

Distribution, identity and fate of Fgf18-expressing cells in the adult mouse Subventricular zone



Giada Vanacore

School of Biological Science
University of East Anglia

A thesis submitted for the degree of
MSc by Research
in Biomolecular Science

October 18, 2022

This copy of the thesis has been supplied on condition that anyone who consults it is understood to recognise that its copyright rests with the author and that use of any information derived there-from must be in accordance with current UK Copyright Law. In addition, any quotation or extract must include full attribution

TABLE OF CONTENTS

ABSTRACT	4
List of Figures	5
List of Tables	6
Abbreviations	7
1 – INTRODUCTION	8
1.1 – Adult Neurogenesis	9
1.1.2 The Concept of Neuroplasticity	9
1.1.3 Fundamental Principles of Neurogenesis	10
1.2 – The Ventricular-Subventricular Zone: A Canonical Neurogenic Niche	13
1.2.1 Organization of the Niche	13
1.2.2 Identification of a Unique Migratory Path	16
1.2.3 The Origin and Heterogeneity of the NSCs in the adult V-SVZ	18
1.2.4 The Niche Microenvironment influences V-SVZ Activity	20
1.3 – The Fibroblast Growth Factor (FGF) signalling pathway	21
1.3.1 FGFs: An Overview	21
1.3.2 The Role of FGF18 in Brain Development	23
1.3.3 The Role of FGF18 in the Adult CNS	25
1.4 – Aims and Objectives	27
2 – MATERIAL & METHODS	28
2.1 – Animal Models	29
2.1.1 FGF18 CreERT2 +/-	29
2.1.2 Rosa26 tdTomato	29
2.2 – Genotyping	30
2.3 – Tamoxifen Treatment	30
2.4 – Tissue Isolation and Preparation	33
2.5 – Immunolabelling Protocol	33
2.5.1 Immunolabelling on Vibratome-Generated Sections	33
2.5.2 Antigen Retrieval	34
2.5.3 Immunolabelling on Cryostat-Generated Sections	34
2.6 – Image Capture and Analysis	37
2.6.1 Cell Counting	37

3 – RESULTS	38
3.1 – FGF18 expression is restricted to the dorsal and medial walls of the lateral ventricles in the adult FGF18-CreERT2::Rosa26-tdTomato (FGF18 ^{Lineage}) double transgenic mice	39
3.2 – Characterisation of the FGF18-expressing cells in the V-SVZ Niche	39
3.3 – FGF18 ⁺ cells in the adult V-SVZ may be progenitors	41
3.4 – FGF18 has a potential role in barrier function in the adult V-SVZ niche	43
3.5 – FGF18 lineage traced cells are detected in the RMS and OB of Long Chase mice only	45
3.5.1 –The morphology of Tomato ⁺ cells in the RMS relates to migrating neuroblasts	45
3.6 – V-SVZ FGF18 ⁺ cells will give rise to granule interneurons of OB	49
3.7 – An age-related decline in FGF18 ⁺ cell number and proliferation	52
4 – DISCUSSION	58
4.1 – FGF18 contributes to the compartmentalisation and regulation of the V-SVZ in the adult mouse brain	59
4.1.1 – NSCs quiescence/activation	59
4.1.2 –FGF18 may signal through the CSF	60
4.1.3 – Barrier Function	61
4.2 – FGF18: a potential role in the neuronal supply to the OB	62
4.3 – Age-Dependent changes in FGF18 is the regulation of V-SVZ neurogenesis	63
CONCLUSION	66
REFERENCES	67
APPENDIX	78
ACKNOWLEDGMENTS	83

ABSTRACT

In the adult mammalian brain, neurogenesis occurs in specific anatomic regions such as the ventricular-subventricular zone (V-SVZ) and the dentate gyrus of the hippocampus. In the V-SVZ, NSC activation and differentiation is responsible for the generation of inhibitory interneurons in the olfactory bulbs (OBs) that contribute to odour discrimination and related behaviours in mice. Fibroblast Growth Factor (FGF) signalling through their receptors (FGFRs) influence the neurogenic activity of the adult V-SVZ. However, the cohort of FGF members participating in this process has yet to be firmly established. This study investigates the expression of the FGF18-expressing cells in the V-SVZ and follows their fate to determine their involvement in the V-SVZ neurogenic activity. In order to do so, adult FGF18-CreERT2::Rosa26-tdTomato mice were pulsed with tamoxifen for two consecutive days and their brains harvested after either 2 or 12 days later to enable lineage tracing analyses. These findings reveal that, in short chase mice, FGF18-expressing cells were restricted to the dorsal and medial walls of the ventricles whilst in the long chase mice, Tomato⁺ cells were additionally found in the Rostral Migratory Stream (RMS) and OBs where they resembled migrating neuroblasts and maturing neurons, respectively. In the V-SVZ, subsets of FGF18-expressing cells co-expressed the stem cell markers GFAP and Sox2, suggesting that they may be NSCs/progenitor cells and regulate neurogenesis. Eventually, they might have a role in barrier function at the ependymal layer of the niche. Analyses of early postnatal mice revealed an age-dependent expression and activity of the FGF18-expressing cells in the V-SVZ niche and daughter cells. Together, this study points to a dynamic participation of FGF18 in the neurogenic activity and homeostasis preservation of the adult mouse V-SVZ, opening new scenarios on the regulatory role of FGF signalling in this region.

Access Condition and Agreement

Each deposit in UEA Digital Repository is protected by copyright and other intellectual property rights, and duplication or sale of all or part of any of the Data Collections is not permitted, except that material may be duplicated by you for your research use or for educational purposes in electronic or print form. You must obtain permission from the copyright holder, usually the author, for any other use. Exceptions only apply where a deposit may be explicitly provided under a stated licence, such as a Creative Commons licence or Open Government licence.

Electronic or print copies may not be offered, whether for sale or otherwise to anyone, unless explicitly stated under a Creative Commons or Open Government license. Unauthorised reproduction, editing or reformatting for resale purposes is explicitly prohibited (except where approved by the copyright holder themselves) and UEA reserves the right to take immediate 'take down' action on behalf of the copyright and/or rights holder if this Access condition of the UEA Digital Repository is breached. Any material in this database has been supplied on the understanding that it is copyright material and that no quotation from the material may be published without proper acknowledgement.

List of Figures

1 – Introduction

Figure 1-1. Neurogenesis in the Adult mouse Brain

Figure 1-2. The Adult V-SVZ Niche

Figure 1-3. The NSCs in the Adult V-SVZ are Specified during Embryonic Development

Figure 1-4. The Fibroblast Growth Factor Family

2 – Methods

Figure 2-1. Transgenic Mouse Alleles

Figure 2-2. Schematic representation of FGF18^{Lineage} mice tamoxifen treatment

3 – Results

Figure 3-1. FGF18-expressing cells are restricted to the dorsal and medial V-SVZ

Figure 3-2. FGF18⁺ cells may be progenitor cells in the V-SVZ niche

Figure 3-3. FGF18 could have a role in barrier function at the medial V-SVZ

Figure 3-4. Lineage traced cells are detected in the RMS of Long Chase mice only

Figure 3-5. Tomato⁺ neuroblasts are found in the RMS of long chase FGF18^{Lineage} adult mice

Figure 3-6. Distribution of Tomato⁺ cells in the OBs of Short chase mice

Figure 3-7. Characterisation of Tomato expression in the OBs of Long chase mice

Figure 3-8. Characterisation of Tomato⁺ cells in the Anterior OB Domain

Figure 3-9. FGF18⁺ radial glia-like cells are found in the ventral SVZ of young FGF18^{Lineage} mice.

Figure 3-10. Characterisation of Tomato expression in young FGF18^{Lineage} mice brains

Figure 3-11. FGF18⁺ cells are not actively proliferating cells in the Adult V-SVZ

Figure 3-12. FGF18⁺ cells do proliferate in early postnatal brains

4 – Discussion

Figure 4-1. Distribution of FGF18-expressing cells in the V-SVZ of adult and young mice

Figure 4-2. Putative roles of FGF18 in the adult SVZ.

APPENDIX

Figure S1. Tomato expression is not detected in untreated FGF18^{Lineage} mice

List of Tables

2 – Methods

Table 2-1. Primers used for genotyping the transgenic mouse allele

Table 2-2. PCR conditions for transgenic mouse genotyping

Table 2-3. Primary antibodies

Table 2-4. Secondary antibodies

Appendix

Table S1. List of the FGF18-CreERT2::Rosa26-tdTomato mice used

Table S2. Tomato⁺ cell number in the OBs of Short chase mice

Table S3. Tomato⁺ cell number in the OBs of Long chase mice

Table S4. Tomato⁺ radial glia-like cells in the ventral SVZ of young long chase and adult long chase mice.

ABBREVIATIONS

A Cells: Neuroblasts	GFAP: Glial Fibrillary Acidic Protein
ANR: Anterior Neural Ridge	GFP: Green Fluorescent Protein
aNSCs: Active Neural Stem cells	LV: Lateral Ventricle
AON: Anterior Olfactory Nucleus	³H-TdR: Tritiated Thymidine
BMP: Bone Morphogenetic Protein	NDS: Normal Donkey Serum
BrdU: Bromodeoxyuridine	NGS: Normal Goat Serum
CalR: Calretinin	NSCs: Neural Stem Cells
CB: Calbindin	OB: Olfactory Bulb
CNS: Central Nervous System	PCNA: Proliferating Cell Nuclear Antigen
CP: Cortical Plate	PGC: Periglomerular Cell
CSF: Cerebrospinal Fluid	qNSCs: Quiescent Neural Stem Cells
Cy2: Streptavidin Cy2	RGC: Radial Glia Cell
E Cells: Ependymal Cells	RMS: Rostral Migratory Stream
ECM: Extracellular Matrix	SGZ: Subgranular Zone
EGFR: Epidermal Growth Factor Receptor	SVZ: Subventricular Zone
FGF: Fibroblast Growth Factor	TAPCs: Transient Amplifying Progenitor Cells
FGFR: Fibroblast Growth Factor Receptor	TH: Tyrosine Hydroxylase
GC: Granule Cell	TX: Triton X100
	V-SVZ: Ventricular-Subventricular Zone

1 – INTRODUCTION

1.1 – Adult Neurogenesis

1.1.1 The Concept of Neuroplasticity

The regeneration of specific organs and tissues, under distinct physiological and pathological conditions, contributes to the cellular and structural preservation, as well as the homeostasis of an organism. This process is mediated by a unique cell population called adult stem cells which, through their ability to self-renew and differentiate, generate the primary cells of the specific organ they reside within. Although still little is known about the potential application of these cells in future biological therapies, extensive work has been conducted over the years to understand their regenerative competence in several somatic tissues such as the intestine and the skin (Barker et al 2010, Hong et al 2017, Yang et al 2019). In this context, the central nervous system (CNS) was long considered incapable of generating new neural cells after its full development. Santiago Ramon y Cajal, the father of modern neuroscience, referred to the mature mammalian nervous system and its nerve path as “something fixed and immutable” where ‘everything may die and nothing may be regenerated’ (Stahnisch & Nitsch 2002). This assumption relied on the limited resources of the time to investigate neuroregeneration, as well as, on the belief that new neurons could negatively interfere with the pre-existing circuits and be incompatible with the process of learning and memory (Colucci-D'Amato et al 2006). The concept of the immutability of the mammalian CNS dominated the field of neurobiology for most of the twentieth century until discoveries on the generation of new neurons – referred to as the process of neurogenesis – in specific areas of the adult brain challenged this doctrine and redefined the structural and functional plasticity of the brain. In the 1960s, with the use of tritiated thymidine (^3H -TdR), Joseph Altman showed for the first time that new neurons are generated in the cortex, the dentate gyrus of the hippocampus and the olfactory bulb (OB) of adult rats (ALTMAN 1962, Altman 1969, Altman & Das 1966). Subsequent studies confirmed these observations (Kaplan & Hinds 1977) but, it was not until Nottebohm investigations in songbirds that the process of adult neurogenesis started to be accepted (Paton & Nottebohm 1984). However, the real turning point was represented by the characterisation of the neurospheres derived from the isolation of multipotent neural stem cells from the adult brain and the identification of epigenetic factors that stimulate neurogenesis (Reynolds & Weiss 1992). The continuous discoveries done since the 1960s *in vitro* and *in vivo* have raised aspirations on the

generation of state-of-the-art approaches that target the introduction of functional new neurons into pre-existing circuits to repair the system's damages. While the presence of neurogenic activity in the postnatal human brain is still under debate, the identification of a process through which new neurons integrate into the mammalian nervous system naturally suggests that understanding the cellular and signalling mechanisms involved in adult neurogenesis is crucial to tackle potential therapies for neurodegenerative diseases, epilepsy and cognitive impairments caused by stroke, ageing and neuropsychiatric disorders (Gonzalez-Perez 2012, Sailor et al 2006). However, many questions still need to be answered to harness the knowledge of this process for therapeutic applications: how is neurogenesis regulated so that the right number of neurons are produced? What are the additional and unique properties of the regions that mediate this process and the corresponding newborn neurons? To what extent do external signals change the rate and the degree of this process? Understanding neurogenesis, as well as the signalling and cellular components that mediate its activity, is therefore fundamental to know the type of plasticity that exists in the adult brain after the developmental period is over.

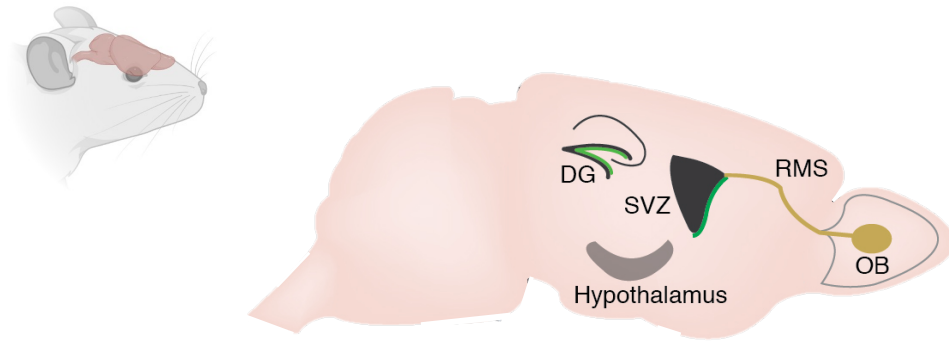
1.1.2 The Fundamental Principles of Adult Neurogenesis

It is now well established that the process of neurogenesis persists in specific anatomic regions of the adult mammalian brain called neurogenic niches. Two main features define a neurogenic niche: (1) the constant influence of its environment, that serves to accommodate the different phases of neurogenesis and changes in the process and (2) the unique architecture of the niche which allows it to generate the appropriate cell progeny (Ferraro et al 2010). Indeed, the interactions between the cellular components of the niche and the microenvironment, to which it is exposed, regulate the activation, maintenance and differentiation of the adult neural stem cells (NSCs) and their progenitors (Bjornsson et al 2015). Therefore, the mechanisms regulating adult neurogenesis mainly rely on two principles. First and foremost, it is a constitutive process that requires several stages to provide neuronal supply. The activation of the quiescent NSCs retained within the niche generates a progenitor cell population that differentiates into neuroblasts (undifferentiated neural precursors). Neuroblasts' migration to a destined area of the adult brain eventually results in the production of new neurons. The second trait of this process is its degree of neuroplasticity. Indeed, the activity of the different cell populations comprising these

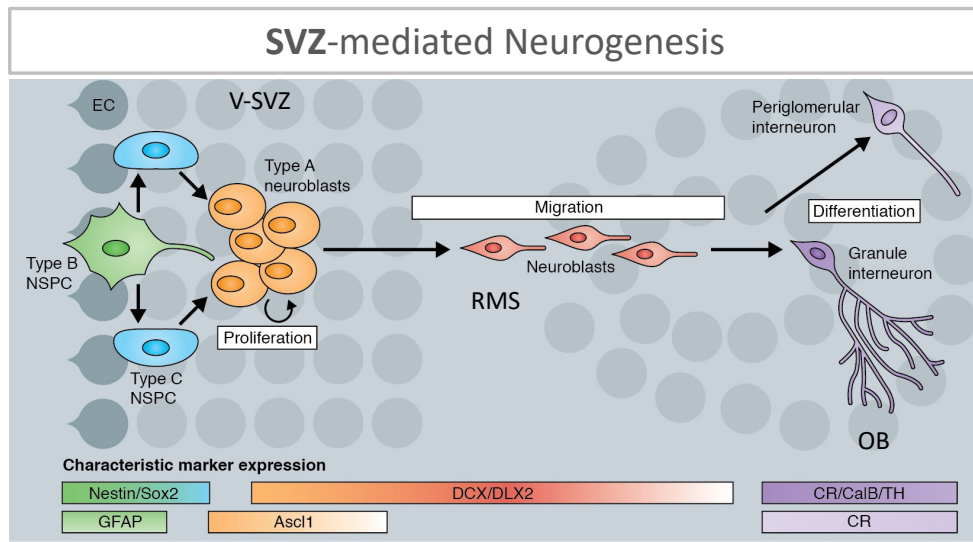
stages can be influenced in quantity and rate of production by external stimuli or fluctuations in the level of the intrinsic factors (e.g., hormones, neurotransmitters) (Lenington et al 2003). The neurovasculature, cerebrospinal fluid (CSF), neurotransmitters and the extracellular matrix (ECM) are among the modulators that have been shown to arbitrate these interactions (Bjornsson et al 2015, Lim & Alvarez-Buylla 2016, Obernier & Alvarez-Buylla 2019), which will be discussed in detail in Chapter 1.2.2. However, how the variation in the signals regulating the niche activity is caused remains unknown.

In the adult animal brain, *de novo* neurogenic activity is mainly restricted to the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the dentate gyrus in the hippocampus (Kempermann et al 2015, Obernier & Alvarez-Buylla 2019) (**Fig.1-1**) However, the cell fate potential differs between these niches (Colangelo et al 2019). The SGZ harbours adult NSCs, referred to as radial glia-like cells, that mediate the generation of excitatory neurons and astroglia while, the NSCs in the SVZ are responsible for the production of inhibitory neurons and a small percentage of glial cells (Kempermann et al 2015, Obernier & Alvarez-Buylla 2019). The difference in neuronal supply is coupled to a variation in the mechanisms required for the integration of new neural cells. *De facto*, the SVZ-mediated neurogenesis involves the migratory maturation of neuroblasts to the OBs and subsequent integration and survival in the laminar structure of OB layers (**Fig. 1-1A**). On the contrary, the new born neurons in the dentate gyrus do not migrate extensively and integrate into the packed granule cell layer from which they are produced (Kempermann et al 2015) (**Fig.1-1B**). Despite the presence of these canonical germinal zones, other regions of the adult brain have been associated with low levels of neurogenesis (non-canonical niches) such as the amygdala, striatum and hypothalamus of rodent (Bernier et al 2002, Cheng 2013, Goodman & Hajihosseini 2015, Luzzati et al 2006), in which the extent of neurogenic activity is still poorly understood.

For the purpose of this thesis, in the following chapters, I will focus on adult neurogenesis in the SVZ and dissect out different aspects of this niche, from the heterogeneity and origin of its cellular components to the factors influencing/mediating its activity and the neuronal fates of the generated neuroblasts.



(A)



(B)

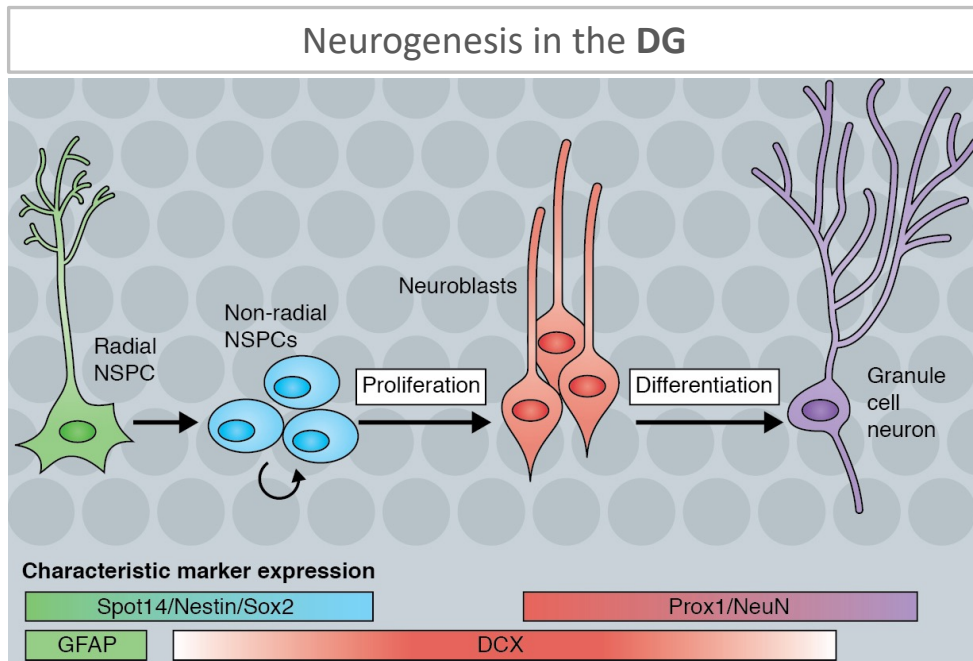


Figure 1-1. Neurogenesis in the Adult mouse Brain.

Neurogenic activity is observed in the SVZ, DG and Hypothalamus. (A) In the SVZ, NSCs (B cells) generate progenitor cells (C cells) that ultimately differentiate into neuroblasts (A cells). These migrate through the RMS and differentiate into inhibitory interneurons. (B) Neurogenesis in the DG is restricted to the subgranular zone and it does not require an extensive migration of neuroblasts. DG = dentate gyrus; OB = olfactory bulb; RMS = rostral migratory stream. Image adapted from (Braun et al., 2014).

1.2 – The Ventricular–Subventricular Zone: Canonical Neurogenic Niche

1.2.1 Organisation of the Niche

The SVZ represents the largest germinal zone in the adult mammalian brain that is formed during embryonic development, adjacent to the lateral ventricles (LV) (Alvarez-Buylla & Garcia-Verdugo 2002). The compact cellular arrangement of this niche adds complexity to the distinction between its ventricular and subventricular compartments. For the following reason, I will refer to this niche as the ventricular-subventricular zone (V-SVZ) in the remaining chapters of this thesis.

To conceptualize the adult mammalian V-SVZ as a reservoir of progenitors, it is fundamental to define the key cellular components and related mechanisms which unveil unique structural and functional properties to mediate the neuronal supply. The activation of the quiescent NSCs, referred to as B₁ cells, along the walls of the ventricles induces symmetrical division and subsequent generation of transient amplifying intermediate progenitors (TAPCs; also named C cells) (Bjornsson et al 2015, Gonzalez-Perez 2012, Obernier & Alvarez-Buylla 2019) (**Fig.1-1A**). It is fundamental to note that stem cells can adopt both an asymmetric and symmetric division mode. A single stem cell undergoing asymmetrical division generates another stem cell and a differentiated daughter cell. Here, while the asymmetric division model regulates the stem cell pool homeostasis at a single cell level, the symmetric division model supports the homeostatic control of the stem cell pool at the population level by either generating two stem cells that will replenish the pool (process of self-renewal) or by following the lineage path and generating two differentiated cells (Shahriyari & Komarova 2013) (**Fig. 1-2B**). Over time, the prevailing symmetrical division model of B₁ cells over the asymmetric one leads to a drastic decline in NSCs number. However, this NSCs depletion doesn't affect the number of newborn neurons because, although the process of neurogenesis is reduced with age, its decline occurs at a slower rate than the B₁ cell number decline (Obernier et al 2018). The choice of a symmetrical division, over an asymmetric one may preserve the integrity of the neurogenic process by making the NSCs less prone to genetic defects and allowing self-renewal with a minimum number of divisions. Indeed, following asymmetrical division, mathematical models have shown that stem cells may eventually start to accumulate mutations or

epigenetic changes that could impact their behaviour and drive ageing and carcinogenesis (Dingli et al 2007, Hormoz 2013). Through symmetrical cell division, the balance between proliferation and differentiation is maintained at the population level with the generation of two differentiated cells being coupled to another division in two stem cells. In particular, with an increase in the cycling rate of the stem cells, new slow-dividing cells are generated to compensate changes in the proliferation rate of the stem cell pool, suggesting a mechanism whereby symmetric division reduces the replicative ageing of a stem cell (Hormoz 2013). While alterations in genes regulating proliferation, differentiation and tissue organisation like *Lgl1* induce neoplasms (Klezovitch et al 2004), other mutations are not tumorigenic and may represent future therapeutic strategies. An example is *PTEN*, a phosphatase whose manipulation in adult NSCs may represent a future therapeutic approach to enhance stem cell self-renewal upon injury without leading to the pool exhaustion (Gregorian et al 2009). Therefore, symmetric division may delay not only the onset of cancer (Shahriyari & Komarova 2013) but also support the change in cell responsiveness. The secondary B_1 cells generated from the symmetrical division of one B_1 cell can either symmetrically self-renew one to two times or remain quiescent. However, the molecular factors that trigger their self-renewal remain unknown. The newly formed TAPCs divide three to four times to amplify their population before differentiating into neuroblasts (A cells) (Ponti et al 2013). These migrate through the rostral migratory stream (RMS) to the OB where they mature and differentiate into GABA-energetic interneurons: granule cells (GCs) and periglomerular cells (PGCs) (**Fig. 1-1A**).

