-37.

Rakic, P., 1988b. Specification of cerebral cortical areas. Science 241, 170-176.

Rallu, M., Machold, R., Gaiano, N., Corbin, J.G., McMahon, A.P., Fishell, G., 2002. Dorsoventral patterning is established in the telencephalon of mutants lacking both Gli3 and Hedgehog signaling. Development 129, 4963-4974.

Ramirez-Castillejo, C., Sanchez-Sanchez, F., Andreu-Agullo, C., Ferron, S.R., Aroca-Aguilar, J.D., Sanchez, P., Mira, H., Escribano, J., Farinas, I., 2006. Pigment epithelium-derived factor is a niche signal for neural stem cell renewal. Nat Neurosci 9, 331-339.

Ross, R.A., Biedler, J.L., Spengler, B.A., 2003. A role for distinct cell types in determining malignancy in human neuroblastoma cell lines and tumors. Cancer letters 197, 35-39.

Rubin, A.N., Alfonsi, F., Humphreys, M.P., Choi, C.K., Rocha, S.F., Kessaris, N., 2010. The germinal zones of the basal ganglia but not the septum generate GABAergic interneurons for the cortex. The Journal of neuroscience : the official journal of the Society for Neuroscience 30, 12050-12062.

Rudy, B., Fishell, G., Lee, S., Hjerling-Leffler, J., 2010. Three groups of interneurons account for nearly 100% of neocortical GABAergic neurons. Developmental neurobiology.

Ryder, E.F., Snyder, E.Y., Cepko, C.L., 1990. Establishment and characterization of multipotent neural cell lines using retrovirus vector-mediated oncogene transfer. Journal of neurobiology 21, 356-375.

Schmahl, W., Knoedlseder, M., Favor, J., Davidson, D., 1993. Defects of neuronal migration and the pathogenesis of cortical malformations are associated with Small eye (Sey) in the mouse, a point mutation at the Pax-6-locus. Acta Neuropathol 86, 126-135.

Schofield, R., 1978. The relationship between the spleen colony-forming cell and the haemopoietic stem cell. Blood Cells 4, 7-25.

Shen, Q., Wang, Y., Kokovay, E., Lin, G., Chuang, S.M., Goderie, S.K., Roysam, B., Temple, S., 2008. Adult SVZ stem cells lie in a vascular niche: a quantitative analysis of niche cell-cell interactions. Cell Stem Cell 3, 289-300.

Shibata, M., Gulden, F.O., Sestan, N., 2015. From trans to cis: transcriptional regulatory networks in neocortical development. Trends in genetics : TIG 31, 77-87.
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. J Neurosci 31, 3683-3695.

Smart, I.H., 1976. A pilot study of cell production by the ganglionic eminences of the developing mouse brain. Journal of anatomy 121, 71-84.

Stenman, J., Yu, R.T., Evans, R.M., Campbell, K., 2003. Tlx and Pax6 co-operate genetically to establish the pallio-subpallial boundary in the embryonic mouse telencephalon. Development 130, 1113-1122.

Sturrock, R.R., Smart, I.H., 1980. A morphological study of the mouse subependymal layer from embryonic life to old age. Journal of anatomy 130, 391-415.

Sugimori, M., Nagao, M., Bertrand, N., Parras, C.M., Guillemot, F., Nakafuku, M., 2007. Combinatorial actions of patterning and HLH transcription factors in the spatiotemporal control of neurogenesis and gliogenesis in the developing spinal cord. Development 134, 1617-1629.

Sugino, K., Hempel, C.M., Miller, M.N., Hattox, A.M., Shapiro, P., Wu, C., Huang, Z.J., Nelson, S.B., 2006. Molecular taxonomy of major neuronal classes in the adult mouse forebrain. Nature neuroscience 9, 99-107.

Sultan, K.T., Brown, K.N., Shi, S.H., 2013. Production and organization of neocortical interneurons. Frontiers in cellular neuroscience 7, 221.

Sussel, L., Marin, O., Kimura, S., Rubenstein, J.L., 1999. Loss of Nkx2.1 homeobox gene function results in a ventral to dorsal molecular respecification within the basal telencephalon: evidence for a transformation of the pallidum into the striatum. Development 126, 3359-3370.

Taglialatela, P., Soria, J.M., Caironi, V., Moiana, A., Bertuzzi, S., 2004. Compromised generation of GABAergic interneurons in the brains of Vax1-/- mice. Development 131, 4239-4249.

Tamamaki, N., Nakamura, K., Okamoto, K., Kaneko, T., 2001. Radial glia is a

progenitor of neocortical neurons in the developing cerebral cortex. Neurosci Res 41, 51-60.