Electron microscopy and immunohistochemical analyses have defined the structural and genetic profile of the cells that constitute the adult V-SVZ niche and the neurogenic lineage of the adult brain (Doetsch et al 1997). While the TAPCs and A cells display a spherical and elongated cell body, respectively, B_1 cells present an astrocytic phenotype, sharing fundamental structural features with radial glia cells (RGCs), responsible for embryonic neurogenesis (Götz & Barde 2005). Indeed, they interact with the CSF, through their small apical end (**Fig. 1-2A**), and with the blood vessels through the basal process (Mirzadeh et al 2008). However, differently to the embryonic niche, the organisation of the adult V-SVZ is mainly imposed by the large ependymal cells that surround the apical processes of B_1 cells, forming a “pinwheel” structure (Doetsch et al 1999b) (**Fig.1-2A**). The ependymal cells are post mitotic cells

with a multiciliate (E1) or biciliate (E2) phenotype that serve to mobilise the CSF and modulate the cellular proliferation within the niche through polarity signals and hydrodynamic cues (Gonzalez-Perez 2012, Guirao et al 2010, Sawamoto et al 2006). Interestingly, B₁ cells present a non-motile primary cilium on their apical end that extends into the lumen of the ventricle and it is suggested to mediate the transduction of signalling molecules found in the CSF (Tong et al 2014). B₁ cells are heterogeneous, as it will be discussed later, but another type of B cells with a more compact chromatin and reduced number of cytoplasm (Doetsch et al 1997) is found in the niche: the B₂ astrocytes. Indeed, during the symmetrical division, some B₁ cells can also generate B₂ astrocytes that lack a neurogenic potential (Obernier et al 2018) (**Fig. 1-2B**). Although they share similar features such as the astrocytic phenotype and the interaction with the neurovasculature, B₂ cells do not contact the lateral ventricle as they are found in the parenchyma, surrounding A cells (**Fig. 1-2A**). This potentially suggests that they function as an insulating barrier that separates the neurogenic niche from the surrounding mature tissue. It is known that during development of gyrencephalic species, some RGCs lack apical contact and still display a progenitor potential (Betizeau et al 2013). However, whether the B₂ cells compensate for the loss of B₁ cells during ageing is still unknown.

The genetic profile of the individual cell population had revealed the expression of several RGCs markers like GFAP, Nestin and Sox2 in type B cells (Doetsch et al 1997). This different combination of markers is mainly dependent on the activation state of these cells from quiescent to active. Quiescence is a form of reversible dormancy that the cells adopt to prevent any DNA damage even when they are exposed to environmental stress such as hypoxia or oxidative stress (Urbán & Cheung 2021). Quiescent NSCs downregulate the transcription of genes related to the cell cycle progression while upregulating genes involved in cell adhesion and mitochondrial function. Single cell analysis of the cells populating the adult V-SVZ niche has recently revealed elevated expression of GFAP but not EGFR in quiescent B cells, Ascl1 and EGFR expression in active B cells and Notum gene expression in activating quiescent cells (Cebrian-Silla et al 2021). Ascl1 represented the main marker expressed by TAPCs, which also present low GFAP expression, while A cells were characterised by high levels of Dcx gene, suggesting that they continue to divide following their generation. However, the transcriptional regulation of the V-SVZ-related genes is still ambiguous as markers expression is shared across the niche's cell

population. Therefore, further investigations on the characterisation of these cells can amplify the markers' selection used to identify them.

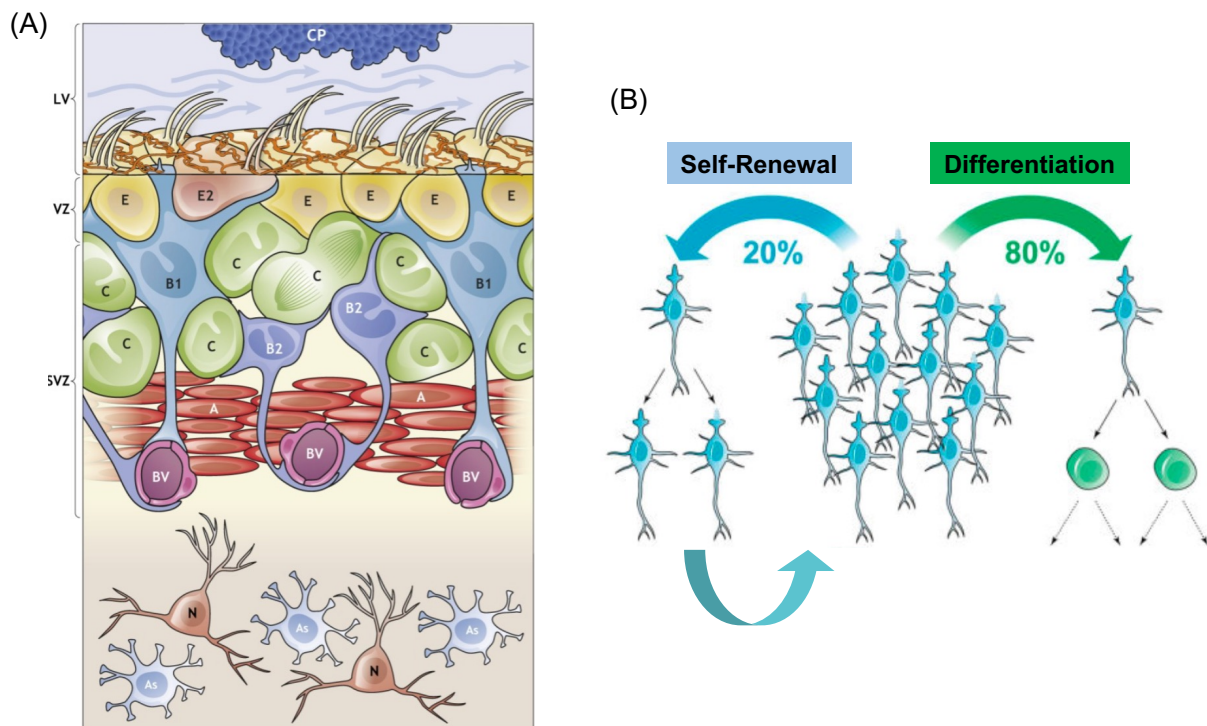


Figure 1-2. The Adult V-SVZ Niche

(A) Cellular organization of the adult V-SVZ niche. The B1 stem cells organize into a pinwheel structure surrounded by endymal cells (E/E2 cells)

(B) Only 20% of the NSCs in the adult V-SVZ divide into other stem cells. 80% of the NSCs undergo symmetric division and differentiate into progenitor cells.

Images adapted from (Obernier et al., 2018; Obernier and Alvarez-Buylla, 2019)

1.2.2 Identification of a Unique Migratory Path

Another distinctive feature related to the process of neurogenesis mediated by this niche is the neuroblasts' migratory pattern. Subsequent to the TAPCs deafferentation, the newly-generated neuroblasts migrate a long distance through the RMS at roughly 20-30 $\mu\text{m}/\text{h}$ in order to reach the OB (Alvarez-Buylla 1997). Along the way, they divide one to two times even at the OBs proximity where they start to express neuronal markers. Neuroblasts present an elongated leading process and a short trailing edge that allows the migratory movement in a "saltatory" manner with the extension of the leading process and a subsequent translocation of the cell body towards the growth tip (Kaneko et al 2017). During development, the neural precursors migrate through radial or tangential migration, using RGCs as scaffolds (Tan & Shi 2013). In the adult V-SVZ, A cells aggregate as chains and move along each other tangentially through

the RMS (Wichterle et al 1997). This chain migratory movement of A cells is ensheathed by GFAP-positive glial cells that are concentrated along the migratory pathway (Lois et al 1996). Once the precursors reach the OB, they dissociate from this chain structure and migrate radially in the OB layer to eventually differentiate into interneurons (Alvarez-Buylla 1997).

Many intrinsic and environmental factors contribute to the migratory competence of the neuroblasts and the maintenance of their movement. The expression of the cell surface molecule PSA-NCAM in the A cells guarantees the integrity of the chain migration (Ono et al 1994) and, at the same time, ECM components found in the RMS such as laminin, integrins and sulfate proteoglycan produce a permissive environment for neuroblasts movement (Thomas et al 1996). Additionally, the neurotransmitter GABA inhibits the proliferation and differentiation of the B cells and TAPCs, leading to a downregulation of the A cells production, while decreasing the speed of their migration (Bolteus & Bordey 2004, Liu et al 2005). On the contrary, the chemorepulsive molecules present in the CSF and sensed by the cells in the niche, are involved in the initiation of neuroblast migration. Indeed, the astrocytes in the V-SVZ express the Robo-2/-3 transmembrane receptors while the choroid plexus produces Slit2, a diffusible ligand for Robo-2, that secretes in the CSF (Borrell et al 2012, Kaneko et al 2010). The directional flow of the CSF is mediated by the cilia on the ependymal cells which generate a chemorepulsive gradient of the Slit2 ligand that ultimately guides the rostral migration of the neuroblasts (Sawamoto et al 2006). Moreover, neuroblasts express the Slit1 ligand which controls the distribution of the astrocytes in the niche to allow the formation of spaces for their migration. The assumption that the OB functions as a source of chemoattractants has also been debated. Although the presence of proteins like Reelin in its layer has shown to be involved in the disassembly of the A cells chains and initiation of the radial migration (Hack et al 2002), surgical removal of the OB has not proved to disrupt the neuroblasts' rostral migration (Jankovski et al 1998). Several other proteins have been investigated, like netrin-1 (Astic et al 2002) kineticin-2 (Ng et al 2005), for their role to attract A cells *in vitro* but their function *in vivo* remains still unclear. Future studies need to evaluate the molecular clues that drive neuroblasts migration from the niche through the RMS and to the OB, as well as what factors mediate the survival of these newly generated neurons in the OB.

1.2.3 The Origin and Heterogeneity of the NSCs in the adult V-SVZ

The identification of neurogenic activity in specific niches of the brain, beyond embryonic development, has redefined the understanding of the molecular events contributing to brain development, more precisely to embryonic neurogenesis. The formation of the neural tube, through the invagination of the neural plate along the dorsal surface of the embryo, represents the starting point of CNS development (Tan & Shi 2013). However, prior to the neural tube closure, the CNS is patterned along the rostral-caudal axis into the forebrain, midbrain and hindbrain. These will generate structures like the telencephalon, diencephalon and mesencephalon that will contribute to distinct features of the mature brain (**Fig. 1-3A**). It is not until the stage of the neural tube's closure that the NSCs are restricted to the ventricular zone in the telencephalon and initiate the process of neurogenesis. Originating from a population of neuroepithelial cells, the RGCs are the stem cell population that generates different neuronal subtypes and acts as a scaffold for their migration (Götz & Barde 2005). Indeed, the newly produced neurons migrate radially into the cortical plate (CP) in an "inside-out" manner, with the 'oldest' neurons placed close to the ventricle. The identification of shared morphological and epigenetic features between the B₁ cells and RGCs in the adult and embryonic V-SVZ, respectively, has introduced different explanatory models on the origin of the B₁ cells. The first accepted theory was a "sequential" model (Kriegstein & Alvarez-Buylla 2009) which attested that the RGCs produce all the neuronal subtypes during embryogenesis and, in postnatal development, the glial cells. At the end of gliogenesis, residual RGCs are transformed into adult neural progenitors to generate specific neurons in adulthood. However, this theory has been substituted with the currently accepted model: the "set-aside" model (Fuentealba et al 2015). Through BrdU labelling and GFP-retroviral injections in utero, it was discovered that, already at E11.5 of mouse development, the neurogenic potential/multipotency of the embryonic progenitors is regionally specified (**Fig. 1-3B**). However, between E13.5 and E15.5, the RGCs in the developing V-SVZ generate cortical, striatal or septal neurons and, at E15, this lineage path diverges. As a result, one path continues to generate neurons necessary for completing the CNS development while the other (pre-B1 cells) remains quiescent until adulthood to generate olfactory interneurons.

NSCs reside along the walls of the lateral ventricle at the dorsal wall, lateral wall (adjacent to the striatum) and medial wall (adjacent to the septum). However, depending on their location, they generate specific subtypes of interneurons. Genetic-fate maps and viral infections revealed regionalisation of the B₁ cells in the adult V-SVZ (Fiorelli et al 2015, Fuentealba et al 2015) (**Fig. 1-3C**). Indeed, the dorsal region is specified to generate either tyrosine hydroxylase (TH)-expressing PGCs and superficial GCs in a 1:2 ratio, while the lateral wall generates calbindin-positive (CB⁺) PGCs and Deep GCs. On the other hand, the B₁ cells in the medial wall produce calretinin-positive (CalR⁺) PGCs and GCs. Single cell RNA sequencing of the cells populating the niche confirmed region-dependent heterogeneity of A cells and cycle-dependent heterogeneity of TAPCs with a unique cellular behaviour between dorsally and more ventrally located B cells (Cebrian-Silla et al 2021). Indeed, the V-SVZ itself is patterned along the dorso-ventral axis as the medial wall is more prone to generate PGCs than the lateral wall that generates more GCs (Fiorelli et al 2015). At the same time, it is also patterned along the rostro-caudal axis as the anteriorly located NSCs generate GCs that are more superficial than the ones located more posteriorly.

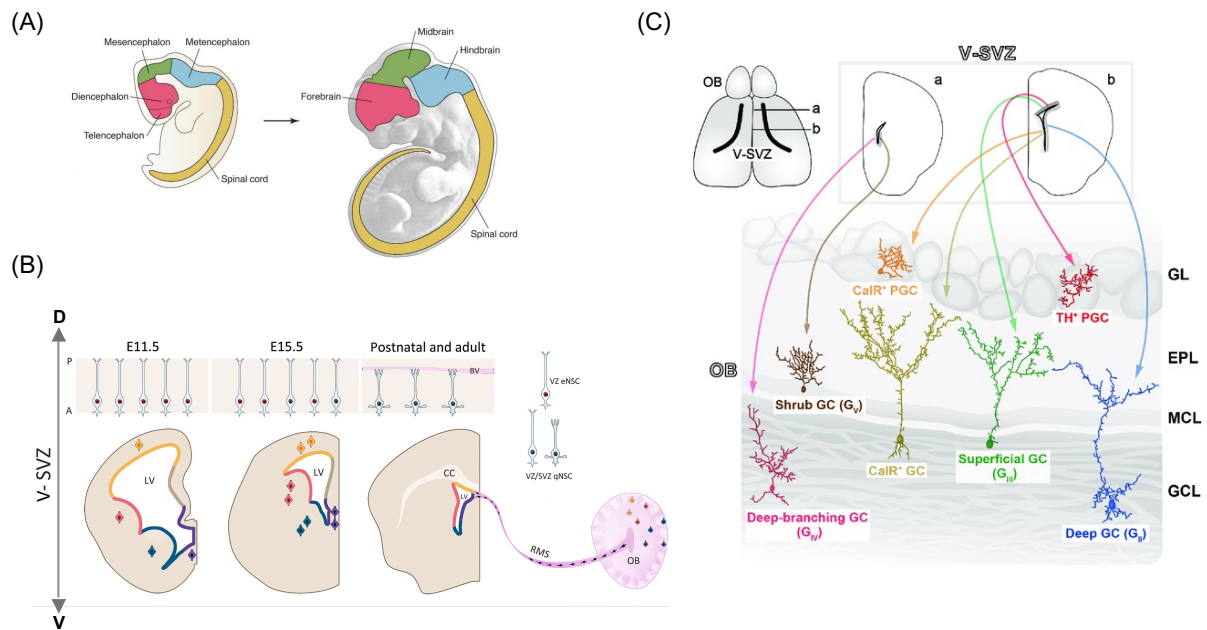


Figure 1-3. The NSCs in the Adult V-SVZ are Specified during Embryonic Development. (A) The embryonic CNS is patterned along the rostral-caudal axis into forebrain (red), midbrain (green) and hindbrain (blue) that will be subdivided into telencephalon, diencephalon, mesencephalon and metencephalon structures. Image from (Sanes et al., 2019). (B) At E11.5 the NSCs populating the embryonic SVZ are regionalized. Before E15.5 the progenitor pool diverges with one pool guaranteeing the correct CNS development and one pool remaining quiescent until adulthood when it generates olfactory interneurons. Dorsal SVZ (yellow); Medial SVZ (purple); Ventral SVZ (green); Lateral SVZ (red). Image from (Fuentetaja et al., 2015). (C) Olfactory interneuron subtypes generated from the adult V-SVZ according to the NSCs location along the walls of the ventricle. Image from (Takashi et al., 2018)

1.2.4 The Niche Microenvironment influences the V-SVZ Activity

The activity of B₁ cells is regulated by a variety of factors which are expressed by the niche components and its surrounding environment or distant neural sources. An example are the modulatory signals received by the close proximity to the blood vessels and the CSF. Circulating hormones in the vasculature interacting with the niche are actively involved in the regulation of the process. Prolactin, produced during early pregnancy and lactation, increases the rate of neurogenesis to prepare the OB for a finer olfactory discrimination in recognising and rearing the offspring (Shingo et al 2003). Conversely, estrogen inhibits the proliferation of the cells in the niche and affects the survival of the newly generated neurons in the OB (Ponti et al 2018). While the blood vessels represent a source for circulating hormones, the CSF represents the

source of several signalling molecules. Ependymal cells release noggin, an antagonist of the bone morphogenetic pathway (BMP) (Lim et al 2000). GFAP-positive cells express BMP4 and the receptors associated with it. BMP4 normally inhibits cell proliferation and favours glial cell generation over neurons. A subset of B1 cells has been shown to generate new oligodendrocytes especially after a brain injury, proving that these cells can respond to the changes in the surrounding environment (Nait-Oumesmar et al 1999). BMP4 has been shown to interact with Fibroblast Growth Factor – 2 (FGF2) (Woodbury & Ikezu 2014) which is present in the CSF and is recruited by ECM structures called fractones (Mercier 2016) to support the proliferation in the V-SVZ. Release from NSC quiescence has recently revealed the presence of multiple oligodendrogenic domains which are dormant at homeostasis but activate, divide and initiate gliogenesis in response to physiological demands, such as injury or PDGFR β increase (Delgado et al 2021). As a result, the coexistence of neurogenesis and gliogenesis at the V-SVZ niche contributes to brain plasticity. On the other hand, among the mediators of neurogenesis are the neurotransmitters. Evidence from rodent models have shown that the release of choline acetyltransferase from the neurons surrounding the niche modulates the progenitors' proliferation and increases the production of neurons (Paez-Gonzalez et al 2014). Still debatable is the role of dopamine in the regulation of the V-SVZ as the TAPCs express its receptor (Kim et al 2010). The regulatory factors discussed are only a small component of the past and ongoing investigations reporting the importance of maintaining a permissive neurogenic environment. Dysfunctions in this process have been associated with different conditions including Alzheimer disease (Haughey et al 2002). The identification of these regulators can support our understanding of how this process works while providing potential therapeutic strategies.

1.3— The Fibroblast Growth Factor (FGF) signalling Pathway

1.3.1 The FGF pathway: An Overview

The regulation and maintenance of different biological processes, both in development and physiological homeostasis, requires the specific spatiotemporal expression and cooperation of several growth factors. The Fibroblast Growth Factor (FGF) family is widely implicated in a variety of cell behaviours during embryonic and postnatal development, as well as physiological processes like tissue repair and regeneration (Maddaluno et al 2017, Ornitz & Itoh 2022, Turner & Grose 2010, Yun et al 2010). The mammalian FGF family comprises 22 members which, according to the phylogenetic

analysis (Ornitz & Itoh 2015), have been arranged into seven subfamilies (**Fig. 1–4A**). Except for the intracellular FGFs (FGF11-14), most FGFs operate as autocrine or paracrine factors (canonical FGFs) and mediate their response through the binding to and activation of one of the four cell-surface tyrosine kinase FGF receptors (FGFR1-4) (Goldfarb 2005). Different factors have been shown to influence FGFR activation and function. An example is the participation of heparan sulfate in facilitating the efficient signal transduction and stabilising the FGF/FGFR complex (Ornitz 2000). Further modulation in the ligand-binding affinity is determined by the alternative splicing of the mRNA encoding the third immunoglobulin-like domain of FGFR-1, -2 and -3 hence generating the receptor IIIb and IIIc variants (Zhang et al 2006). FGF binding in a receptor- and cell-dependent manner activates distinct signalling cascades such as Ras-Erk1/2 signalling, PI3 kinase/Akt pathway and PLC γ pathway (Ornitz & Itoh 2015). The ability to induce a variety of downstream intracellular pathways and to exert different biological functions is the reason why FGFs are referred to as pleiotropic factors. Among the canonical FGFs, the pleiotropic protein FGF18 has diverse regulatory functions, primarily in osteogenesis and chondrogenesis and in the induction and patterning of several tissues such as limb buds, lungs and the CNS (Hajihosseini & Heath 2002, Hung et al 2016, Liu et al 2003, Ohuchi et al 2000, Usui et al 2004). FGF18 is part of the paracrine-acting FGF8 subfamily that contains FGF8, FGF17 and FGF18. This is confirmed by a 52.7% similarity in the amino acid sequence to FGF8 and FGF17 (Katoh 2005, Ohbayashi et al 1998). A genetic profile of FGF18 has further unveiled a typical signalling sequence constituted by a hydrophobic amino terminus that characterises it as a secreted protein. In particular, the presence of two N-linked glycosylation sites defines FGF18 as a secreted glycoprotein. Like all the members of its subfamily, FGF18 lacks affinity for FGFR b-isoforms but it has a strong affinity for FGFR-3c, -2c and, -1c in a cell type-dependent manner (Hoshikawa et al 2002, Olsen et al 2006). This selective receptor affinity derives from its structural conformation. In a proposed model, the identification of a hydrophobic β 4- β 5 loop in FGF18 structure has shown to confer stability and specificity in the interaction with the hydrophobic groove in the D3 domain of the FGFR c-isoforms (Olsen et al 2006) (**Fig.1–4B**) This would be repelled in b-isoforms due to the presence of hydrophilic residues in the domain. In the following section, I will focus on the role of FGF18 in CNS development and eventually explore the potential of a novel FGF18-lineage mouse line to investigate its implication in adult neurogenesis.

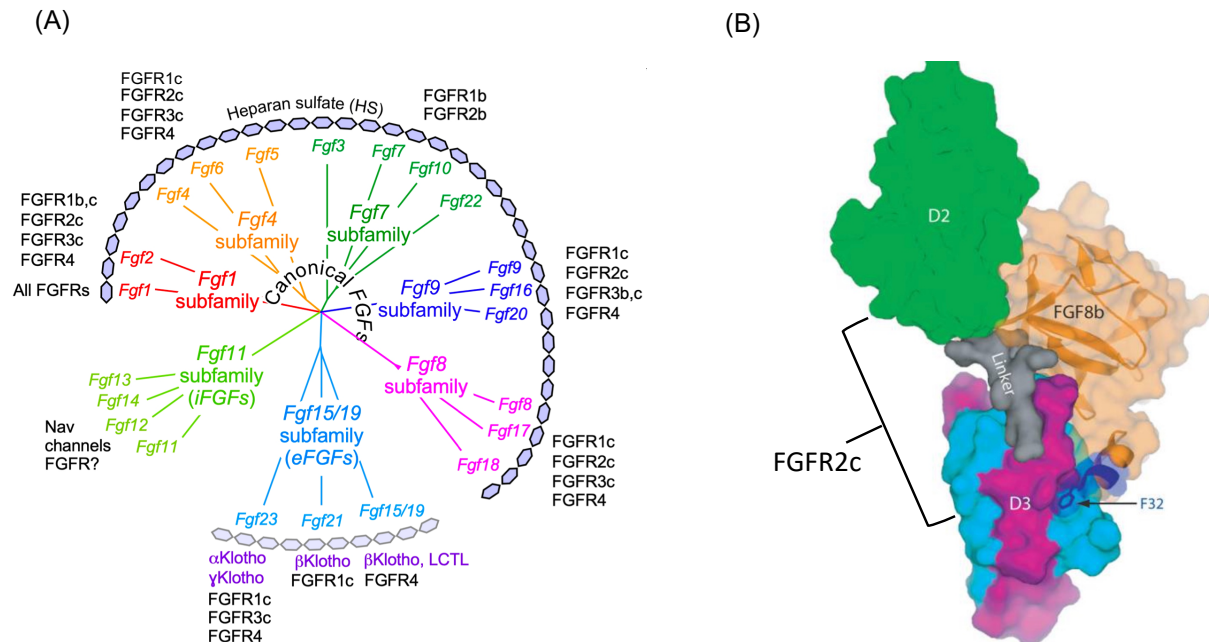


Figure 1-4. The Fibroblast Growth Factor Family.(A) The FGF family members and corresponding receptors. Image from (Ornitz & Itoh 2022) (B) The β 4- β 5 loop in the FGF8b structure has shown to interact through its phenylalanine 32 (F32) with the hydrophobic groove in the D3 domain of the FGFR2c-isoforms. This binding specificity is also observed with FGF17b and FGF18. Image from (Olsen et al 2006).

1.3.2 The Role of FGF18 in Brain Development

The expression and regulatory function of FGF18 has been largely investigated in relation to osteogenesis and chondrogenesis, primarily because the initial studies on FGF18^{-/-} mice attributed the embryonic lethality to the disruption of these two processes (Liu et al 2002, Ohbayashi et al 2002). Over time, further analyses on mouse models have unveiled additional roles of FGF18 in the formation of other tissues such as lung alveolar and neuromuscular junction development (Ito et al 2018, Usui et al 2004). Therefore, it is still unclear what might cause the lethality.

The neural tube marks the beginning of the CNS development. At this stage, the contribution of the FGF8 subfamily, particularly of FGF8, has been to some extent explored but still little is known about the function of FGF18 in this process. Prior to its closure, FGF8 family ligands are expressed in a neuroepithelial layer of the caudal region of the neural tube called isthmus (Crossley & Martin 1995). The isthmus defines the midbrain-hindbrain boundary and acts as an organiser for the differentiation of neuronal subtypes. Studies on chick embryos, using FGF18-soaked beads and whole mount *in situ* hybridisation, revealed a repressive activity of FGF18 within the node

region where it influences *Pitx2* expression by acting as a right determinant in left-right asymmetry (Ohuchi et al 2000). In a similar pattern to the one observed with FGF8, *in situ* hybridisation has revealed a lower expression of FGF18 shortly after the onset of FGF8 transcription in the isthmus region of both mice and chick (Maruoka et al 1998, Ohuchi et al 2000). Moreover, induction of FGF18 using FGF8-soaked beads implanted in the caudal diencephalon of stage 10 chick embryos showed that FGF8 induces FGF18 expression during the development of the second mesencephalon and that FGF18 is involved in midbrain development (Ohuchi et al 2000). Together these results suggest a functional relationship between FGF8 and FGF18 in patterning the midbrain-hindbrain junction. Indeed, FGF18 is induced by FGF8 in order to amplify the FGF domain and gradient during midbrain and cerebellum development (Liu et al 2003). At E8.5, FGF8 and FGF18 are also observed in the anterior neural ridge (ANR), a region located between the neural ectoderm and non-neural ectoderm of the neural plate that serves as a second organiser for the anterior-posterior patterning, growth and maintenance of the forebrain (Ohuchi et al 2000). *In situ* hybridisation in chicks revealed FGF18 activity in the anterior dorsolateral hinge points, proposing a potential contribution of this factor in the closure of the anterior neural plate (Karabagli et al 2002). However, this expression is yet to be investigated in mammals.

Later in development, as the telencephalon patterning continues, the neocortex is specified into distinct layers as a result of V-SVZ embryonic neurogenesis. FGFs have been reported to play fundamental roles in brain development and particularly corticogenesis. One example is FGF10, a mesenchymal-epithelial factor part of the FGF7 subfamily that has shown a peculiar expression pattern both during embryogenesis and in adulthood (Hajihosseini et al 2008, Watson & Francavilla 2018). Particularly during embryonic neurogenesis, FGF10 has shown to be transiently expressed in the neuroepithelial cells where it controls their differentiation into RGCs and the onset of neurogenesis by signalling through FGFR2-b (Sahara & O'Leary 2009). Indeed, FGF10 null mice exhibit a delay in brain development that affects the size of the cortex, which exhibits a greater rostral surface, as well as its lamination, which appears thicker than wild type mice, because of a delay in RGCs differentiation and a subsequent overproduction of progenitors. Interestingly, while FGF10 signals through FGFR2-b during corticogenesis, this receptor is absent in the postnatal hypothalamus suggesting that FGF10 may also act in a non-canonical fashion

(Hajihosseini et al 2008). At the stage of embryonic neurogenesis, FGF18 is expressed in the cortical plate (CP) where it regulates proper neuron's positioning and migratory behaviour (Hasegawa et al 2004). FGF18-mediated signal through FGFR3 in cortical progenitors regulates the transcription of Pea3-Ets factors in order to coordinate the migration of early-generated cortical neurons and cell body positioning in mid-to-late corticogenesis. Indeed, in the model proposed, electroporation of FGF18 in VZ cells at E11.5 resulted in a significant induction of Pea3-Ets transcription factors at E13.5, when they are normally mildly expressed. However, FGF18 knockout mice didn't show any lamination defect at birth when they died. Therefore, a possible genetic compensation mediated by FGF factors expressed in the VZ (e.g., FGF8, FGF15, FGF17) could act on progenitors expressing FGFRs and rescue the radial migration defect in FGF18^{-/-} mice. Taking these considerations together, FGF18 activity appears to be closely related to the FGF8 function during early stages of embryonic development. Indeed, although it has a weaker signalling capability, FGF18 seems to initially support FGF8 function in patterning the primordial CNS structures and giving a positional identity to the NSCs. However, as development proceeds, FGF18 expression becomes more prominent during laminar patterning than the one of its subfamily members, indicating its primarily role in radial migration of cortical neurons.

1.3.3 The Role of FGF18 in the Adult CNS

The prenatal or early postnatal lethality that derives from the knockout of the FGF18 gene has not facilitated the analysis of this factor in the adult brain. Although the function of FGF18 has been discussed in relation to the CNS development, the signalling activity mediated by this protein postnatally and in adulthood is yet to be fully investigated and understood. Early studies have characterised FGF18 as a neurotrophic factor first with the PC12 cell line (Ohbayashi et al 1998), where it mediates neurite outgrowth in neuronal-derived tissues, and later, in the postnatal rat brain (Hoshikawa et al 2002). Neurotrophic factors represent a specific class of growth factors that promotes the initial growth and development of neurons, as well as the neuronal survival, synaptic plasticity and long-term memories in the mature brain through the activation of tyrosine kinases. In P10 rat brain, the predominant expression of FGF18 observed in the cortex was largely related to neurons with a preferential activity on glial cells (Hoshikawa et al 2002). Indeed, the strong affinity for

FGFR-3c isoform, which is expressed by astrocytes and microglia, indicates a neuron-derived nature of this mitogenic glial cell growth factor. The FGF18 activity has been also associated to the learning and memory processes (Cavallaro et al 2002). Hippocampal expression of FGF18 showed to be upregulated during the maze running training with no effects on the physical activity itself but an improvement in the spatial learning behaviour and spatial memory of normal rats. This neuroprotective function of FGF18 finds further confirmation in the attenuation of the symptoms of stroke in rat models with induced cerebral artery occlusion (Ellsworth et al 2004). Here, the administration of FGF18 in the first 2-3 hours or 24 hours post-ischemia reduced the infarct volume and behavioural deficits such as unbalanced walk, apraxia and memory disruption. The recovery of the damaged tissue and behavioural alterations is mediated locally through vasodilation of the cerebral vasculature with a close resemblance to FGF2-mediated neuroprotection. FGF2 and FGF18 present a different binding affinity as FGF2 favours signalling through FGFR1 while FGF18 through FGFR3 in a cell type-dependent manner and according to cofactors' activity and the structural conformation of the protein whereby FGF18 binds IIIc receptor variants only while FGF2 IIIb variants too but in a negligible manner (Belov & Mohammadi 2013, Ornitz et al 1996). The neuroprotective activity shared by these two FGF ligands could indeed function as a therapeutic strategy against neurodegenerative disorders like Alzheimer, Huntington, Amyotrophic Lateral Sclerosis or epilepsy and ischemic stroke which exhibit a reduced GLT1 astrocytic expression (Savchenko et al 2019). The studies performed to date open new therapeutic horizons where FGF18 can be implicated, but they also actively seek for a clearer understanding of its fully function and expression in the mature brain. Initial studies showed FGF18 mRNA expression to be mostly confined to the cerebral cortex and hippocampus region with reduced levels in the OB, hypothalamus and thalamus of P1 and P10 rat brain (Hoshikawa et al 2002). The generation of a FGF18^{Cre} reporter mouse line has verified this expression pattern but also identified new regions where FGF18 could exhibit a neurogenic function such as in the V-SVZ and hypothalamus (Hagan et al 2019). Indeed, the presence of lineage traced cells in the OBs of adult mice doesn't exclude the involvement of FGF18 in the process of adult neurogenesis mediated by the V-SVZ. Here, the contribution of the FGF ligand, FGF2, in this niche has already suggested to influence the quiescence of NSCs comprising it and their progenitors' proliferation (Leeman et al 2018, Woodbury & Ikezu 2014). Although the general

function of FGFs is still unclear, the expression of FGFRs along the walls of the ventricles could support this hypothesis. Indeed, FGFR3 is expressed by the NSCs lining the ventricular zone where it regulates their quiescence and activation (Young et al 2010). The contribution of FGFR3 in this process, in combination with the identification of FGF18-positive cells in the adult V-SVZ and OB, could potentially imply a role of FGF18 in the process of adult neurogenesis.

1.4 – Aims and Objectives

Taking this information together, this study investigates the expression and potential function of FGF18 in the postnatal mouse brain. With the use of the double transgenic mouse line FGF18-CreERT2/+::Rosa26-TdTomato (FGF18^{Lineage}), previously described (Hagan et al 2019), and a tamoxifen Pulse-Chase system, it can be hypothesized that FGF18-expressing (FGF18⁺) cells in the adult V-SVZ regulate the neurogenic activity of the niche and contribute towards the compartmentalization of this region. Additionally, this study aims to investigate the expression domain of the FGF18⁺ cell population and their potential activity within the V-SVZ niche from birth to adulthood in order to identify an age-dependent FGF18 mechanism influencing the function of the niche.

2 – MATERIAL & METHODS

2.1 – Animal Models

In the following study, tamoxifen inducible Fgf18-CreERT2/+::Rosa26-tdTomato transgenic mice – hereby referred as Fgf18^{Lineage} – were used (Hagan et al 2019) (**Table S1**). As previously mentioned, the splice acceptor (SA) eGFP:CreERT2 vector was introduced in the intron between exon 1B and 1C at the Fgf18 genomic locus along with a PGK-neo selection cassette. FGF18^{CreERT2/+} heterozygous mice were subsequently crossed with Cre-dependent Rosa^{tdTomato/+} heterozygous mice to generate Fgf18^{Lineage} mice.

Mice of both sexes were used, with *n* numbers provided in the main text. All mice were maintained on a mixed C57BL6/129Ola genetic background and raised on Chow diet under a 12-h light/dark cycle in accordance with UK and UEA local regulations governing work with transgenic animals.

2.1.1 – FGF18^{CreERT2/+}

Homozygous mice (Fgf18^{CreERT2/CreERT2}) exhibited perinatal lethality caused by a disruption in the Fgf18 function (Hagan et al 2019). Indeed, they displayed all the skeletal abnormalities previously described in Fgf18^{-/-} mice. The Fgf18^{CreERT2} allele exhibits the deletion of the 1C exon (**Fig. 2–1A**), however, heterozygous mice were fertile and viable demonstrating that the targeted allele behaves as a functional null allele.

2.1.2 – Rosa26^{tdTomato}

A LoxP-flanked STOP cassette is inserted at the *Gt (ROSA)26Sor* locus to prevent the transcription of the CAG-promoter driven tdTomato fluorescent protein (**Fig. 2–1B**). Crossing Rosa26^{tdTomato} mice with FGF18^{CreERT2} mice enables the constitutive expression of the tdTomato protein and lineage tracing of FGF18 expressing cells in the resulting offspring after tamoxifen administration. As a result, the FGF18^{Lineage} transgenic mouse line enables to examine FGF18 expression and lineage trace Tomato expression at multiple time points.

2.2 – Genotyping

PCR genotyping with forward and reverse primers was used to identify the FGF18-CreERT2 and Rosa26-tdTomato alleles (**Table 2-1**). The CreERT2 allele was identified using primers against the GFP sequence. In **Table 2-2**, tailored PCR conditions are shown. The resulting PCR fragments, measured in base pairs (bp), were resolved on 1% agarose gel and visualized with ethidium bromide.

2.3 – Tamoxifen Treatment

Tamoxifen was administered via oral gavage at 80-100 mg/kg bodyweight, from a 20 mg/ml stock solution, diluted in 10% ethanol/corn oil.

For adult mice, 90-day-old (P90) Fgf18^{Lineage} mice were treated for 2 consecutive days with tamoxifen and brains were harvested at P94 (short chase, $n=3$) and P104 (long chase, $n=5$), respectively (**Fig. 2–2A**).

For early postnatal mice, P4 Fgf18^{Lineage} litter mice were treated for 2 consecutive days with tamoxifen via oral gavage and the brains were harvested at P7 (short chase, $n=2$) and P16 (long chase, $n=3$), respectively (**Fig. 2–2B**).

Table 2-1. Primers used for genotyping the transgenic mouse line.

Forward (Fwd) and Reverse (Rev) primers used to identify the FGF18^{CreERT2/+} allele and Tomato expression in FGF18-CreERT2::Rosa26-tdTomato double transgenic mice. PCR Product size is in base pairs.

	Primer Sequences (5' – 3')	PCR Product size (bp)
Fgf18^{CreERT2/+}	Fwd: AAG TTC ATC TGC ACC ACC G	173
	Rev: TCC TTG AAG AAG ATG GTG CG	
Rosa26	Fwd: CCGAAAATCTGTGGGAAGTC	297
	Rev: AAGGGAGCTGCAGTGGAGTA	
Rosa26^{tdTomato/+}	Fwd: CTGTTCTGTACGGCATGG	196
	Rev: GGCATTAAAGCAGCGTATCC	

Table 2-2. PCR conditions for transgenic mouse genotyping.

PCR cycles utilised in genotyping PCR reactions to detect the FGF18^{CreERT2} allele and Tomato expression at the Rosa26 allele.

Alleles	Temperature (°C)	Time (min:sec)	Number of cycles
FGF18 ^{CreERT2/+} and Rosa26 ^{tdTomato/+}	94	2:00	x15
	94	0:30	
	60 (58*)	0:30	
	68	1:10	
	94	0:30	x17
	60 (58*)	0:30	
	68	1:10	
	(+ 0:20/cycle)		
	68	7:00	x1
	6	15:00	x1

* Temperature condition used in the PCR cycle to detect Tomato expression

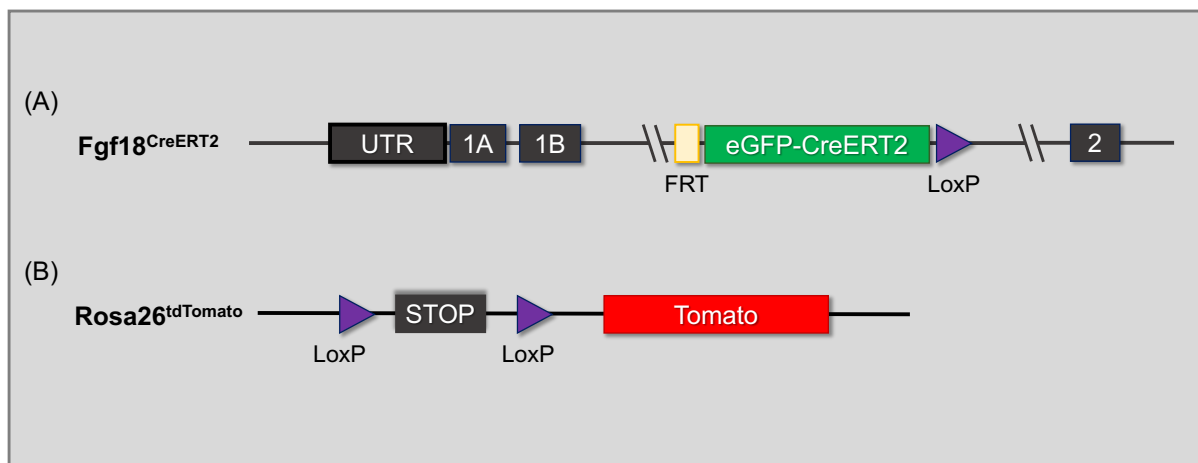


Fig 2-1. Transgenic Mouse Alleles. Schematic representations of the transgenic alleles used in *Fgf18^{CreERT2/+::Rosa26^{tdTomato/+}}* (*Fgf18^{Lineage}*) double transgenic mice models for lineage tracing experiments.

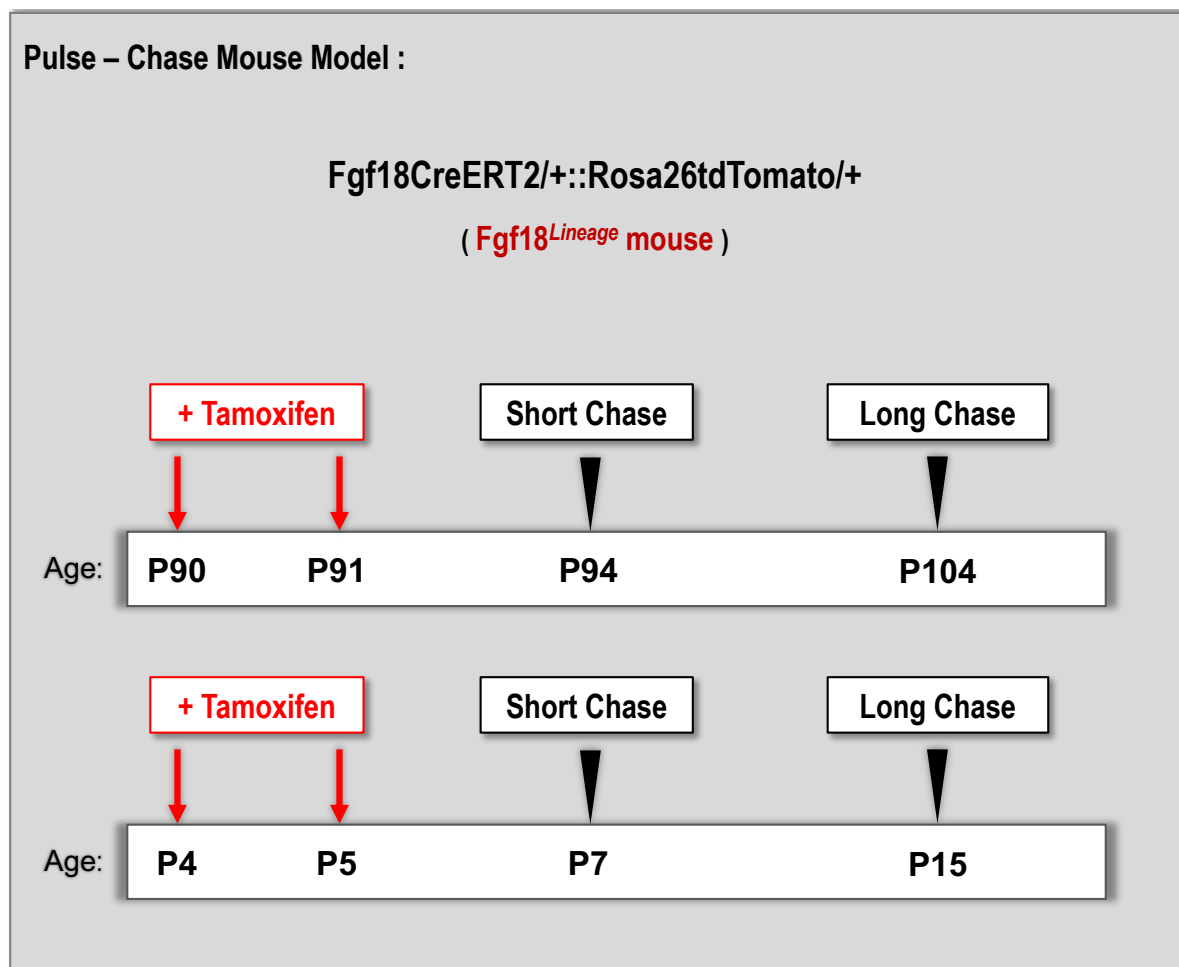


Figure 2-2. Schematic representation of *Fgf18^{Lineage}* mice tamoxifen treatment.

(A) 90-day-old (P90) mice were pulsed for 2 consecutive days (short pulse) with tamoxifen through oral gavage. For lineage tracing analysis, brains were harvested at P94 (short chase) and P104 (long chase). (B) P4 mice were pulsed with tamoxifen for 2 consecutive days and sacrificed at P7 and P15

2.4 – Tissue Isolation and Preparation

Mice were killed by CO₂ asphyxiation and perfused with 4% paraformaldehyde (PFA) prior to brain harvesting. Brains were subsequently subject to different storage conditions based on their predestined sectioning method.

Following PFA perfusion, to generate vibratome sections, brains were fixed overnight in 4% PFA, pH 7.0, at 4°C. The next day, fixed brains were de-hydrated in ascending concentrations (30%, 50%, 70%, 90%; 1 hour per dilution) of ethanol, before being stored at 4°C in absolute ethanol. Just before use, brains were re-hydrated back to PBS and embedded in 3% w/v agar overnight at 4°C. Coronal and sagittal sections were generated using a vibratome microtome (Leica Microsystems, Wetzlar, Germany) at 60 µm thickness and stored in PBS until use in 48 well plates.

Subsequent to dissection, to generate cryostat sections, brains were frozen fresh on dry ice in foiled cryomolds with the ventral side facing upwards and, stored overnight at – 80°C. The following day, brains were embedded in double-foiled cryomolds filled with optimal cutting temperature (OCT) compound, allowed to set on dry ice and stored at - 80°C until use. Coronal sections in a range between 16 - 25 µm thickness were generated using a freezing microtome (MicroM HM560, Thermo Fisher Scientific, Waltham, MA, USA) and thaw-mounted on Superfrost slides (Thermo Fisher Scientific) then stored at - 80°C until use.