Tammela, T., Zarkada, G., Wallgard, E., Murtomaki, A., Suchting, S., Wirzenius, M., Waltari, M., Hellstrom, M., Schomber, T., Peltonen, R., Freitas, C., Duarte, A., Isoniemi, H., Laakkonen, P., Christofori, G., Yla-Herttuala, S., Shibuya, M., Pytowski, B., Eichmann, A., Betsholtz, C., Alitalo, K., 2008. Blocking VEGFR-3 suppresses angiogenic sprouting and vascular network formation. Nature 454, 656-660.

Tan, S.S., Kalloniatis, M., Sturm, K., Tam, P.P., Reese, B.E., Faulkner-Jones, B., 1998. Separate progenitors for radial and tangential cell dispersion during development of the cerebral neocortex. Neuron 21, 295-304.

Tan, X., Shi, S.H., 2013. Neocortical neurogenesis and neuronal migration. Wiley interdisciplinary reviews. Developmental biology 2, 443-459.

Tanaka, D., Nakaya, Y., Yanagawa, Y., Obata, K., Murakami, F., 2003. Multimodal tangential migration of neocortical GABAergic neurons independent of GPI-anchored proteins. Development 130, 5803-5813.

Tavazoie, M., Van der Veken, L., Silva-Vargas, V., Louissaint, M., Colonna, L., Zaidi, B., Garcia-Verdugo, J.M., Doetsch, F., 2008. A specialized vascular niche for adult neural stem cells. Cell Stem Cell 3, 279-288.

Temple, S., 2001. The development of neural stem cells. Nature 414, 112-117.

Thommes, K., Lennartsson, J., Carlberg, M., Ronnstrand, L., 1999. Identification of Tyr-703 and Tyr-936 as the primary association sites for Grb2 and Grb7 in the c-Kit/stem cell factor receptor. Biochem J 341 (Pt 1), 211-216.

Troyanovsky, S.M., 1999. Mechanism of cell-cell adhesion complex assembly. Current opinion in cell biology 11, 561-566.

Tsao, C.Y., Mendell, J.R., Rusin, J., Luquette, M., 1998. Congenital muscular dystrophy with complete laminin-alpha2-deficiency, cortical dysplasia, and cerebral white-matter changes in children. Journal of child neurology 13, 253-256.

Valcanis, H., Tan, S.S., 2003. Layer specification of transplanted interneurons in developing mouse neocortex. J Neurosci 23, 5113-5122.

Valiente, M., Ciceri, G., Rico, B., Marin, O., 2011. Focal adhesion kinase modulates radial glia-dependent neuronal migration through connexin-26. The Journal of neuroscience : the official journal of the Society for Neuroscience 31, 11678-11691.

Vasudevan, A., Bhide, P.G., 2008. Angiogenesis in the embryonic CNS: a new twist on an old tale. Cell adhesion & migration 2, 167-169.

Vasudevan, A., Long, J.E., Crandall, J.E., Rubenstein, J.L., Bhide, P.G., 2008. Compartment-specific transcription factors orchestrate angiogenesis gradients in the embryonic brain. Nat Neurosci 11, 429-439.

Walsh, C., Cepko, C.L., 1992. Widespread dispersion of neuronal clones across functional regions of the cerebral cortex. Science 255, 434-440.

Walsh, C., Cepko, C.L., 1993. Clonal dispersion in proliferative layers of developing cerebral cortex. Nature 362, 632-635.

Wang, X.J., Tegner, J., Constantinidis, C., Goldman-Rakic, P.S., 2004. Division of labor among distinct subtypes of inhibitory neurons in a cortical microcircuit of working memory. Proceedings of the National Academy of Sciences of the United States of America 101, 1368-1373.

Ware, M.L., Tavazoie, S.F., Reid, C.B., Walsh, C.A., 1999. Coexistence of widespread clones and large radial clones in early embryonic ferret cortex. Cereb Cortex 9, 636-645.

Whittington, M.A., Traub, R.D., 2003. Interneuron diversity series: inhibitory interneurons and network oscillations in vitro. Trends in neurosciences 26, 676-682.
Wichterle, H., Garcia-Verdugo, J.M., Herrera, D.G., Alvarez-Buylla, A., 1999. Young neurons from medial ganglionic eminence disperse in adult and embryonic brain. Nat Neurosci 2, 461-466.

Wichterle, H., Turnbull, D.H., Nery, S., Fishell, G., Alvarez-Buylla, A., 2001. In utero fate mapping reveals distinct migratory pathways and fates of neurons born in the mammalian basal forebrain. Development 128, 3759-3771.