2.5 – Immunolabeling Protocol

2.5.1 – Immunolabelling on Vibratome Sections

To permeabilize and block non-specific antigen binding sites, vibratome-generated sections were incubated in a blocking solution of 20% normal goat serum (NGS) and 1% Triton X100 (TX) in PBS for 2 h at room temperature (RT) on the rocker. Subsequently, sections were incubated overnight at 4°C with primary antibodies (**Table 2–3**) diluted in 0.2% NGS and 0.1% TX in PBS. Following the overnight incubation, sections were washed five times (1 h per wash) in 0.2% NGS and 0.1% TX diluted in PBS and incubated overnight at 4°C with the relevant secondary antibodies in 0.2% NGS and 0.5% NP-40 in PBS on the rocker (**Table 2–4**). The next day, sections were washed six times (30 min per wash) with PBS, counterstained with Hoechst 33342 (1:2000, Invitrogen) and mounted with Vectashield (Vector Laboratories). Where biotinylated secondary antibodies

were used, after overnight incubation sections were washed five times (1 h per wash) in 0.2% NGS and 0.1% TX in PBS. Sections were then incubated in a solution containing Streptavidin conjugated Cy2 (1:700, Jackson ImmunoResearch), in 0.2% NGS and 0.5% NP-40 diluted in PBS at 4°C on the rocker overnight. The following day, sections were washed six times with PBS (30 min per wash), counterstained with Hoechst and mounted with Vectashield (Vector Laboratories).

2.5.1.1 – Antigen retrieval

Prior to PCNA immunolabelling, vibratome-generated sections were incubated in a solution containing 5% glacial acetic acid and 95% absolute ethanol (*Acid Alcohol treatment*) for 25 min on a rocker, previously cooled at – 20°C.

Prior to S100 β immunolabelling, sections were exposed to an antigen unmasking treatment with a 10 mM Citrate buffer containing 0.05% Tween-2, pH 6.0, for 10 min at RT on the rocker and then for 30 min circa in the water bath at 70°C with pre-warmed Citrate Buffer at 70°C.

Following either one of these treatments, sections were washed twice (10 min per wash) in PBS before blocking.

2.5.2 – Immunolabelling on Cryostat Sections

Cryostat sections were permeabilized with prewarmed 10 mM Citrate Buffer containing 0.05% Tween-20 (pH 6.0) at 84°C for circa 35 min and washed three times (10 min per wash) with 0.1% TX in PBS (PBT). Subsequently, sections were incubated in a blocking buffer (0.15% glycine, 0.2% bovine serum albumin and 0.1% TX diluted in PBS) containing 10% normal donkey serum (NDS) for 1 h in a humid chamber at RT. Primary antibodies (**Table 2–3**) were diluted in the blocking buffer solution containing 1% NDS and, incubated overnight at 4°C in a humid chamber. The next day, sections were washed three times (10 min per wash) with PBT. Secondary antibodies (**Table 2–4**) were diluted in the blocking buffer solution containing 1% NDS for 2 hours in a humid chamber at RT. To guarantee a uniform exposure of the antibody, sections within the humid chamber were placed on the rocker for the last incubation hour. Following the incubation, sections were washed with PBT three times (10 min per wash), counterstained with Hoechst and mounted with Vectashield (Vector Laboratories).

Table 2–3. Primary antibodies. Details of the primary antibodies used with corresponding dilutions and conditions, determined by optimisation studies. SA = signal amplification.

Antibody (<i>anti</i> -)	Host	Dilution	Pre-treatment	SA	Sectioning Method	Manufacturer
DsRed	Rabbit	1:2000	No	No	Vibratome	Clontech
GFAP	Mouse	1:1000	No	No	Vibratome	Abcam
PCNA	Mouse	1:250	Acid Alcohol	IgG2A-Biotin	Vibratome	Millipore
S100 β	Mouse	1:100	Citrate Buffer	IgG2A-Biotin	Vibratome	Abcam
NeuN	Mouse	1:1000	No	IgG1-Biotin	Vibratome	Millipore
Sox2	Goat	1:25	Citrate Buffer	No	Cryostat	R&D System
E-Cadherin	Mouse	1:250	Citrate Buffer	No	Cryostat	BD Transduction lab.

Table 2–4. Secondary antibodies. Details of the secondary antibodies and conjugates used with corresponding dilutions and conditions, determined by optimisation studies.

Antibody (<i>anti</i> -)	Host	Conjugate	Dilution	Manufacturer
Rabbit	Goat	Alexa 568	1:1000	Abcam
Mouse	Goat	Alexa 488	1:1000	Invitrogen
Mouse IgG1	Goat	Biotin	1:300	Millipore
Mouse IgG2A	Goat	Biotin	1:300	Jackson Immunoresearch
Goat	Donkey	Alexa 488	1:250	Strattech Scientific
Rabbit	Donkey	Alexa 488	1:250	Strattech Scientific

2.6 – Image Capture and Analysis

Images were captured with the Zeiss Axioplan 2 microscope and the Axiovision software (version 4.8). Sections were also imaged as Z-stacks (optical sections), within the range of 0.5 and 1.0 μm , using an apotome attachment and analysed by 3D reconstructions to examine cell distribution and colocalizations. The apotome attachment enabled the generation of images characterised by high contrast and sharpness. Images were post-processed (adjustment of brightness/contrast levels) using Fiji (<https://fiji.sc>) Axiovision and Photoshop (Adobe Systems Inc., San Jose, CA, USA) software packages. The anatomical location of the sections was defined by Bregma coordinates (Franklin & Paxinos 2007).

2.6.1 – Cell Counting

Tomato⁺ cells were counted under a x20 objective using vibratome sections of 60 μm thickness. Average values were plotted as Bar graphs. Variation in cell number is indicated as Mean $\pm$ Standard Deviation, per brain, within the text. Quantification of Tomato⁺ cells in the anterior and posterior OB domains are indicated as percentage of the total cell number as Pie charts.

For cell counting in the OBs of adult long chase ($n=3$) and short chase mice ($n=3$), sections were grouped according to Bregma coordinates. To ensure consistency within the dataset, sections corresponding to Bregma coordinates +4.28; +3.92 and +3.56 were referred as 'Anterior OB' ($n=3$ sections per brain), while sections corresponding to Bregma coordinates +3.20 and +2.96 as 'Posterior OB' ($n=2$ sections per brain) (**Table S2;S3**).

For cell counting of Tomato⁺ glia-like cells in the ventral SVZ of young ($n=3$) and adult ($n=3$) mice, only long chase mice were analysed (**Table S4**). To avoid any bias in the analysis (e.g., tamoxifen consumption and regime schedule), SVZ sections from the same set of FGF18^{Lineage} adult mice were used. Only sections with the same Bregma coordinates were analysed to ensure consistency and reliability of the dataset ($n=4$ sections per brain).

3 – RESULTS

3.1–FGF18 expression is restricted to the dorsal and medial walls of the lateral ventricles in the adult FGF18-CreERT2:Rosa26-tdTomato (FGF18^{Lineage}) double transgenic mice

As previously described (Hagan et al 2019), the tamoxifen inducible FGF18^{Lineage} reporter mouse line expresses the CreERT2 protein under the influence of the FGF18 promoter. Upon its administration, tamoxifen binds to the CreERT2 fusion protein, located within the FGF18 allele, and translocate from the cytoplasm to the nucleus of FGF18-expressing cells. A LoxP-flanked STOP cassette is placed upstream the tdTomato reporter in the Rosa26 allele. This induces constitutive tdTomato expression in FGF18-expressing (FGF18⁺) cells and their daughter (Tomato⁺) cells. To report and characterise FGF18 expression within the adult V-SVZ niche, P90 mice were used in the current study. This is because developmental programs have ceased at this age and senescence has not started yet. To perform lineage tracing analyses, mice were pulsed for 2 consecutive days (short pulse) and sacrificed around P94 (short chase, *n*=3) or P104 (long chase, *n*=5). Brains were serially sectioned and immunolabelling against the tdTomato protein revealed FGF18 expression at the dorsal and medial walls of the ventricle in both sets of mice (Bregma + 1.34 to – 0.10 mm) (**Fig. 3-1**). A lower intensity of Tomato fluorescence was observed in all sections deriving from short chase brains (**Fig. 3-1A-D**).

3.2– Characterisation of the FGF18-expressing cells in the V-SVZ Niche

Primary analyses identified irregular or spherical shaped FGF18⁺ (Tomato⁺) cells, occasionally characterised by a small extending process, at the dorsal wall (**Fig. 3-1G'**). FGF18⁺ cells with spherical or elongated flat contours, arranged in a compact manner, were detected at the medial wall (**Fig. 3-1G''**). Scattered cells were also observed at the lateral V-SVZ, adjacent to the striatum, but only in long chase brains (**Fig. 3-1E-H**). This peculiar FGF18 pattern was likewise identified in the anterior ventricular region (Bregma + 1.54 to + 1.34) (**Fig. 3-1E**) where it was less prevalent and mainly restricted to the medial wall of the ventricle in short chase mice (**Fig. 3-1A**). Tomato expression was also observed in the ependymal layer of the choroid plexus (**Fig. 3-1D, 3-1H**) and in the cerebral cortex of long chase mice only, where it labelled isolated pyramidal neurons. Together, these results suggest a dynamic role of the FGF18 growth factor in the adult mouse brain.

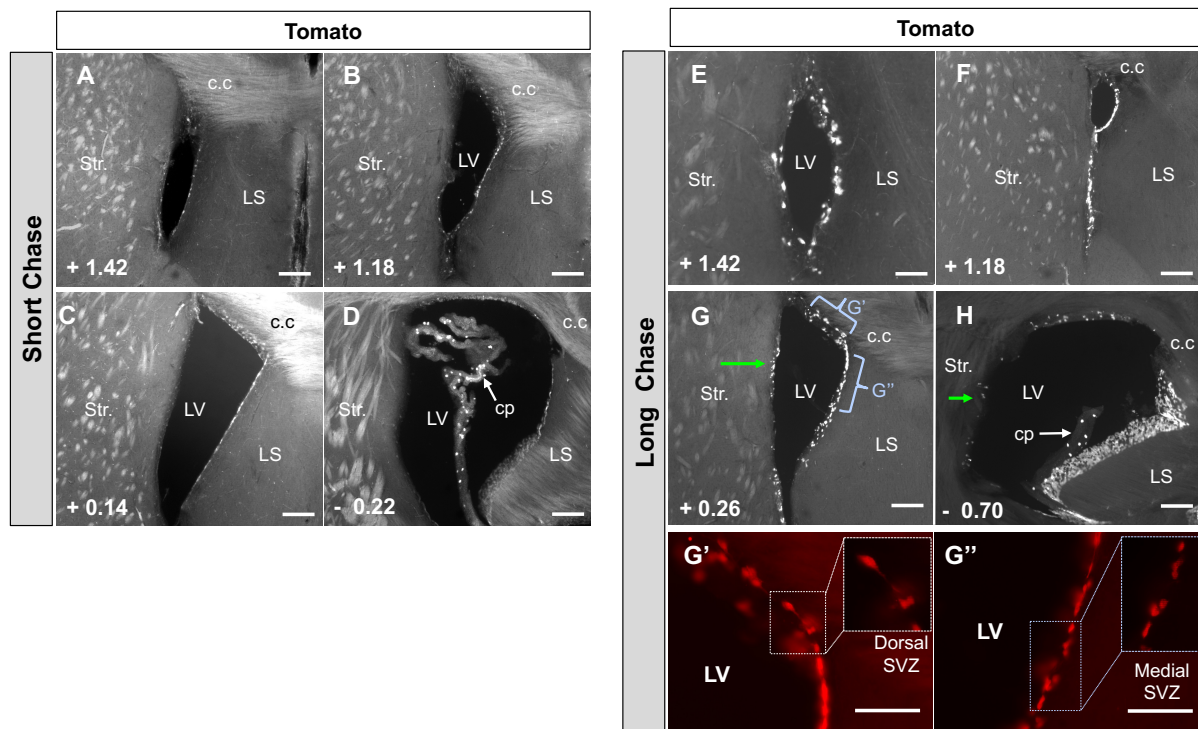


Figure 3–1. FGF18-expressing cells are restricted to the dorsal and medial V-SVZ. Adult *Fgf18-CreERT2/+::Rosa26-tdTomato* (*Fgf18^{Lineage}*) mice were treated with tamoxifen at P90 and P91 with subsequent brain harvesting at P94 (short chase) and P104 (long chase). (A–D) In short chase mice, FGF18⁺ cells are distributed along the dorsal wall and medial wall, adjacent to the septum (LS), of the lateral ventricle (E–H) In long chase mice, FGF18⁺ cells are distributed along the dorsal wall and medial wall of the lateral ventricle along the anterior-posterior brain axis. Scattered FGF18⁺ cells were observed at the lateral V-SVZ, adjacent to the striatum (Str), green arrow. (G'–G'') Morphological features and cellular arrangements of the FGF18⁺ cells at the dorsal and medial V-SVZ. Approximate Bregma co-ordinates are defined in bottom right corner with Bregma equal to zero representing the intersection of the sagittal and coronal sutures of the skull. Bregma coordinates with positive values indicate regions closer to the anterior aspect of the brain (e.g., OBs) while the coordinates with negative values indicate regions towards the posterior end of the brain. Images are taken from brains coronally sectioned with the vibratome. Scale bars= 50 μm and 100 μm. C.C., corpus callosum; C.P, choroid plexus; LS, Lateral Septum; LV, Lateral ventricle; Str., Striatum.

3.3 – FGF18⁺ cells in the adult V-SVZ niche may be progenitor cells

Previous studies have profiled the genetic expression of the cells within the adult V-SVZ while defining their neurogenic lineage progression (Doetsch et al 1999a, Lim & Alvarez-Buylla 2016). In the current study, immunostaining with several lineage markers was used to characterise FGF18 expression along the walls of the ventricles. In this niche, Glial fibrillary acidic protein (GFAP) is one marker for NSCs, together with Nestin and Sox2. GFAP expression in the adult V-SVZ also includes parenchymal astrocytes, progenitors, neuroblasts and ependymal cells (Doetsch et al 1999a, Doetsch et al 1997, Merkle et al 2004). After just 2 days from the last tamoxifen treatment, immunostaining of sections from short chase brains ($n=3$) showed double-labelled Tomato⁺ GFAP⁺ cells at the dorsal and medial V-SVZ (**Fig. 3-2A-B**). This expression pattern was also observed in long chase mice ($n=5$) with double labelled cells mainly found along the medial wall (**Fig. 3-2C-D**). These results suggest that FGF18 may label putative NSCs or progenitors in the adult V-SVZ niche. Staining of sections from long chase brains ($n=2$) with the stem cell and progenitor marker Sox2 revealed the presence of scattered double-labelled Tomato⁺ Sox2⁺ cells within the dorsal wall of the ventricle (**Fig. 3-2F-F''**). However, Sox2 expression prevailed at the medial wall where it co-stained with all of the Fgf18⁺ cells (**Fig. 3-2G-G''**), hence supporting the hypothesis that FGF18⁺ cells may be progenitor cells within the niche. Here, 3D images prospective of the medial V-SVZ, confirmed the distribution of the FGF18⁺ cells in three cellular layers of the niche (**Fig. 3-2E**).

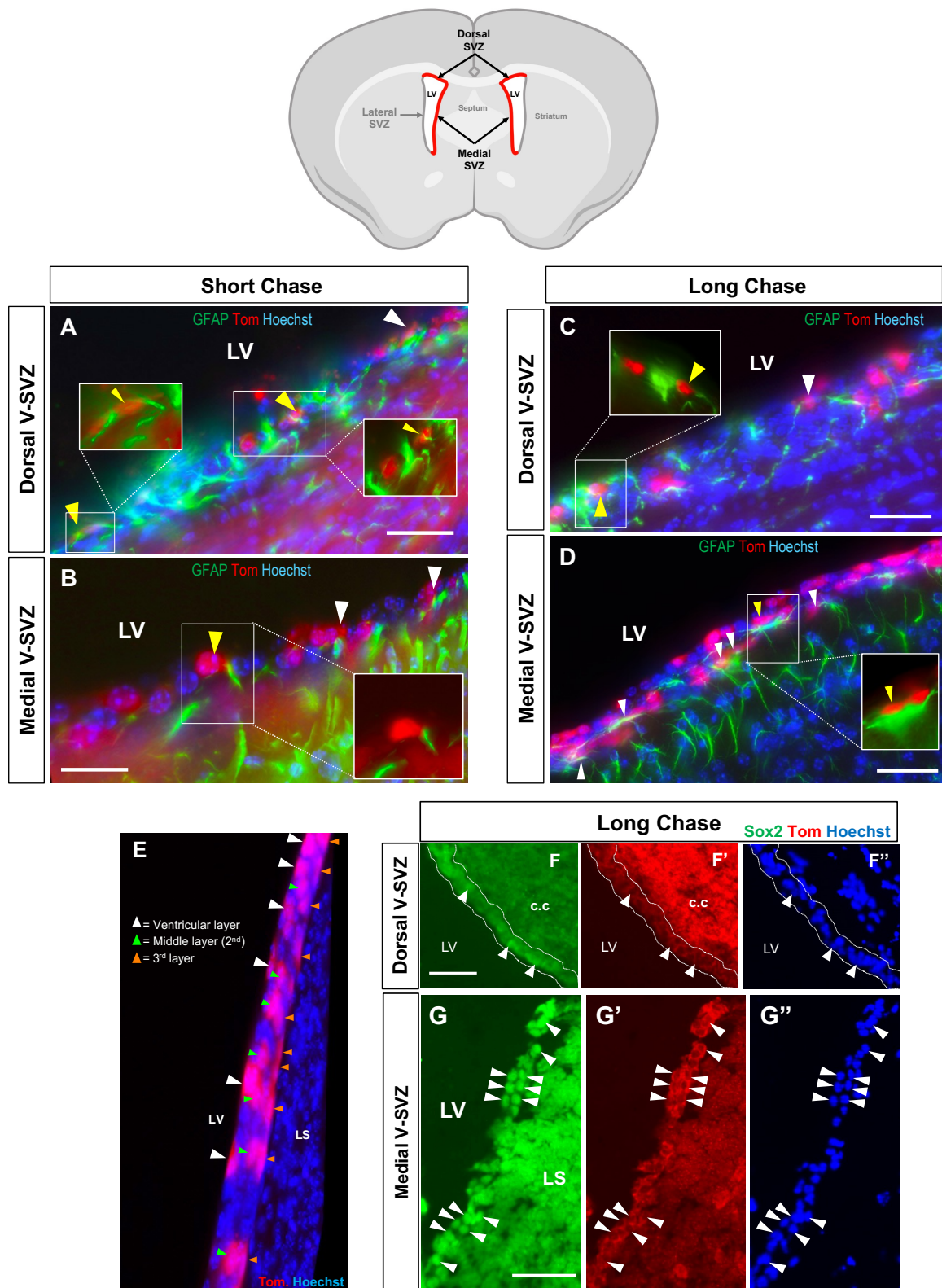


Figure 3–2. FGF18⁺ cells may be progenitor cells in the V-SVZ niche. Double labelled (GFAP⁺ Tomato⁺) cells are detected at the dorsal and medial wall of the ventricle in adult short chase (A–B) and long chase mice (C–D). (E) FGF18⁺ cells are arranged in three cellular layers at the medial V- SVZ, adjacent to the septum. Double labelled (Sox2⁺ Tomato⁺) cells are detected in the dorsal V- SVZ (F–F'') and, particularly, at the medial V-SVZ (G–G'') of long chase mice. White arrows indicate double labelled cells. Yellow arrows indicate double labelled cells magnified. Approximate Bregma coordinates in the bottom corners. Scale bars= 100 μ m. C.C., corpus callosum; LS, Lateral Septum; LV, Lateral ventricle; Str., Striatum.

3.4 – FGF18 has a potential role in barrier function in the adult V-SVZ niche

Given their compact distribution in the medial aspect of the ventricle, it was hypothesised that the FGF18⁺ cells could also reside within the ependymal layer of the niche. To test this hypothesis, Tomato expression was investigated in relation to the calcium-binding protein S100 β , whose expression has been previously associated to ependymal cells and astrocytes of the V-SVZ niche (Platel & Bordey 2016). S100 β -expressing cells co-labelled with Tomato expression in both short ($n=3$) and long chase brains ($n=5$) at the medial V-SVZ (**Fig. 3-3B,3D**). Immunostaining with the cell junction protein E-Cadherin allowed to define the ependymal region of the medial V-SVZ (**Fig. 3-3E-E'**) where it seemed to colocalize with FGF18⁺ cells. However, it is important to note that the Tomato protein, expressed under the influence of the FGF18 promoter, is a cytoplasmic protein hence, localized in the cytoplasm of a cell while, E-cadherin is a membrane protein. In the current study, Tomato expression defines the expression of the FGF18 gene but not the localization of the FGF18 protein which is a secreted glycoprotein. Ideally, future studies could test the colocalization of FGF18⁺ cells with E-cadherin using an effective anti-FGF18 antibody. Currently, there are no commercially available anti-FGF18 antibodies that are reliable and effective in immuno-histochemical reactions. Together, these results suggest that FGF18⁺ cells could play a role in barrier function at the ependymal layer of the medial V-SVZ.

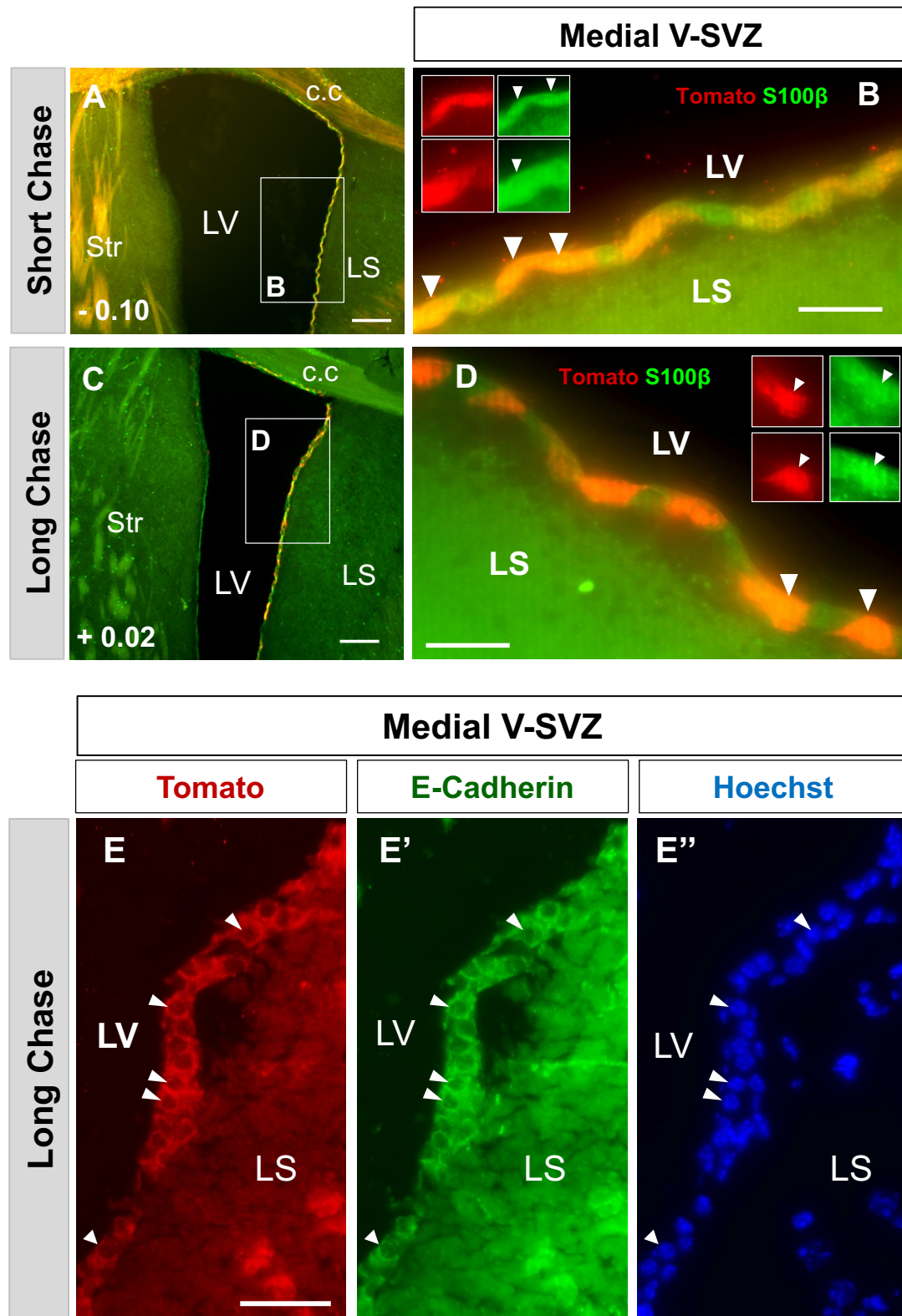


Figure 3–3. FGF18 could have a role in barrier function at the medial V-SVZ.

(A-B) FGF18⁺ cells co-express the ependymal layer marker S100β at the medial V-SVZ of adult short chase and long chase (C-D) mice. White arrows indicate double labelled cells. (E-E'') Immunolabelling on cryostat sections for the cell junction protein E-Cadherin and Tomato. Analysis of the medial wall of the ventricle. Approximate Bregma coordinates in the bottom corners. Scale bars= 50 or 100 μm. C.C., corpus callosum; LS., Lateral Septum; LV, Lateral ventricle; Str., Striatum.

3.5 – FGF18 lineage traced cells are detected in the RMS and OB of Long Chase mice only

De novo neurogenesis, mediated by the adult V-SVZ, results in the extensive migration of neuroblasts from the niche through the RMS to the OB. To address any possible contribution of FGF18⁺ cells to neurogenic activity, Tomato⁺ cells were followed anteriorly through a combination of coronal and sagittal sections of the FGF18^{Lineage} adult mice. In short chase mice ($n=3$), lineage traced cells were not detected in the RMS (**Fig. 3-4A-D**) and OBs (**Fig. 3-6A-D**). However, scattered Tomato⁺ cells were occasionally identified in OB sections from adult short chase mice (3 ± 3 per brain). To guarantee statistical consistency, analyses were performed on sections from brains ($n=3$) with comparable Bregma coordinates (**Table S2**). Quantification showed that 78% of the Tomato⁺ cells are distributed in the posterior OB domain (B. +3.20; +2.96) (**Fig. 3-6F**) where they present a low fluorescence intensity. This indicates that after 2 days from the last tamoxifen treatment Tomato expression is largely confined to the V-SVZ in adult FGF18^{Lineage} mice. On the contrary, in long chase mice ($n=4$), Tomato⁺ cells appeared ensheated by the GFAP⁺ glia cells that characterise the RMS (**Fig. 3-4G-G''**). Here, Tomato⁺ cortical neurons were also observed in the piriform cortex (**Fig. 3-4F**).

3.5.1 – The morphology of Tomato⁺ cells in the RMS relates to migrating neuroblasts

The arrangement and morphology of the lineage traced cells in the RMS was confirmed through sagittal sectioning of the brain ($n=1$). Sagittal view of the ventricular area confirmed the pattern and distribution of FGF18⁺ cells seen in coronal sections (**Fig. 3-5A-A'**). Tomato⁺ cells *en route* to the RMS (**Fig. 3-5B**) appeared to move as aggregates tangentially along the GFAP⁺ glia cells until acquiring a radial migratory pattern in the OB proximity (**Fig. 3-5C-C'**). These resembled migrating neuroblasts (Lois et al 1996) which are characterised by a trailing edge and a leading process orientated towards the OB (**Fig. 3-5C''**). For the statistical analysis, $n=3$ long chase mice were used due to OB sections availability. Contrary to what was observed in short chase mice, Tomato⁺ cells were detected in greater numbers in the OB sections from long chase mice (46 ± 20 per brain) (**Fig. 3-5D, 3-6E**). The variation in the Tomato⁺ cell number, among the different brains analysed, is possibly determined by the tamoxifen treatment mice were exposed to (e.g., amount of tamoxifen effectively absorbed by the mouse) (**Table S3**). As a result, following FGF18⁺ cells of the V-SVZ niche, it can be speculated that they do contribute to the neuronal supply in the OBs.

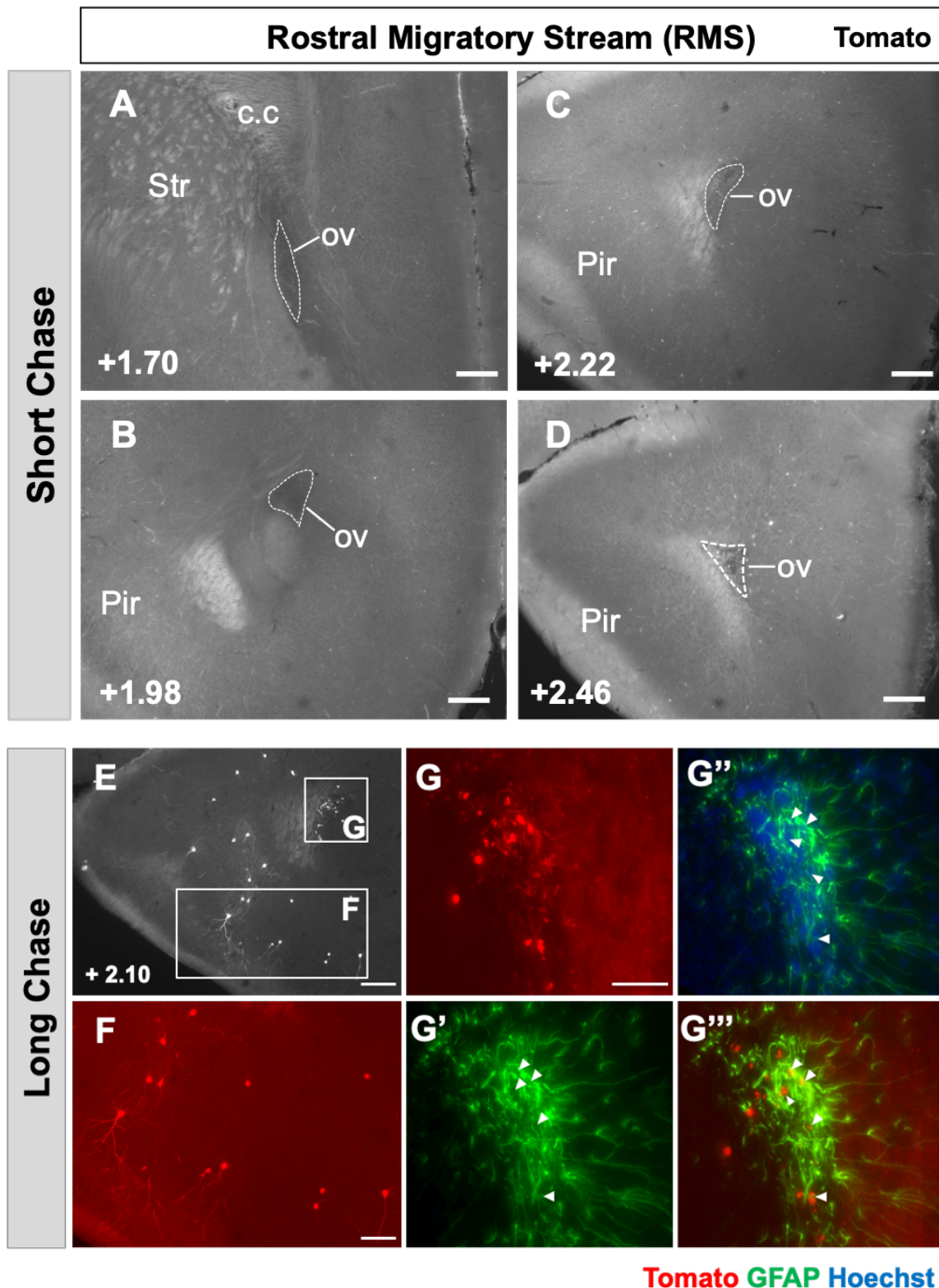


Figure 3–4. Lineage traced cells are detected in the RMS of Long Chase mice only. (A–D) RMS region. The olfactory ventricle (ov) represents the GFAP⁺ glia region of the RMS (E–G''') In long chase mice, lineage traced cells are observed in the RMS, enclosed by the GFAP⁺ glia cells that characterise this region (G–G''', white arrows). (B) Tomato⁺ neurons in the piriform cortex. Bregma coordinates in the bottom corner. Scale Bars= 50 μ m; 100 μ m. Corpus callosum; Pir., Piriform cortex; Str., Striatum.

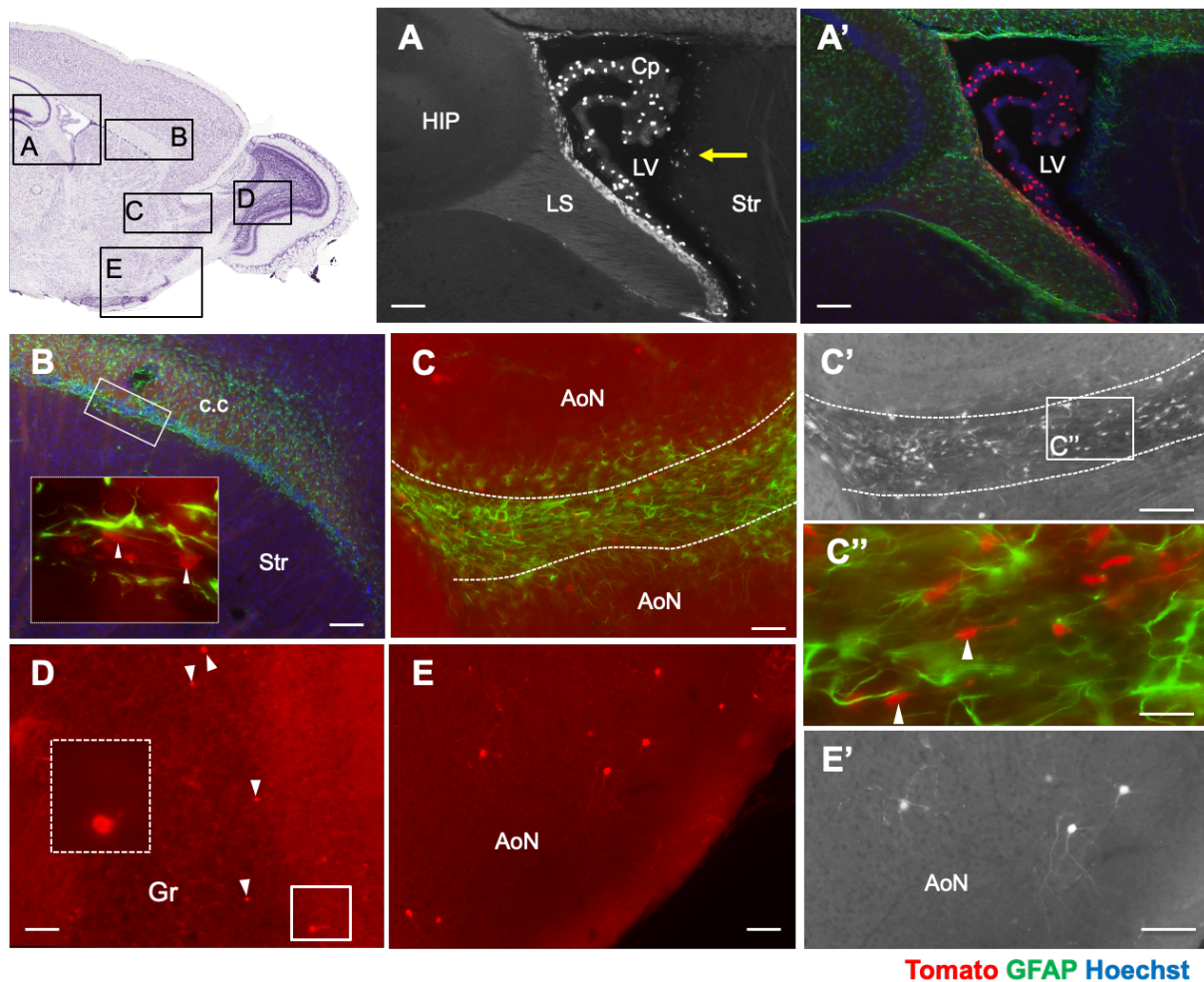


Figure 3–5. Tomato⁺ neuroblast in the RMS of long chase FGF18^{Lineage} adult mice.

Sagittal sectioning of the brain revealed the morphological features of the Tomato⁺ cells in the RMS region (C–C') which are characterised by leading and trailing process and exhibit a radially migrating phenotype in the OB proximity (C'', white arrows). (A–A') Sagittal view of the V-SVZ niche. Yellow arrow indicates FGF18⁺ cells distributed at the lateral wall. (B) Corpus callosum with Tomato⁺ cells migrating towards the OB in the GFAP⁺ glia sheets. (D) Sagittal perspective of the olfactory bulb. (E–E') Tomato⁺ neurons in the anterior olfactory nucleus. Scale Bars= 50 μm; 100μm. Atlas image used as a reference is from Allen Brain Atlas (<https://mouse.brain-map.org/static/atlas>) a., anterior; p., posterior. AoN, anterior olfactory nucleus; c.c., corpus callosum; Cp., choroid plexus; Gr, granule layer; HIP, hippocampus; LS., lateral septum; LV, lateral ventricle; Str., striatum.

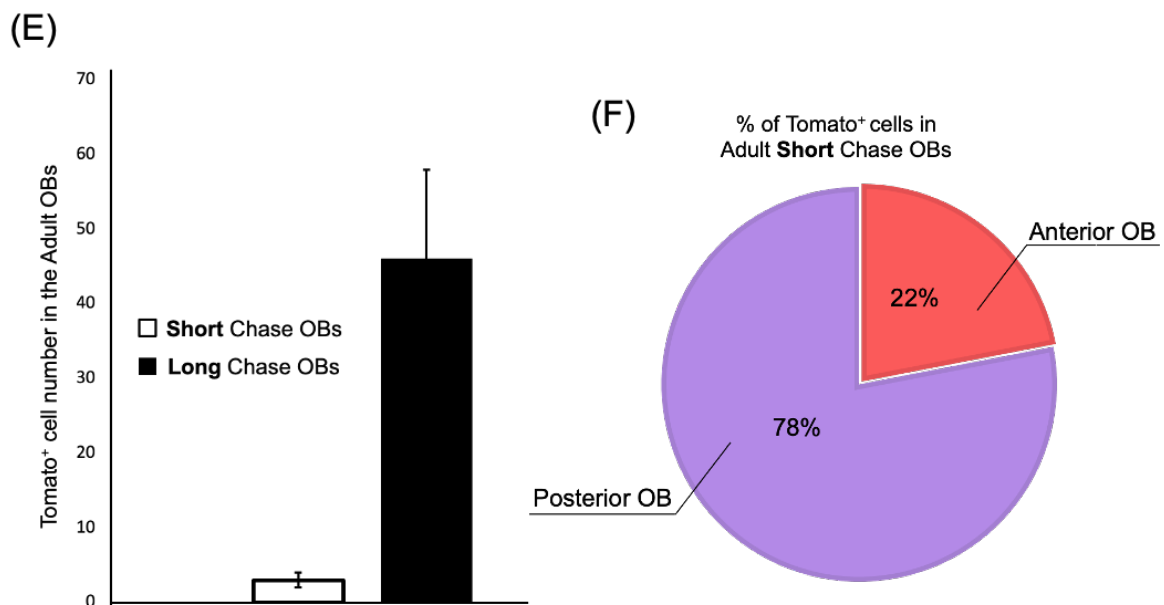
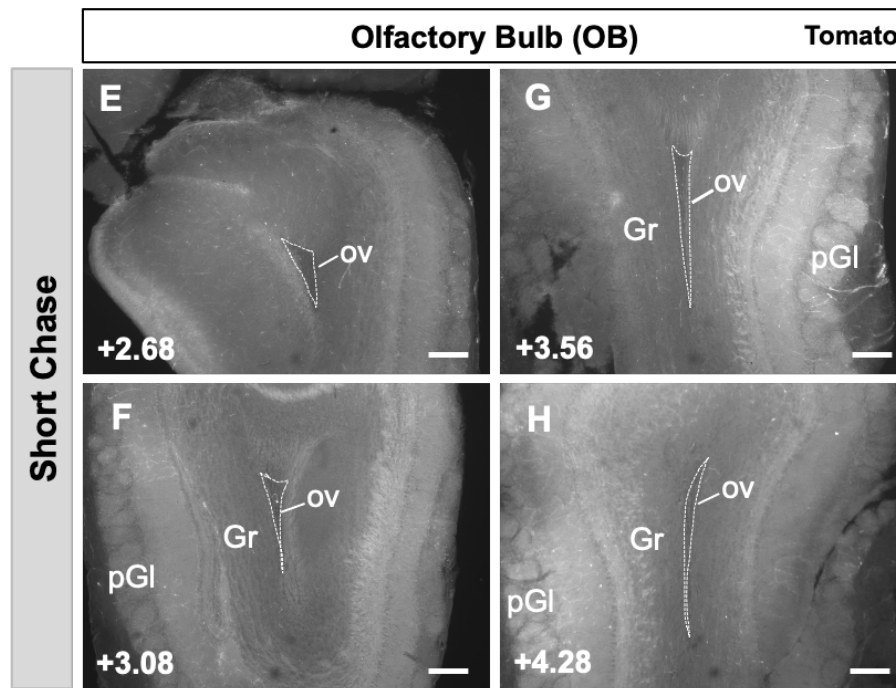


Figure 3–6. Distribution of Tomato⁺ cells in the OBs of Short chase mice.

(A–D) Tomato⁺ cells are not detected in the OBs of short chase mice. (A–C) Posterior OB domain. (B–D) Anterior OB domain. Bregma coordinates in the bottom corner. (E) Comparative quantification shows a greater number of Tomato⁺ cells in the adult long chase OBs ($n=3$; 46 ± 20 per brain) than short chase OBs ($n=3$; 3 ± 3 per brain). Standard error is indicated as error bars. (F) In $n=15$ vibratome sections of short chase OBs, 78% of the Tomato⁺ cells are found in the posterior domain (B. +3.20; +2.96) and the remaining in the anterior domain (B. +4.28 to +3.56). Scale Bars= 50 μm ; 100 μm . Gr., Granule cell layer; o.v., Olfactory ventricle; PGL., Periglomerular cell layer

3.6 – V-SVZ FGF18⁺ cells will give rise to granule interneurons of OB

Detailed quantification of the Tomato⁺ cells in the OBs of long chase mice revealed that 53% of the cells observed (**Fig. 3-6E, Table S3**) were distributed in the posterior olfactory domain (Bregma +3.20; +2.96) and the remaining in the anterior domain (Bregma +4.28 to +3.56) (**Fig. 3-7A**). To characterise these cells, coronal brain sections ($n=3$) were stained for the mature neural marker NeuN. At the posterior domain (**Fig. 3-7B-E'**), Tomato⁺ cells were mostly confined to the olfactory ventricle region. Tomato⁺ NeuN⁺ neurons were observed in the anterior olfactory nucleus (AON) where they exhibited fine arborization (**Fig. 3-7C-C'; 7E-E'**).

Consistent with previous analyses on the different maturation stages of newly generated neurons (Petreanu & Alvarez-Buylla 2002) (**Fig. 3-8A**), the findings from the current study revealed distinct cell morphologies in the anterior domain of the OB as Tomato⁺ cells migrate from the RMS to integrate in its olfactory cell layers (**Fig. 3-8B; 3-8G**). Lineage traced Tomato⁺ cells distributed within the olfactory ventricle region and surrounding commissure (**Fig. 3-8C**) resembled migrating neuroblasts with a short leading process and growth cone (**Fig. 3-8C'**). This immature phenotype was confirmed by NeuN immunostaining which defined these cells as NeuN-negative. Tomato⁺ NeuN⁻ cells were also observed in the granule layer where they displayed a radially migrating phenotype with prominent extending leading and trailing processes, potentially indicating integrating olfactory cells (**Fig. 3-8D; 3-8E**). Eventually, scattered Tomato⁺ NeuN⁺ neurons were also observed in the olfactory granule layer (**Fig. 3-8F; 3-8H**). These exhibited a larger and rounder cell body than Tomato⁺ immature cells, as well as, developing basal dendrites with growing neurites (**Fig. 3-8F'; 3-8H'-H''**). The maturity of these granule neurons is confirmed by their apical dendrites which extended to the mitral layer or branching in the external plexiform layer (EPL) (**Fig. 3-8F''**). Scattered Tomato⁺ neurons were also observed in the periglomerular layer, indicating that granule neurons are generated in a higher number than periglomerular neurons.

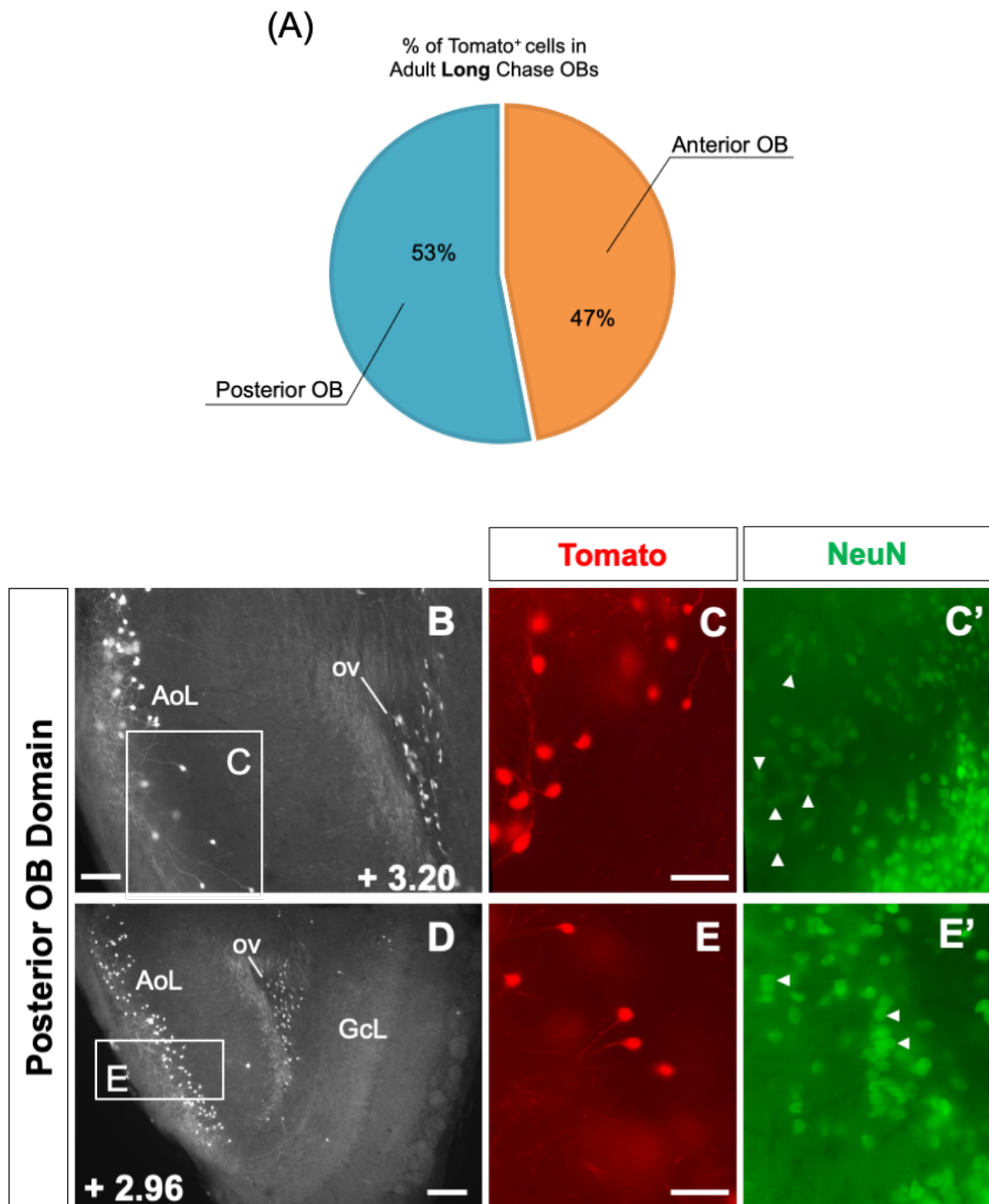


Figure 3–7. Characterisation of Tomato expression in the OBs of Long chase mice. (A) In $n=15$ vibratome sections of long chase OBs, 53% of the Tomato⁺ cells are found in the posterior domain (B. +3.20; +2.96) with the remaining in the anterior domain (B. +4.28 to +3.56). (B–E') Lineage tracing of Tomato expression in the OB of long chase mice revealed Tomato⁺ neurons (NeuN⁺) in the lateral anterior olfactory nucleus (AoL) of the posterior OB domain (white arrows). Bregma coordinates in the bottom corner. Scale Bars= 50 μ m; 100 μ m. GcL., Granule cell layer; o.v., Olfactory ventricle.