Williams, B.P., Price, J., 1995. Evidence for multiple precursor cell types in the embryonic rat cerebral cortex. Neuron 14, 1181-1188.

Williams, K.C., Zhao, R.W., Ueno, K., Hickey, W.F., 1996. PECAM-1 (CD31) expression in the central nervous system and its role in experimental allergic encephalomyelitis in the rat. Journal of neuroscience research 45, 747-757.

Wolf, N.S., 1978. Dissecting the hematopoietic microenvironment. III. Evidence for a positive short range stimulus for cellular proliferation. Cell and tissue kinetics 11, 335-345.

Won, C., Lin, Z., Kumar, T.P., Li, S., Ding, L., Elkhal, A., Szabo, G., Vasudevan, A.,

2013. Autonomous vascular networks synchronize GABA neuron migration in the embryonic forebrain. Nature communications 4, 2149.

Wonders, C.P., Anderson, S.A., 2006. The origin and specification of cortical interneurons. Nature reviews. Neuroscience 7, 687-696.

Wonders, C.P., Taylor, L., Welagen, J., Mbata, I.C., Xiang, J.Z., Anderson, S.A., 2008. A spatial bias for the origins of interneuron subgroups within the medial ganglionic eminence. Developmental biology 314, 127-136.

Xu, Q., Cobos, I., De La Cruz, E., Rubenstein, J.L., Anderson, S.A., 2004. Origins of cortical interneuron subtypes. J Neurosci 24, 2612-2622.

Xu, Q., Guo, L., Moore, H., Waclaw, R.R., Campbell, K., Anderson, S.A., 2010. Sonic hedgehog signaling confers ventral telencephalic progenitors with distinct cortical interneuron fates. Neuron 65, 328-340.

Xu, Q., Tam, M., Anderson, S.A., 2008. Fate mapping Nkx2.1-lineage cells in the mouse telencephalon. The Journal of comparative neurology 506, 16-29.

Xu, Q., Wonders, C.P., Anderson, S.A., 2005. Sonic hedgehog maintains the identity of cortical interneuron progenitors in the ventral telencephalon. Development 132, 4987-4998.

Yang, Y., Connelly, K., Graham, J.J., Detsky, J., Lee, T., Walcarius, R., Paul, G., Wright, G.A., Dick, A.J., 2011. Papillary muscle involvement in myocardial infarction: initial results using multicontrast late-enhancement MRI. Journal of magnetic resonance imaging : JMRI 33, 211-216.

Yilmaz, O.H., Kiel, M.J., Morrison, S.J., 2006. SLAM family markers are conserved among hematopoietic stem cells from old and reconstituted mice and markedly increase their purity. Blood 107, 924-930.

Young, K.M., Fogarty, M., Kessaris, N., Richardson, W.D., 2007. Subventricular zone stem cells are heterogeneous with respect to their embryonic origins and neurogenic fates in the adult olfactory bulb. J Neurosci 27, 8286-8296.

Yozu, M., Tabata, H., Nakajima, K., 2005. The caudal migratory stream: a novel migratory stream of interneurons derived from the caudal ganglionic eminence in the developing mouse forebrain. J Neurosci 25, 7268-7277.

Yu, Y.C., Bultje, R.S., Wang, X., Shi, S.H., 2009. Specific synapses develop preferentially among sister excitatory neurons in the neocortex. Nature 458, 501-504.

Zhadanov, A.B., Provance, D.W., Jr., Speer, C.A., Coffin, J.D., Goss, D., Blixt, J.A., Reichert, C.M., Mercer, J.A., 1999. Absence of the tight junctional protein AF-6 disrupts epithelial cell-cell junctions and cell polarity during mouse development. Current biology : CB 9, 880-888.

Zhang, Z., Galileo, D.S., 1998. Retroviral transfer of antisense integrin alpha6 or alpha8 sequences results in laminar redistribution or clonal cell death in developing brain. J Neurosci 18, 6928-6938.

Zhong, T.P., Childs, S., Leu, J.P., Fishman, M.C., 2001. Gridlock signalling pathway fashions the first embryonic artery. Nature 414, 216-220.

Zhou, Q., Anderson, D.J., 2002. The bHLH transcription factors OLIG2 and OLIG1 couple neuronal and glial subtype specification. Cell 109, 61-73.

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. Cereb Cortex 14, 1408-1420.

Zlokovic, B.V., 2008. The blood-brain barrier in health and chronic neurodegenerative disorders. Neuron 57, 178-201.