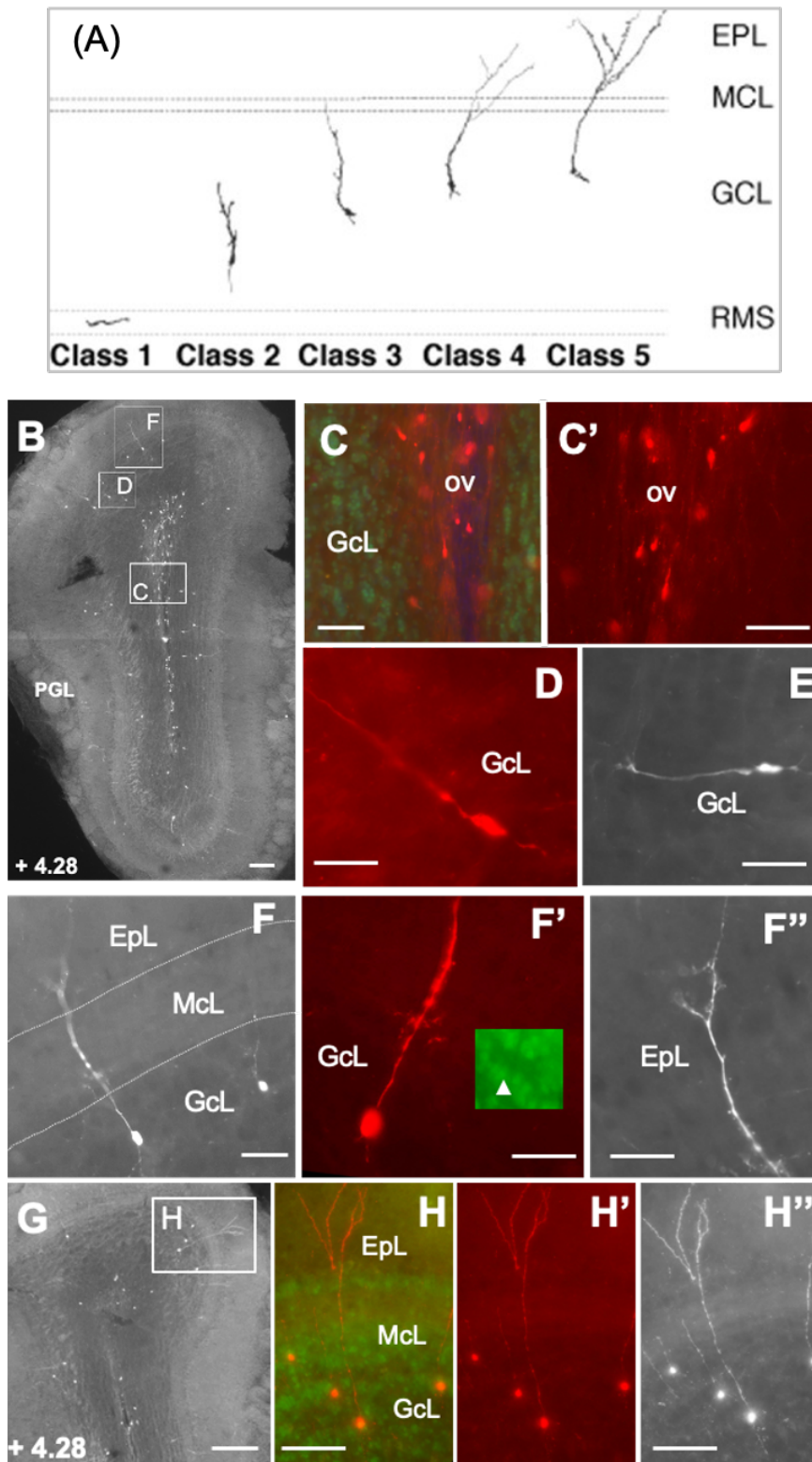


Figure 3–8. Characterization of the Tomato⁺ cells in the Anterior OB Domain.

(A) Maturation stages of newly generated granule cells. Image from (Petraenu et al., 2002). (B,G) Tomato⁺ cells of different morphological phenotypes are detected in the anterior OB domain of long chase mice: migrating neuroblasts (C–C'); immature granule cell, class 2 (D–E); Granule interneuron (F–F',H–H'') with an extending process in the EPL (F''). White arrow indicates co-labelling with the mature neural marker NeuN. Fluorescent images were taken as Z-stacks and oriented within a 3D plan to analyse cells' characteristics. Scale Bars= 50 μ m; 100 μ m. EpL, External plexiform layer; GcL, granule layer; McL, mitral cell layer; ov., olfactory ventricle; PGL, periglomerular layer.

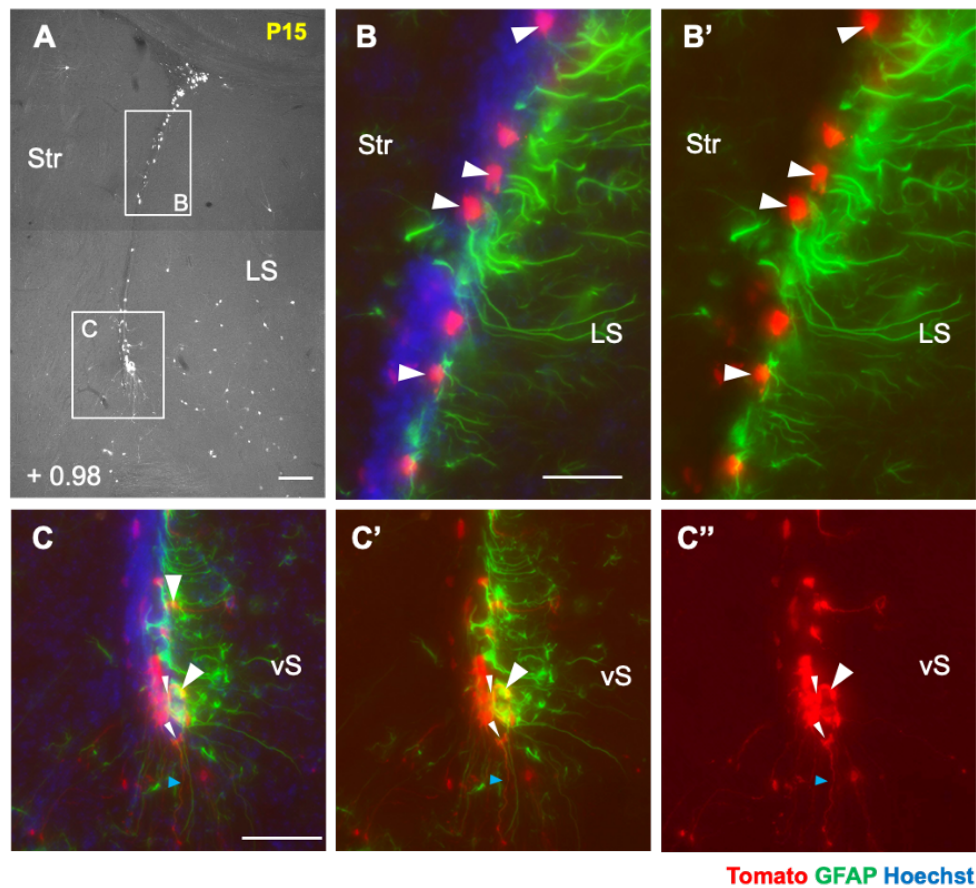
Together, these analyses identify FGF18⁺ cells of the V-SVZ as a source of progenitors involved in the regulation of adult neurogenesis. This is supported by the presence of migrating lineage traced neuroblasts in the RMS and by the different morphological features of Tomato⁺ cells detected in the granule cell layers at the anterior olfactory domain. Future studies are needed to assess the role of Tomato⁺ neurons in the AON and piriform cortex to further elucidate FGF18 contribution to odour discrimination in the adult mice.

3.7 – An age-related decline in FGF18⁺ cell number and proliferation

The interplay of different signalling factors supports and mediates neurogenesis in the V-SVZ niche (Lim & Alvarez-Buylla 2016). However, this process is affected during the lifespan as a response to changes in the niche microenvironment and cellular composition caused by ageing . Studies on the adult V-SVZ niche led to question on whether FGF18 expression in this region was restricted to adulthood. Therefore, to further uncover any possible variation in FGF18 expression caused by ageing, P4 FGF18^{Lineage} mice were pulsed for 2 consecutive days and their brains were harvested at P7 (short chase, $n=2$) or P15 (long chase, $n=3$). A survey of the coronal sections from these young brains revealed the presence of fewer FGF18⁺ cells at the medial and ventral region of the niche (**Fig. 3-9A**) Immunostaining using the neurogenic lineage marker GFAP showed subsets of double labelled Tomato⁺ GFAP⁺ cells at the medial and ventral regions (**Fig. 3-9B'**; **9C'**). The FGF18⁺ cells that occupied the ventral wall exhibited radial glial-like morphologies with apical ends and long basal processes (**Fig. 3-9C''**), suggesting that the Tomato⁺ GFAP⁺ cells lining this region could function as NSCs. Comparative quantification of the FGF18⁺ radial glia-like cell number in the ventral SVZ showed a greater distribution of these cells, about 5 times more, in sections from young long chase mice (11 ± 7 per brain) than in adult long chase mice (2 ± 2 per brain) (**Fig. 3-9D**; **Table S4**). This suggests that, with time, the expression and number of FGF18⁺ radial glia-like cells in this region are subject to ageing. Although not quantified, an increase in the number of Tomato⁺ neurons was generally observed in the cortex of young long chase mice (**Fig. 3-10B**) compared to the one of adult long chase mice (**Fig. 3-10C**). Moreover, differently to the adult FGF18^{Lineage} brains, scattered Tomato⁺ neurons were observed in the lateral septum (**Fig. 3-9A**), striatum and vertical diagonal band (VDB) (**Fig. 3-10A**) potentially as the

result of FGF18 activity occurring during embryonic development. Lineage tracing of Tomato expression anteriorly revealed that Tomato⁺ cells are not observed in the GFAP⁺ glia region of the RMS (**Fig. 3-10B,D**) or the OB (**Fig. 3-10F,8H**) of early postnatal short chase brains. By contrast, Tomato⁺ cells were detected in the long chase brain in both the SVZ and OB (**Fig 3-10E; 10G; 10I**), resembling the expression pattern observed in the adult mice.

Given the characterisation of FGF18 and Tomato expression at early postnatal days, I questioned whether ageing had any effect on the proliferative capacity of the FGF18⁺ cells observed within the niche. In order to test this, immunostaining with the endogenous proliferative marker PCNA (proliferating cell nuclear antigen, PCNA) was performed on both adult and early postnatal brains' sections. To visualize the proliferating state of cells in the adult V-SVZ niche, PCNA expression was analyzed at different Bregma coordinates of the adult brain ($n=5$). As previously described by the literature (Bordiuk et al 2014), the lateral SVZ harbours an elevated density of actively cycling cells than the other aspects of the lateral ventricle (**Fig. 3-11A; 11C, 11E**). Along the anterior-posterior axis, clusters of scattered PCNA⁺ cells were detected at the medial V-SVZ. However, double staining of sections from long chase brains revealed that FGF18⁺ cells in the medial SVZ are located adjacent to or in proximity of PCNA⁺ cells (**Fig. 3-11B-BB''; 11D-D''; 11F-F''**). By contrast, the number of actively proliferating FGF18⁺ cells (Tomato⁺ PCNA⁺) at the medial and lateral V-SVZ were increased in the early postnatal long chase mice ($n=2$) (**Fig. 3-12E''**), with the ventral V-SVZ region showing a greater distribution of proliferating FGF18⁺ cells than the medial wall (**Fig. 3-12F-F''**). However, in short chase mice, the proliferating activity of these cells was mainly restricted to the ventral wall (**Fig. 3-12B-C''**). Taken together, these results suggest a potential age-dependent decline in proliferating activity of FGF18 in the V-SVZ of younger Fgf18^{Lineage} mice compared to the adult mice, hence establishing a dynamic role of FGF18 in the overall activity of this neurogenic niche postnatally.



(D)

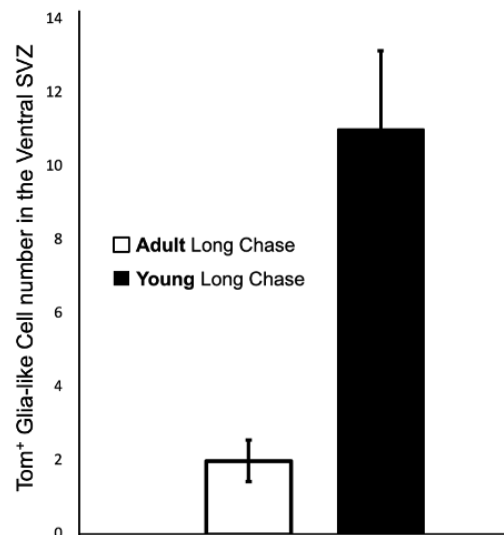


Figure 3–9. FGF18⁺ radial glia-like cells are found in the ventral SVZ of young FGF18^{Lineage} mice brain. A subset of FGF18⁺ cells co-expresses GFAP at the medial (B–B') and ventral wall (C–C') (white arrows). (C'') FGF18⁺ cells in the ventral SVZ exhibit long basal processes extending within the niche (blue arrows) and apical endings. (D) Comparative quantification shows a greater number of Tomato⁺ radial glia-like cells in the ventral SVZ of young long chase mice ($n=3$; 11 ± 7 per brain) than adult long chase mice ($n=3$; 2 ± 2 per brain). Standard error is indicated by error bars. Scale bar: 50 μm ; 100 μm . LS, Lateral septum; LV, Lateral ventricle; Str., Striatum; vS., Ventral septum

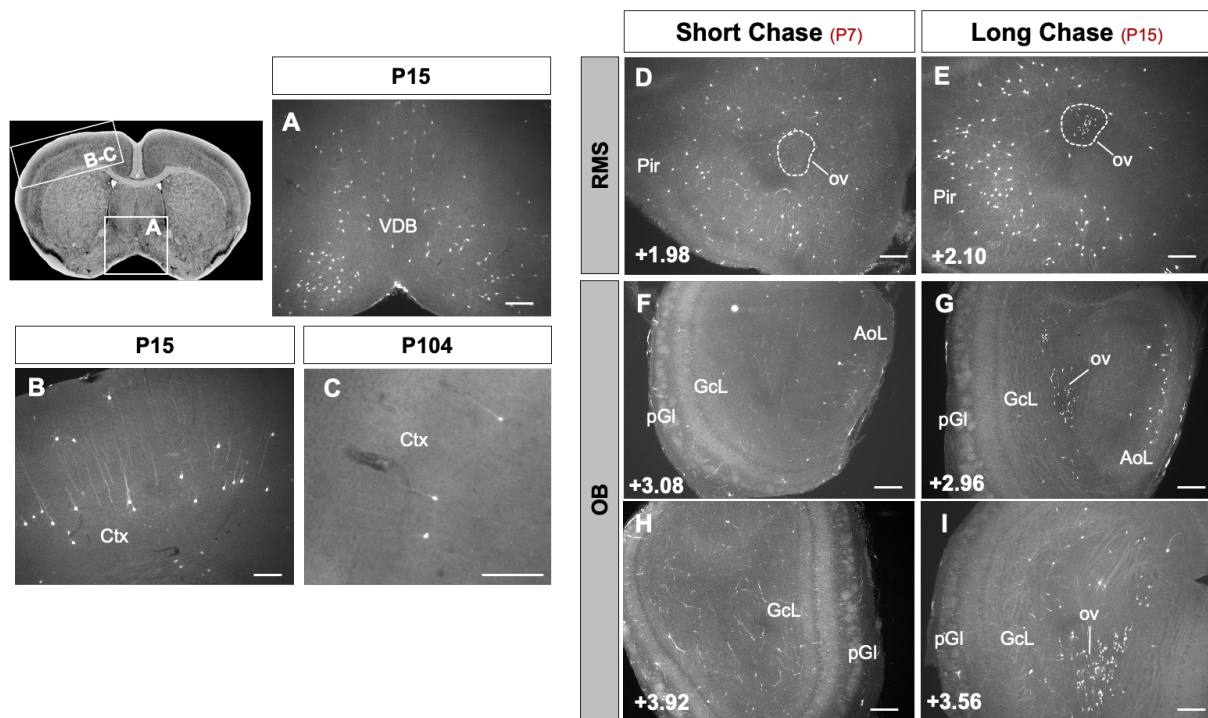


Figure 3–10. Characterisation of Tomato expression in young FGF18^{Lineage} mice brains. (A) Vertical diagonal band. (B-C) The number of Tomato⁺ neurons in the cerebral cortex of long chase young mice appeared to be higher than the number of Tomato⁺ neurons in the cortex of adult long chase mice. (D, F, H) Lineage traced cells are not detected in the GFAP⁺ glia region of the RMS and in the OB of short chase young mice. (E, G, I) Tomato⁺ cells are observed in the RMS and OB of long chase young mice. Atlas images are from the Mouse Brain Library (http://www.mbl.org/mbl_main/atlas.html). Approximate Bregma coordinates in the bottom corners. Scale bars: 50 μ m. AoL., Anterior olfactory nucleus; Ctx., Cortex; GcL., Granule cell layer; ov., Olfactory ventricle; pGl., periglomerular layer; Pir., piriform cortex; VDB., vertical diagonal band.

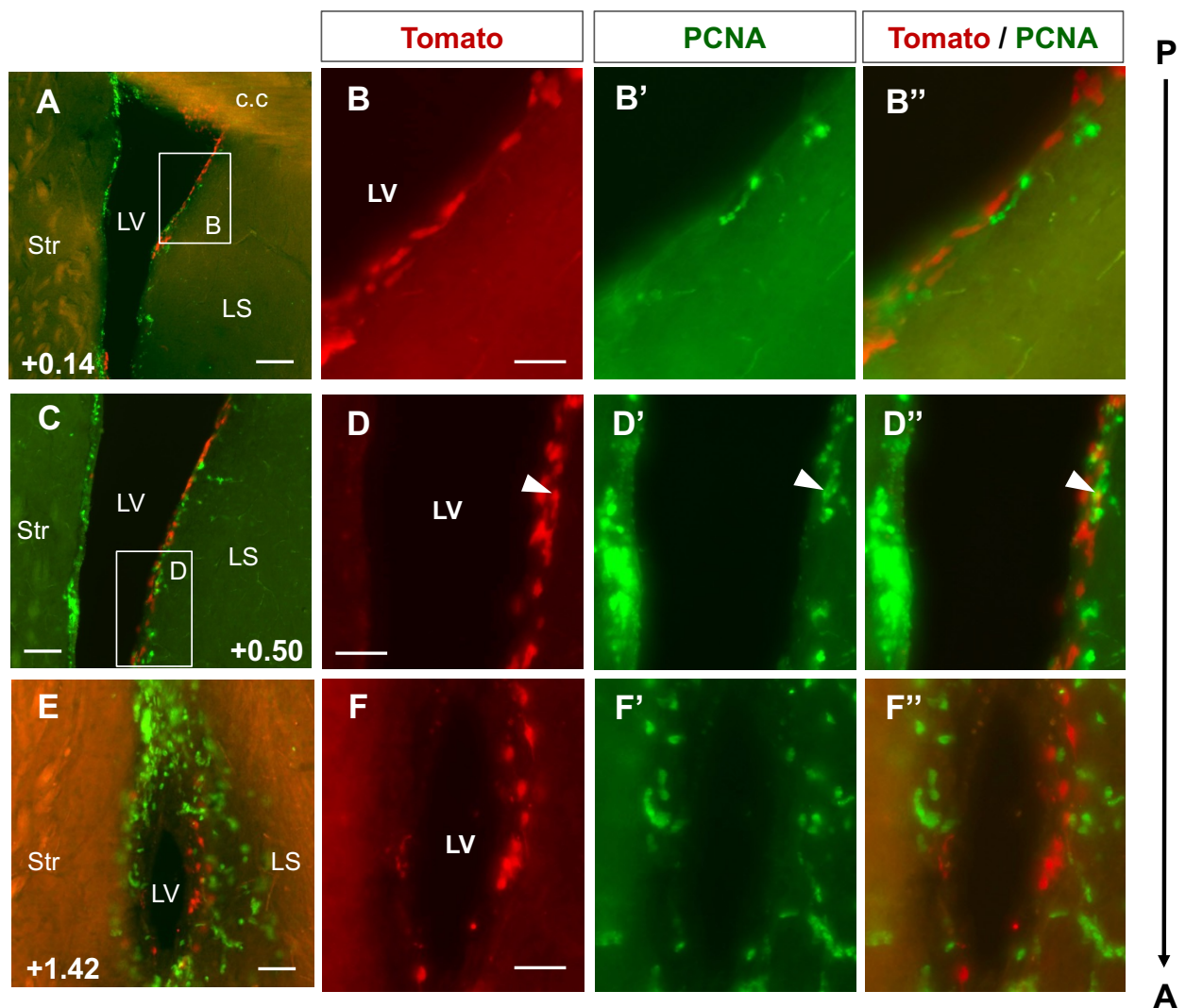


Figure 3–11. FGF18⁺ cells are not actively proliferating cells in the Adult V-SVZ. Immunostaining of adult long chase brains for the endogenous proliferating marker PCNA revealed that, at the medial wall of the V-SVZ, FGF18⁺ cells mingle with or sit adjacent to PCNA⁺ proliferating cells along the posterior-anterior axis. Bregma coordinates in the bottom corners. Scale bars: 50 μ m or 100 μ m. C.C., corpus callosum; LS, Lateral septum; LV, Lateral ventricle; Str., Striatum.

4 – DISCUSSION

The generation of new functional neurons in specific regions of the mammalian nervous system has redefined the structural and functional plasticity of the adult brain. In this context, the FGF members participating in the process of adult neurogenesis have yet to be fully established. For the first time, this study characterises in detail FGF18 expression in the V-SVZ niche of the adult mouse brain, elucidating putative roles that this signalling factor could exert in OB neurogenesis (**Fig. 4-1; 4-2**).

4.1 – FGF18 contributes to the compartmentalisation and regulation of the V-SVZ in the adult mouse brain.

Through the use of a FGF18CreERT2::Rosa26-tdTomato (FGF18^{Lineage}) mouse line (Hagan et al 2019) and a pulse-chase system, the domain of FGF18 expression was shown to be confined to the dorsal and medial walls of the lateral ventricles, both in short and long chase adult mice (**Fig. 4.1**). A first screening of the morphological characteristics and distribution of the FGF18⁺ cells, within the niche, suggests a variation in the FGF18-mediated regulation of the V-SVZ activity postnatally. Clearly, FGF18 expression at the medial wall, adjacent to the septum, generates great interest for being a far less studied region than the lateral wall. As already stated in previous chapters of this thesis, the NSCs populate the lateral, medial and dorsal walls of the lateral ventricle (Chp. 1.2.2). The anatomical location of the NSC pool along the walls of the ventricle highlights differences both in the NSCs' identity and in the mechanisms regulating their behaviour (Fiorelli et al 2015, Fuentealba et al 2015). Identifying the regulatory signalling molecules and their corresponding receptors that support and mediate the neurogenic activity of different aspects of the niche is therefore fundamental.

4.1.1 – NSCs quiescence / activation

It has been demonstrated that FGF2 represents a signalling source for NSCs proliferation and differentiation within the SVZ neurogenic niches (Woodbury & Ikezu 2014, Yoshimura et al 2001). My findings reveal that at the dorsal and, particularly, at the medial walls of the lateral ventricle a subset of FGF18⁺ cells co-express the stem cell markers GFAP and Sox2. Given the lack of functional analyses yet, it can only be speculated that FGF18 regulates the transitory mechanism of NSCs from an actively dormant (quiescence) state to activation. This could be mediated by signalling through the receptor FGFR3 for which FGF18 shows great affinity (Hoshikawa et al 2002,

Olsen et al 2006). Transcriptomic analysis has shown that FGFR3 is implicated in the signalling process regulating quiescence and early activation of NSCs in the adult mice V-SVZ (Dulken et al 2017). This is because FGFR3 correlates with astrocytes markers (Ntsr2, Gja1) but not with early neuronal markers associated with the late-aNSC population (Dlx-1/-2) during the mid- to late-activation period of the NSCs. Future studies using scRNA-sequencing of the FGF18⁺ cells, FGF18 ChIP-sequencing and RT-PCR analyses for the FGF18 ligand and corresponding receptors, could be performed in combination with *in vitro* neurospheres' assays to assess the transcriptional level and program of FGF18 in the adult V-SVZ, as well as, to identify a FGF18-FGFR3 binding activity. Understanding the gene network set regulated by FGF18 will further specify the signalling domain of FGF18 in the adult V-SVZ, its involvement in the quiescence-activation of the NSCs populating this niche and the time window of this process.

4.1.2 – FGF18 may signal through the CSF

It is important to note that FGF18, as part of the FGF8 subfamily, can signal through its corresponding receptor by targeting the cell from which it is secreted (autocrine) or targeting a nearby cell (paracrine) (Ornitz & Itoh 2015). The identification of FGF18⁺ cells in the epithelia of the choroid plexus suggests that it could both work cell autonomously within the V-SVZ or be secreted by the choroid plexus into the lumen of the ventricle, filled with CSF, to regulate the neurogenic activity of the niche. The CSF represents an integral part of the niche microenvironment. It functions not only as a buffer for the brain, but it also influences cells' functionality in the V-SVZ niche and its disruption leads to dysfunctions in cortical development and subsequent neurological deficiencies (Bjornsson et al 2015, Obernier & Alvarez-Buylla 2019, Owen-Lynch et al 2003). FGF2 has previously been demonstrated to regulate the activity of the NSCs in the adult V-SVZ via its secretion from the choroid plexus and paracrine signalling effect through the CSF (Falcão et al 2012, Johanson et al 2004, Porlan et al 2013). Douet and colleagues (Douet et al 2013) defined the details of this process where FGF2 diffuses through the ependymal layer, via the interstitial cleft channels of the ependymocytes, to reach fractones and NSCs. Fractones are fragments of the ECM that sequesters growth factors from the lateral ventricle in the ventricular walls where they are activated via heparin-binding mechanisms. From the data collected in this study, it may be speculated that FGF18 is released by the choroid

plexus in the CSF and contributes to NSCs and progenitors' activity by entering the ependyma layer. Future studies that look at FGF18 secretion into the CSF could confirm this hypothesis.

4.1.3 – Barrier Function

The specialized microenvironment in which the V-SVZ niche is endowed is necessary for maintaining the proliferative and neurogenic state of NSCs and progenitor cells. While GFAP and Sox2 are commonly identified as stem cell markers, their expression domain includes RGCs' derivatives too, such as ependymal cells. Most ependymal cells are post-mitotic non-regenerative cells that guarantee a barrier and filtration system for the CSF. The transcriptional profile of these cells identifies a close association with quiescent NSCs markers, including Sox2 (Stratton et al 2019). Immunolabelling with Sox2 revealed a co-expression with FGF18⁺ cells, particularly at the medial wall. While Sox2 is recognised as a marker for NSCs, it is unlikely that all the FGF18⁺ cells present a 'stem' capacity in this region, leading to the hypothesis that FGF18 could have a dual expression/role in the medial wall as a marker for both ependymal and NSCs. Indeed, the compact arrangement of these cells and their distribution on three cellular layers of the niche suggests FGF18 could also exert a barrier function. This is supported by immunostaining with markers of ependymal cells (Doetsch et al 1999a) (Karpowicz et al 2009).

Previous studies have correlated S100 β expression and activity to the ependymal layer, defining a condition in which cells lose their stem potential and acquire a mature state *in vitro* (Raponi et al 2007). The stem-cell like ability of ependymal cells has always been debated with some considering these as postmitotic cells able to act as dormant NSCs that become activated in response to injury (Johansson 1999, Panayiotou & Malas 2013). At E15.5, GFAP⁺ RGCs commit to an ependymal or B cell fate (Fuentelba et al 2015) but mature ependymal cells lose GFAP expression and develop motile cilia in the postnatal brain (Young et al 2013). However, data have also shown that the ependymal layer doesn't de-differentiate following an injury (e.g., stroke). Instead, it supports regeneration by increasing their level of GFAP expression. As a result, the gene set shared with NSCs purely derives from their ancestral origin and the gene set related to cilia is what distinguishes them from the stem cell and progenitor lineage (Shah et al 2018). It would be interesting to perform future *in vivo* studies that could test ependymal layer damage and loss, usually observed during an

injury and ageing. For example, through intraventricular injections of specific agents that target the ependyma (e.g., neuraminidase denudation) (Luo et al 2008) and a subsequent measurement of the FGF18 levels within the V-SVZ niche microenvironment through RT-PCR.

4.2 – FGF18: a potential role in the neuronal supply to the OB

The implication of FGF18 in the neurogenic activity of the adult V-SVZ is reinforced by the finding of lineage traced FGF18⁺ cells migrating within the GFAP⁺ glia cell tube of the RMS in long but not short chase mice. Consistent with previous analyses characterising the migratory mode of cells populating this region (Lois et al 1996, Martinez-Molina et al 2011), the data show that Tomato⁺ cells are encompassed by GFAP⁺ astrocytes and migrate tangentially as chains until they reach the OB proximity where they acquire a radial orientation (**Fig. 3-5C'-C''**). The morphological identity of these cells characterised by actively extending and retracting leading processes support their locomotory motion to the OBs, suggesting that FGF18-mediated activity in the V-SVZ region is accountable for the generation of migrating neuroblasts that ultimately reach the OBs and differentiate into neurons.

Petreanu and Alvarez-Buylla (Petreanu & Alvarez-Buylla 2002) classified the maturation phases and differentiation timing that the newly generated olfactory neurons undergo, in P60 mice (**Fig.3-8A**). In this study migrating neuroblasts, immature granule cells and maturing neurons were observed in their final phases of maturation, as aforementioned discussed (Chp. 3.4), in the rostral OBs of long chased mice only. It should be mentioned that it cannot be presumed, at this stage, that all the detected neurons will survive and/or build a synaptic connectivity with the pre-existing circuit over time. Indeed, apoptosis is a fundamental homeostatic mechanism adopted by the neural system to balance the integration of neurons and scale their number (Ryu et al 2016). Therefore, apoptosis assays could be implemented in the future studies to investigate Tomato expression in relation to this process.

The survival, integration and maturation of adult newborn neurons requires inputs from surrounding cells such as the mitral cells and sensory neurons (Panzanelli et al 2009) but also connections with other regions such as the AON and the piriform cortex (Arisi et al 2012, Libbrecht et al 2021). Potentially, the identification of Tomato⁺ neurons in the AON is the result diversion of the neuroblasts migratory path to the OB. Future

studies could extend the analysis of the FGF18 expression domain to the olfactory epithelium, important for sending olfactory inputs to the OB, but also use of mouse models lacking the OB to determine whether FGF18 fails to thrive neurogenesis or not.

4.3 – Age-Dependent changes in FGF18 is the regulation of V-SVZ neurogenesis

Jin and colleagues (Jin et al 2003) showed that FGF2 and EGF infusion in the lateral ventricle of aged mice reversed the neurogenic capability of the V-SVZ at a level comparable to untreated young mice. Modifications in the ECM composition also affects FGF signalling (Yamada et al 2017), resulting in an impaired neurogenesis in the aged V-SVZ. In the current study, the increase in Tomato⁺ cell number in the OBs and RMS of early postnatal FGF18^{Lineage} long chase mice, when compared to adult long chase mice, points to a decrease in FGF18 activity over time. In the V-SVZ, FGF18 expression and cell distribution resembles the pattern observed in the adult brain. Interestingly, FGF18⁺ cells were not detected in the dorsal V-SVZ niche of young short and long chase mice. However, the morphological characteristics of the FGF18⁺ cells observed in the ventral region of younger mice correlate with RGC populations which are characterised by extending basal processes and apical endings (Obernier & Alvarez-Buylla 2019) (**Fig. 4-1**). Cells with this radial glia morphology also showed a GFAP⁺ staining thus, indicating that FGF18 labels a population of NSCs in this region which is potentially accountable for the generation of the progenitor and neuroblasts observed in the medial and lateral walls of the ventricle in long chase mice. The dramatic decrease in FGF18⁺ radial glia-like cells in the V-SVZ of adult long chase mice (**Fig. 3-9D; 4-1**) suggests that, with ageing, the NSC pool of the V-SVZ depletes possibly following the NSCs' asymmetric division, steady differentiation into progenitor cells and reduction in cell proliferation (Conover & Shook 2011, Obernier et al 2018). The decrease in the neurogenic activity of the niche detected in the adult brain, compared to the early postnatal brain, is also associated with a reduction in the proliferating activity of FGF18⁺ cells which could be caused by a decrease in proliferation or lengthening of the cell cycle in progenitors (Luo et al 2006). Remarkably, Tomato⁺ neurons were detected in other regions of the brain such as the septum and VDB of younger mice, but not adult mice. The identification of Tomato⁺ neurons in the cortex of early postnatal mice could be the result of FGF18 activity

retained from embryonic development. Indeed, FGF18 has previously shown to support the process of cortical laminar patterning through the coordination of neuronal migration and cell body positioning (Hasegawa et al 2004). As a result, future studies could investigate the expression of FGF18⁺ cells in the SVZ of late embryos or even in younger brains, before P7/P15. This raises more possibilities on the diverse roles of FGF18 in the early postnatal brain and gives foundations for future studies on the role of FGF18 in the V-SVZ-mediated neurogenesis from embryonic development throughout ageing.

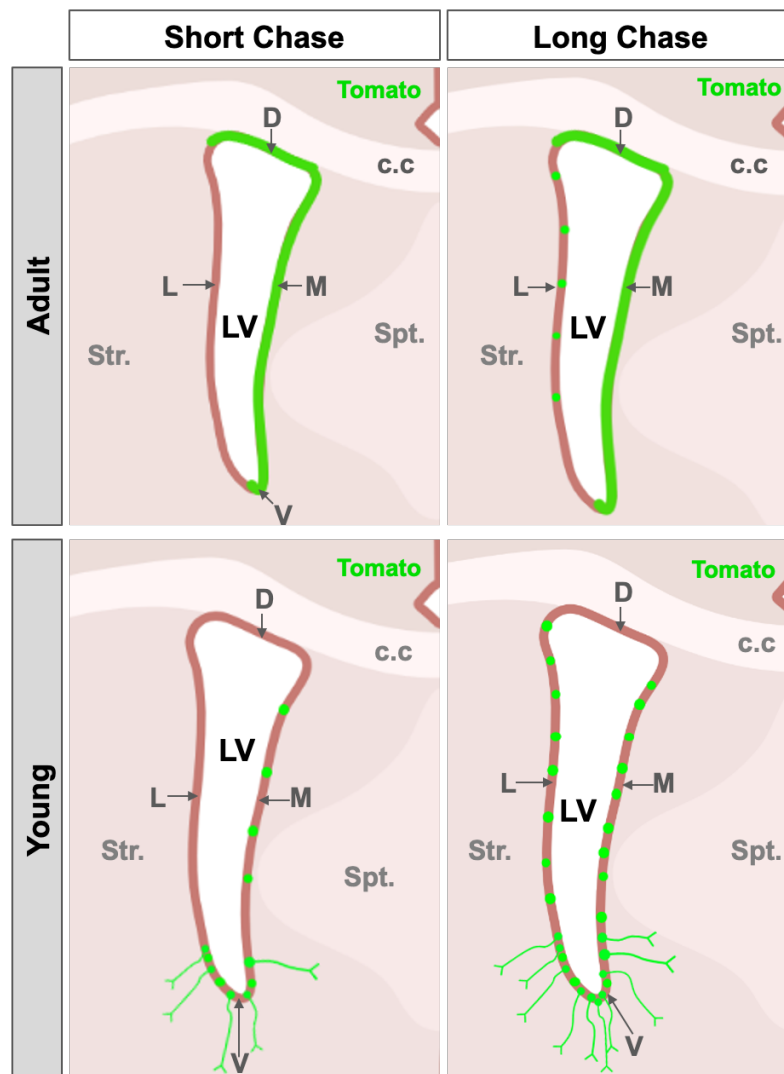


Figure 4–1. Distribution of FGF18-expressing cells in the V-SVZ of adult and young mice. Tomato expression (FGF18⁺ cells) was observed at the dorsal and medial walls of the lateral ventricle in the adult V-SVZ. Scattered FGF18⁺ cells also distributed at the lateral wall in long chase mice only. In young mice, FGF18⁺ cells were observed at the medial and ventral wall of the ventricle in both mice. Tomato⁺ radial glia-like cells were observed at the ventral wall. FGF18⁺ cells distributed at the lateral wall in young long chase mice. c.c., corpus callosum; D, dorsal wall; L, lateral wall; M, medial wall; V, ventral wall; Str., striatum; Spt., septum.

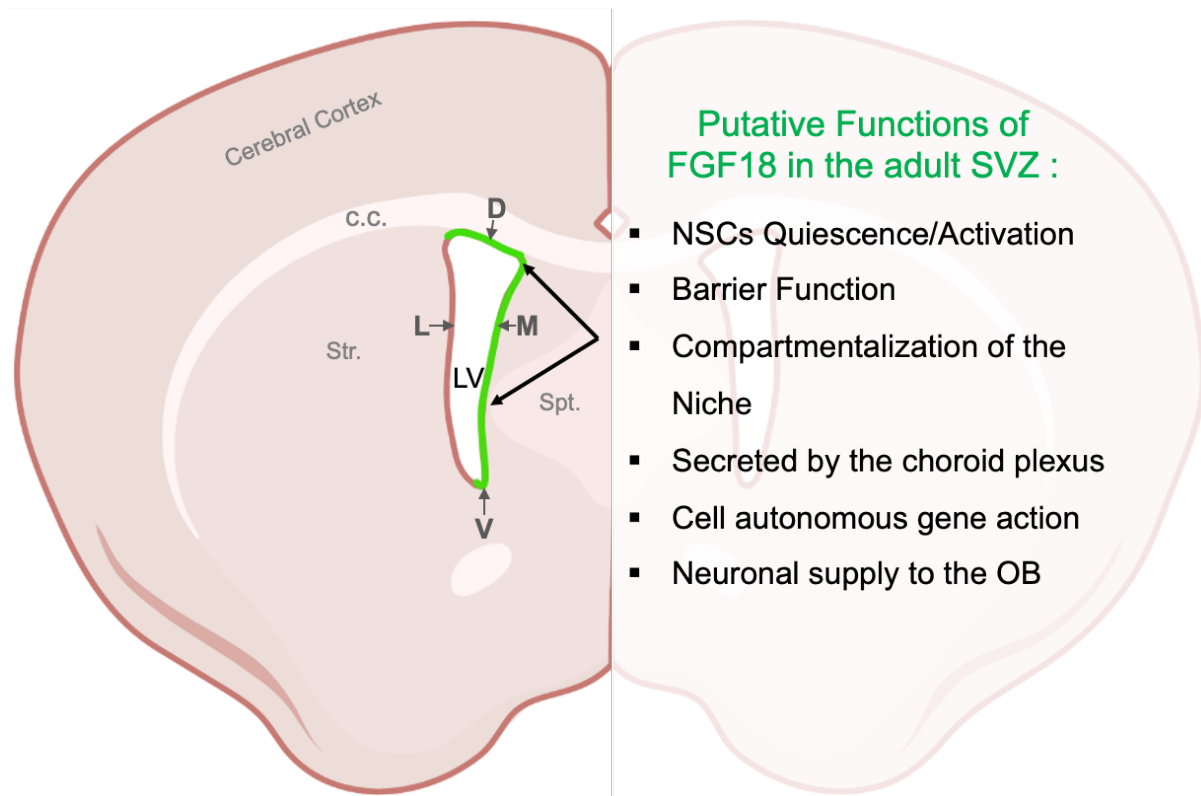


Figure 4–2. Putative roles of FGF18 in the adult SVZ.

According to the data presented in this study, FGF18 may signal through a FGFR (e.g., FGFR3c) to regulate the NSCs quiescence-to-activation process and then, contribute to the neuronal supply to the OB. The identification of FGF18⁺ cells in the ependymal layer of the V-SVZ niche suggests they may have a barrier function at the medial wall. FGF18⁺ cells were also observed in the choroid plexus suggesting that FGF18 could be secreted by the choroid plexus in the CSF and regulate the niche activity. Future studies will investigate the details of FGF18⁺ cells' roles in the adult SVZ niche. c.c., corpus callosum; D, dorsal wall; L, lateral wall; M, medial wall; V, ventral wall; Str., striatum; Spt., septum.

CONCLUSION

In summary, this study highlights an active participation of FGF18 in preserving the integrity of the postnatal V-SVZ niche and in regulating the process of neurogenesis it mediates. In particular, according to these data, FGF18 may contribute towards the NSCs quiescence-to-activation process and progenitors' activity, as well as exerting a barrier function at the ependymal layer. This dynamic participation of FGF18⁺ cells in the V-SVZ-mediated neurogenesis is also observed in the early postnatal mice brains. In light of these findings, it will be necessary to perform *in vivo* conditional FGF18 deletion studies, combined with gain-of-function studies using viral vectors and *in vitro* model culture to consolidate these hypotheses and determine the age-dependent changes in the FGF18 transcriptional program.

REFERENCES

- ALTMAN J. 1962. Are new neurons formed in the brains of adult mammals? *Science* 135: 1127-8
- Altman J. 1969. Autoradiographic and histological studies of postnatal neurogenesis. IV. Cell proliferation and migration in the anterior forebrain, with special reference to persisting neurogenesis in the olfactory bulb. *J Comp Neurol* 137: 433-57
- Altman J, Das GD. 1966. Autoradiographic and histological studies of postnatal neurogenesis. I. A longitudinal investigation of the kinetics, migration and transformation of cells incorporating tritiated thymidine in neonate rats, with special reference to postnatal neurogenesis in some brain regions. *J Comp Neurol* 126: 337-89
- Alvarez-Buylla A. 1997. Mechanism of migration of olfactory bulb interneurons. *Semin Cell Dev Biol* 8: 207-13
- Alvarez-Buylla A, Garcia-Verdugo JM. 2002. Neurogenesis in adult subventricular zone. *J Neurosci* 22: 629-34
- Arisi GM, Foresti ML, Mukherjee S, Shapiro LA. 2012. The role of olfactory stimulus in adult mammalian neurogenesis. *Behav Brain Res* 227: 356-62
- Astic L, Pellier-Monnin V, Saucier D, Charrier C, Mehlen P. 2002. Expression of netrin-1 and netrin-1 receptor, DCC, in the rat olfactory nerve pathway during development and axonal regeneration. *Neuroscience* 109: 643-56
- Barker N, Bartfeld S, Clevers H. 2010. Tissue-resident adult stem cell populations of rapidly self-renewing organs. *Cell Stem Cell* 7: 656-70
- Belov AA, Mohammadi M. 2013. Molecular mechanisms of fibroblast growth factor signaling in physiology and pathology. *Cold Spring Harb Perspect Biol* 5
- Bernier PJ, Bedard A, Vinet J, Levesque M, Parent A. 2002. Newly generated neurons in the amygdala and adjoining cortex of adult primates. *Proc Natl Acad Sci U S A* 99: 11464-9
- Betizeau M, Cortay V, Patti D, Pfister S, Gautier E, et al. 2013. Precursor diversity and complexity of lineage relationships in the outer subventricular zone of the primate. *Neuron* 80: 442-57
- Bjornsson CS, Apostolopoulou M, Tian Y, Temple S. 2015. It takes a village: constructing the neurogenic niche. *Dev Cell* 32: 435-46

- Bolteus AJ, Bordey A. 2004. GABA release and uptake regulate neuronal precursor migration in the postnatal subventricular zone. *J Neurosci* 24: 7623-31
- Bordiuk OL, Smith K, Morin PJ, Semënov MV. 2014. Cell proliferation and neurogenesis in adult mouse brain. *PLoS One* 9: e111453
- Borrell V, Cárdenas A, Ciceri G, Galcerán J, Flames N, et al. 2012. Slit/Robo signaling modulates the proliferation of central nervous system progenitors. *Neuron* 76: 338-52
- Cavallaro S, D'Agata V, Manickam P, Dufour F, Alkon DL. 2002. Memory-specific temporal profiles of gene expression in the hippocampus. *Proc Natl Acad Sci U S A* 99: 16279-84
- Cebrian-Silla A, Nascimento MA, Redmond SA, Mansky B, Wu D, et al. 2021. Single-cell analysis of the ventricular-subventricular zone reveals signatures of dorsal and ventral adult neurogenesis. *Elife* 10
- Cheng MF. 2013. Hypothalamic neurogenesis in the adult brain. *Front Neuroendocrinol* 34: 167-78
- Colangelo AM, Cirillo G, Alberghina L, Papa M, Westerhoff HV. 2019. Neural plasticity and adult neurogenesis: the deep biology perspective. *Neural Regen Res* 14: 201-05
- Colucci-D'Amato L, Bonavita V, di Porzio U. 2006. The end of the central dogma of neurobiology: stem cells and neurogenesis in adult CNS. *Neurol Sci* 27: 266-70
- Conover JC, Shook BA. 2011. AGING OF THE SUBVENTRICULAR ZONE NEURAL STEM CELL NICHE. *Aging Dis* 2: 149-63
- Crossley PH, Martin GR. 1995. The mouse Fgf8 gene encodes a family of polypeptides and is expressed in regions that direct outgrowth and patterning in the developing embryo. *Development* 121: 439-51
- Delgado AC, Maldonado-Soto AR, Silva-Vargas V, Mizrak D, von Känel T, et al. 2021. Release of stem cells from quiescence reveals gliogenic domains in the adult mouse brain. *Science* 372: 1205-09
- Dingli D, Traulsen A, Michor F. 2007. (A)symmetric stem cell replication and cancer. *PLoS Comput Biol* 3: e53
- Doetsch F, Caillé I, Lim DA, García-Verdugo JM, Alvarez-Buylla A. 1999a. Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. *Cell* 97: 703-16
- Doetsch F, García-Verdugo JM, Alvarez-Buylla A. 1997. Cellular composition and three-dimensional organization of the subventricular germinal zone in the adult mammalian brain. *J Neurosci* 17: 5046-61

- Doetsch F, García-Verdugo JM, Alvarez-Buylla A. 1999b. Regeneration of a germinal layer in the adult mammalian brain. *Proc Natl Acad Sci U S A* 96: 11619-24
- Douet V, Kerever A, Arikawa-Hirasawa E, Mercier F. 2013. Fractone-heparan sulphates mediate FGF-2 stimulation of cell proliferation in the adult subventricular zone. *Cell Prolif* 46: 137-45
- Dulken BW, Leeman DS, Boutet SC, Hebestreit K, Brunet A. 2017. Single-Cell Transcriptomic Analysis Defines Heterogeneity and Transcriptional Dynamics in the Adult Neural Stem Cell Lineage. *Cell Rep* 18: 777-90
- Ellsworth JL, Garcia R, Yu J, Kindy MS. 2004. Time window of fibroblast growth factor-18-mediated neuroprotection after occlusion of the middle cerebral artery in rats. *J Cereb Blood Flow Metab* 24: 114-23
- Falcão AM, Marques F, Novais A, Sousa N, Palha JA, Sousa JC. 2012. The path from the choroid plexus to the subventricular zone: go with the flow! *Front Cell Neurosci* 6: 34
- Ferraro F, Celso CL, Scadden D. 2010. Adult stem cells and their niches. *Adv Exp Med Biol* 695: 155-68
- Fiorelli R, Azim K, Fischer B, Raineteau O. 2015. Adding a spatial dimension to postnatal ventricular-subventricular zone neurogenesis. *Development* 142: 2109-20
- Franklin KB, Paxinos G. 2007. *The Mouse Brain in Stereotaxic Coordinates*. Elsevier.
- Fuentealba LC, Rompani SB, Parraguez JI, Obernier K, Romero R, et al. 2015. Embryonic Origin of Postnatal Neural Stem Cells. *Cell* 161: 1644-55
- Goldfarb M. 2005. Fibroblast growth factor homologous factors: evolution, structure, and function. *Cytokine Growth Factor Rev* 16: 215-20
- Gonzalez-Perez O. 2012. Neural stem cells in the adult human brain. *Biol Biomed Res* 2: 59-69
- Goodman T, Hajihosseini MK. 2015. Hypothalamic tanycytes-masters and servants of metabolic, neuroendocrine, and neurogenic functions. *Front Neurosci* 9: 387
- Gregorian C, Nakashima J, Le Belle J, Ohab J, Kim R, et al. 2009. Pten deletion in adult neural stem/progenitor cells enhances constitutive neurogenesis. *J Neurosci* 29: 1874-86
- Guirao B, Meunier A, Mortaud S, Aguilar A, Corsi JM, et al. 2010. Coupling between hydrodynamic forces and planar cell polarity orients mammalian motile cilia. *Nat Cell Biol* 12: 341-50
- Götz M, Barde YA. 2005. Radial glial cells defined and major intermediates between embryonic stem cells and CNS neurons. *Neuron* 46: 369-72

- Hack I, Bancila M, Loulier K, Carroll P, Cremer H. 2002. Reelin is a detachment signal in tangential chain-migration during postnatal neurogenesis. *Nat Neurosci* 5: 939-45
- Hagan AS, Boylan M, Smith C, Perez-Santamarina E, Kowalska K, et al. 2019. Generation and validation of novel conditional flox and inducible Cre alleles targeting fibroblast growth factor 18 (Fgf18). *Dev Dyn* 248: 882-93
- Hajihosseini MK, De Langhe S, Lana-Elola E, Morrison H, Sparshott N, et al. 2008. Localization and fate of Fgf10-expressing cells in the adult mouse brain implicate Fgf10 in control of neurogenesis. *Mol Cell Neurosci* 37: 857-68
- Hajihosseini MK, Heath JK. 2002. Expression patterns of fibroblast growth factors-18 and -20 in mouse embryos is suggestive of novel roles in calvarial and limb development. *Mech Dev* 113: 79-83
- Hasegawa H, Ashigaki S, Takamatsu M, Suzuki-Migishima R, Ohbayashi N, et al. 2004. Laminar patterning in the developing neocortex by temporally coordinated fibroblast growth factor signaling. *J Neurosci* 24: 8711-9
- Haughey NJ, Liu D, Nath A, Borchard AC, Mattson MP. 2002. Disruption of neurogenesis in the subventricular zone of adult mice, and in human cortical neuronal precursor cells in culture, by amyloid beta-peptide: implications for the pathogenesis of Alzheimer's disease. *Neuromolecular Med* 1: 125-35
- Hong SN, Dunn JC, Stelzner M, Martín MG. 2017. Concise Review: The Potential Use of Intestinal Stem Cells to Treat Patients with Intestinal Failure. *Stem Cells Transl Med* 6: 666-76
- Hormoz S. 2013. Stem cell population asymmetry can reduce rate of replicative aging. *J Theor Biol* 331: 19-27
- Hoshikawa M, Yonamine A, Konishi M, Itoh N. 2002. FGF-18 is a neuron-derived glial cell growth factor expressed in the rat brain during early postnatal development. *Brain Res Mol Brain Res* 105: 60-6
- Hung IH, Schoenwolf GC, Lewandoski M, Ornitz DM. 2016. A combined series of Fgf9 and Fgf18 mutant alleles identifies unique and redundant roles in skeletal development. *Dev Biol* 411: 72-84
- Ito K, Ohkawara B, Yagi H, Nakashima H, Tsushima M, et al. 2018. Lack of Fgf18 causes abnormal clustering of motor nerve terminals at the neuromuscular junction with reduced acetylcholine receptor clusters. *Sci Rep* 8: 434

- Jankovski A, Garcia C, Soriano E, Sotelo C. 1998. Proliferation, migration and differentiation of neuronal progenitor cells in the adult mouse subventricular zone surgically separated from its olfactory bulb. *Eur J Neurosci* 10: 3853-68
- Jin K, Sun Y, Xie L, Batteur S, Mao XO, et al. 2003. Neurogenesis and aging: FGF-2 and HB-EGF restore neurogenesis in hippocampus and subventricular zone of aged mice. *Aging Cell* 2: 175-83
- Johanson C, McMillan P, Tavares R, Spangenberg A, Duncan J, et al. 2004. Homeostatic capabilities of the choroid plexus epithelium in Alzheimer's disease. *Cerebrospinal Fluid Res* 1: 3
- Johansson MW. 1999. Cell adhesion molecules in invertebrate immunity. *Dev Comp Immunol* 23: 303-15
- Kaneko N, Marín O, Koike M, Hirota Y, Uchiyama Y, et al. 2010. New neurons clear the path of astrocytic processes for their rapid migration in the adult brain. *Neuron* 67: 213-23
- Kaneko N, Sawada M, Sawamoto K. 2017. Mechanisms of neuronal migration in the adult brain. *J Neurochem* 141: 835-47
- Kaplan MS, Hinds JW. 1977. Neurogenesis in the adult rat: electron microscopic analysis of light radioautographs. *Science* 197: 1092-4
- Karabagli H, Karabagli P, Ladher RK, Schoenwolf GC. 2002. Comparison of the expression patterns of several fibroblast growth factors during chick gastrulation and neurulation. *Anat Embryol (Berl)* 205: 365-70
- Karpowicz P, Willaime-Morawek S, Balenci L, DeVeale B, Inoue T, van der Kooy D. 2009. E-Cadherin regulates neural stem cell self-renewal. *J Neurosci* 29: 3885-96
- Katoh M. 2005. Comparative genomics on FGF8, FGF17, and FGF18 orthologs. *Int J Mol Med* 16: 493-6
- Kempermann G, Song H, Gage FH. 2015. Neurogenesis in the Adult Hippocampus. *Cold Spring Harb Perspect Biol* 7: a018812
- Kim Y, Wang WZ, Comte I, Pastrana E, Tran PB, et al. 2010. Dopamine stimulation of postnatal murine subventricular zone neurogenesis via the D3 receptor. *J Neurochem* 114: 750-60
- Klezovitch O, Fernandez TE, Tapscott SJ, Vasioukhin V. 2004. Loss of cell polarity causes severe brain dysplasia in Lgl1 knockout mice. *Genes Dev* 18: 559-71
- Kriegstein A, Alvarez-Buylla A. 2009. The glial nature of embryonic and adult neural stem cells. *Annu Rev Neurosci* 32: 149-84

- Leeman DS, Hebestreit K, Ruetz T, Webb AE, McKay A, et al. 2018. Lysosome activation clears aggregates and enhances quiescent neural stem cell activation during aging. *Science* 359: 1277-83
- Lenington JB, Yang Z, Conover JC. 2003. Neural stem cells and the regulation of adult neurogenesis. *Reprod Biol Endocrinol* 1: 99
- Libbrecht S, Van den Haute C, Welkenhuysen M, Braeken D, Haesler S, Baekelandt V. 2021. Chronic chemogenetic stimulation of the anterior olfactory nucleus reduces newborn neuron survival in the adult mouse olfactory bulb. *J Neurochem* 158: 1186-98
- Lim DA, Alvarez-Buylla A. 2016. The Adult Ventricular-Subventricular Zone (V-SVZ) and Olfactory Bulb (OB) Neurogenesis. *Cold Spring Harb Perspect Biol* 8
- Lim DA, Tramontin AD, Trevejo JM, Herrera DG, García-Verdugo JM, Alvarez-Buylla A. 2000. Noggin antagonizes BMP signaling to create a niche for adult neurogenesis. *Neuron* 28: 713-26
- Liu A, Li JY, Bromleigh C, Lao Z, Niswander LA, Joyner AL. 2003. FGF17b and FGF18 have different midbrain regulatory properties from FGF8b or activated FGF receptors. *Development* 130: 6175-85
- Liu X, Wang Q, Haydar TF, Bordey A. 2005. Nonsynaptic GABA signaling in postnatal subventricular zone controls proliferation of GFAP-expressing progenitors. *Nat Neurosci* 8: 1179-87
- Liu Z, Xu J, Colvin JS, Ornitz DM. 2002. Coordination of chondrogenesis and osteogenesis by fibroblast growth factor 18. *Genes Dev* 16: 859-69
- Lois C, García-Verdugo JM, Alvarez-Buylla A. 1996. Chain migration of neuronal precursors. *Science* 271: 978-81
- Luo J, Daniels SB, Lenington JB, Notti RQ, Conover JC. 2006. The aging neurogenic subventricular zone. *Aging Cell* 5: 139-52
- Luo J, Shook BA, Daniels SB, Conover JC. 2008. Subventricular zone-mediated ependyma repair in the adult mammalian brain. *J Neurosci* 28: 3804-13
- Luzzati F, De Marchis S, Fasolo A, Peretto P. 2006. Neurogenesis in the caudate nucleus of the adult rabbit. *J Neurosci* 26: 609-21
- Maddaluno L, Urwyler C, Werner S. 2017. Fibroblast growth factors: key players in regeneration and tissue repair. *Development* 144: 4047-60
- Martinez-Molina N, Kim Y, Hockberger P, Szele FG. 2011. Rostral migratory stream neuroblasts turn and change directions in stereotypic patterns. *Cell Adh Migr* 5: 83-95

- Maruoka Y, Ohbayashi N, Hoshikawa M, Itoh N, Hogan BL, Furuta Y. 1998. Comparison of the expression of three highly related genes, Fgf8, Fgf17 and Fgf18, in the mouse embryo. *Mech Dev* 74: 175-7
- Mercier F. 2016. Fractones: extracellular matrix niche controlling stem cell fate and growth factor activity in the brain in health and disease. *Cell Mol Life Sci* 73: 4661-74
- Merkle FT, Tramontin AD, García-Verdugo JM, Alvarez-Buylla A. 2004. Radial glia give rise to adult neural stem cells in the subventricular zone. *Proc Natl Acad Sci U S A* 101: 17528-32
- Mirzadeh Z, Merkle FT, Soriano-Navarro M, Garcia-Verdugo JM, Alvarez-Buylla A. 2008. Neural stem cells confer unique pinwheel architecture to the ventricular surface in neurogenic regions of the adult brain. *Cell Stem Cell* 3: 265-78
- Nait-Oumesmar B, Decker L, Lachapelle F, Avellana-Adalid V, Bachelin C, Baron-Van Evercooren A. 1999. Progenitor cells of the adult mouse subventricular zone proliferate, migrate and differentiate into oligodendrocytes after demyelination. *Eur J Neurosci* 11: 4357-66
- Ng KL, Li JD, Cheng MY, Leslie FM, Lee AG, Zhou QY. 2005. Dependence of olfactory bulb neurogenesis on prokineticin 2 signaling. *Science* 308: 1923-7
- Obernier K, Alvarez-Buylla A. 2019. Neural stem cells: origin, heterogeneity and regulation in the adult mammalian brain. *Development* 146
- Obernier K, Cebrian-Silla A, Thomson M, Parraguez JI, Anderson R, et al. 2018. Adult Neurogenesis Is Sustained by Symmetric Self-Renewal and Differentiation. *Cell Stem Cell* 22: 221-34.e8
- Ohbayashi N, Hoshikawa M, Kimura S, Yamasaki M, Fukui S, Itoh N. 1998. Structure and expression of the mRNA encoding a novel fibroblast growth factor, FGF-18. *J Biol Chem* 273: 18161-4
- Ohbayashi N, Shibayama M, Kurotaki Y, Imanishi M, Fujimori T, et al. 2002. FGF18 is required for normal cell proliferation and differentiation during osteogenesis and chondrogenesis. *Genes Dev* 16: 870-9
- Ohuchi H, Kimura S, Watamoto M, Itoh N. 2000. Involvement of fibroblast growth factor (FGF)18-FGF8 signaling in specification of left-right asymmetry and brain and limb development of the chick embryo. *Mech Dev* 95: 55-66
- Olsen SK, Li JY, Bromleigh C, Eliseenkova AV, Ibrahimi OA, et al. 2006. Structural basis by which alternative splicing modulates the organizer activity of FGF8 in the brain. *Genes Dev* 20: 185-98

- Ono K, Tomasiewicz H, Magnuson T, Rutishauser U. 1994. N-CAM mutation inhibits tangential neuronal migration and is phenocopied by enzymatic removal of polysialic acid. *Neuron* 13: 595-609
- Ornitz DM. 2000. FGFs, heparan sulfate and FGFRs: complex interactions essential for development. *Bioessays* 22: 108-12
- Ornitz DM, Itoh N. 2015. The Fibroblast Growth Factor signaling pathway. *Wiley Interdiscip Rev Dev Biol* 4: 215-66
- Ornitz DM, Itoh N. 2022. New developments in the biology of fibroblast growth factors. *WIREs Mech Dis*: e1549
- Ornitz DM, Xu J, Colvin JS, McEwen DG, MacArthur CA, et al. 1996. Receptor specificity of the fibroblast growth factor family. *J Biol Chem* 271: 15292-7
- Owen-Lynch PJ, Draper CE, Mashayekhi F, Bannister CM, Miyan JA. 2003. Defective cell cycle control underlies abnormal cortical development in the hydrocephalic Texas rat. *Brain* 126: 623-31
- Paez-Gonzalez P, Asrican B, Rodriguez E, Kuo CT. 2014. Identification of distinct ChAT⁺ neurons and activity-dependent control of postnatal SVZ neurogenesis. *Nat Neurosci* 17: 934-42
- Panayiotou E, Malas S. 2013. Adult spinal cord ependymal layer: a promising pool of quiescent stem cells to treat spinal cord injury. *Front Physiol* 4: 340
- Panzanelli P, Bardy C, Nissant A, Pallotto M, Sassoè-Pognetto M, et al. 2009. Early synapse formation in developing interneurons of the adult olfactory bulb. *J Neurosci* 29: 15039-52
- Paton JA, Nottebohm FN. 1984. Neurons generated in the adult brain are recruited into functional circuits. *Science* 225: 1046-8
- Petreaanu L, Alvarez-Buylla A. 2002. Maturation and death of adult-born olfactory bulb granule neurons: role of olfaction. *J Neurosci* 22: 6106-13
- Platel JC, Bordey A. 2016. The multifaceted subventricular zone astrocyte: From a metabolic and pro-neurogenic role to acting as a neural stem cell. *Neuroscience* 323: 20-8
- Ponti G, Farinetti A, Marraudino M, Panzica G, Gotti S. 2018. Sex Steroids and Adult Neurogenesis in the Ventricular-Subventricular Zone. *Front Endocrinol (Lausanne)* 9: 156
- Ponti G, Obernier K, Guinto C, Jose L, Bonfanti L, Alvarez-Buylla A. 2013. Cell cycle and lineage progression of neural progenitors in the ventricular-subventricular zones of adult mice. *Proc Natl Acad Sci U S A* 110: E1045-54

- Porlan E, Perez-Villalba A, Delgado AC, Ferrón SR. 2013. Paracrine regulation of neural stem cells in the subependymal zone. *Arch Biochem Biophys* 534: 11-9
- Raponi E, Agenes F, Delphin C, Assard N, Baudier J, et al. 2007. S100B expression defines a state in which GFAP-expressing cells lose their neural stem cell potential and acquire a more mature developmental stage. *Glia* 55: 165-77
- Reynolds BA, Weiss S. 1992. Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science* 255: 1707-10
- Ryu JR, Hong CJ, Kim JY, Kim EK, Sun W, Yu SW. 2016. Control of adult neurogenesis by programmed cell death in the mammalian brain. *Mol Brain* 9: 43
- Sahara S, O'Leary DD. 2009. Fgf10 regulates transition period of cortical stem cell differentiation to radial glia controlling generation of neurons and basal progenitors. *Neuron* 63: 48-62
- Sailor KA, Ming GL, Song H. 2006. Neurogenesis as a potential therapeutic strategy for neurodegenerative diseases. *Expert Opin Biol Ther* 6: 879-90
- Savchenko E, Teku GN, Boza-Serrano A, Russ K, Berns M, et al. 2019. FGF family members differentially regulate maturation and proliferation of stem cell-derived astrocytes. *Sci Rep* 9: 9610
- Sawamoto K, Wichterle H, Gonzalez-Perez O, Cholfín JA, Yamada M, et al. 2006. New neurons follow the flow of cerebrospinal fluid in the adult brain. *Science* 311: 629-32
- Shah PT, Stratton JA, Stykel MG, Abbasi S, Sharma S, et al. 2018. Single-Cell Transcriptomics and Fate Mapping of Ependymal Cells Reveals an Absence of Neural Stem Cell Function. *Cell* 173: 1045-57.e9
- Shahriyari L, Komarova NL. 2013. Symmetric vs. asymmetric stem cell divisions: an adaptation against cancer? *PLoS One* 8: e76195
- Shingo T, Gregg C, Enwere E, Fujikawa H, Hassam R, et al. 2003. Pregnancy-stimulated neurogenesis in the adult female forebrain mediated by prolactin. *Science* 299: 117-20
- Stahnisch FW, Nitsch R. 2002. Santiago Ramón y Cajal's concept of neuronal plasticity: the ambiguity lives on. *Trends Neurosci* 25: 589-91
- Stratton JA, Shah P, Sinha S, Crowther E, Biernaskie J. 2019. A tale of two cousins: Ependymal cells, quiescent neural stem cells and potential mechanisms driving their functional divergence. *FEBS J* 286: 3110-16
- Tan X, Shi SH. 2013. Neocortical neurogenesis and neuronal migration. *Wiley Interdiscip Rev Dev Biol* 2: 443-59

- Thomas LB, Gates MA, Steindler DA. 1996. Young neurons from the adult subependymal zone proliferate and migrate along an astrocyte, extracellular matrix-rich pathway. *Glia* 17: 1-14
- Tong CK, Han YG, Shah JK, Obernier K, Guinto CD, Alvarez-Buylla A. 2014. Primary cilia are required in a unique subpopulation of neural progenitors. *Proc Natl Acad Sci U S A* 111: 12438-43
- Turner N, Grose R. 2010. Fibroblast growth factor signalling: from development to cancer. *Nat Rev Cancer* 10: 116-29
- Urbán N, Cheung TH. 2021. Stem cell quiescence: the challenging path to activation. *Development* 148
- Usui H, Shibayama M, Ohbayashi N, Konishi M, Takada S, Itoh N. 2004. Fgf18 is required for embryonic lung alveolar development. *Biochem Biophys Res Commun* 322: 887-92
- Watson J, Francavilla C. 2018. Regulation of FGF10 Signaling in Development and Disease. *Front Genet* 9: 500
- Wichterle H, Garcia-Verdugo JM, Alvarez-Buylla A. 1997. Direct evidence for homotypic, glia-independent neuronal migration. *Neuron* 18: 779-91
- Woodbury ME, Ikezu T. 2014. Fibroblast growth factor-2 signaling in neurogenesis and neurodegeneration. *J Neuroimmune Pharmacol* 9: 92-101
- Yamada T, Kerever A, Yoshimura Y, Suzuki Y, Nonaka R, et al. 2017. Heparan sulfate alterations in extracellular matrix structures and fibroblast growth factor-2 signaling impairment in the aged neurogenic niche. *J Neurochem* 142: 534-44
- Yang R, Liu F, Wang J, Chen X, Xie J, Xiong K. 2019. Epidermal stem cells in wound healing and their clinical applications. *Stem Cell Res Ther* 10: 229
- Yoshimura S, Takagi Y, Harada J, Teramoto T, Thomas SS, et al. 2001. FGF-2 regulation of neurogenesis in adult hippocampus after brain injury. *Proc Natl Acad Sci U S A* 98: 5874-9
- Young CC, van der Harg JM, Lewis NJ, Brooks KJ, Buchan AM, Szele FG. 2013. Ependymal ciliary dysfunction and reactive astrogliosis in a reorganized subventricular zone after stroke. *Cereb Cortex* 23: 647-59
- Young KM, Mitsumori T, Pringle N, Grist M, Kessaris N, Richardson WD. 2010. An Fgfr3-iCreER(T2) transgenic mouse line for studies of neural stem cells and astrocytes. *Glia* 58: 943-53

Yun YR, Won JE, Jeon E, Lee S, Kang W, et al. 2010. Fibroblast growth factors: biology, function, and application for tissue regeneration. *J Tissue Eng* 2010: 218142

Zhang X, Ibrahimi OA, Olsen SK, Umemori H, Mohammadi M, Ornitz DM. 2006. Receptor specificity of the fibroblast growth factor family. The complete mammalian FGF family. *J Biol Chem* 281: 15694-700

APPENDIX

Table S1. List of the FGF18-CreERT2::Rosa26-tdTomato mice used. Adult mice and Litter mice were subject to the first tamoxifen treatment at postnatal-day-90 (P90) and P4, respectively. Brains n° 927 and n° #2 were used as controls in this study.

Brain n°	FGF18-CreERT2 allele	Mode of Sectioning	Age	Tamoxifen Regime (days)	Chase system
800	Positive	Vibratome	Adult	2 d. gavage + 12 d.	Long
801	Positive	Vibratome	Adult	2 d. gavage + 12 d.	Long
804	Positive	Vibratome	Adult	2 d. gavage + 2 d.	Short
815	Positive	Vibratome	Adult	2 d. gavage + 2 d.	Short
816	Positive	Vibratome	Adult	2 d. gavage + 2 d.	Short
919	Positive	Vibratome	Adult	2 d. gavage + 12 d.	Long
922	Positive	Vibratome	Adult	2 d. gavage + 12 d.	Long
924	Positive	Vibratome	Adult	2 d. gavage + 12 d.	Long
925	Positive	Vibratome (sagittal)	Adult	2 d. gavage + 12 d.	Long
1066	Positive	Cryostat	Adult	2 d. gavage + 12 d.	Long
1073	Positive	Cryostat	Adult	2 d. gavage + 12 d.	Long
927	Negative	Vibratome	Adult	No Tamoxifen pulse	N/A
#2	Negative	Vibratome	Litter	No Tamoxifen pulse	N/A
2	Positive	Vibratome	Litter	2 d. gavage + 15 d.	Long
3	Positive	Vibratome	Litter	2 d. gavage + 2 d.	Short
4	Positive	Vibratome	Litter	2 d. gavage + 2 d.	Short
7	Positive	Vibratome	Litter	2 d. gavage + 12 d.	Long
8	Positive	Vibratome	Litter	2 d. gavage+12 d.	Long

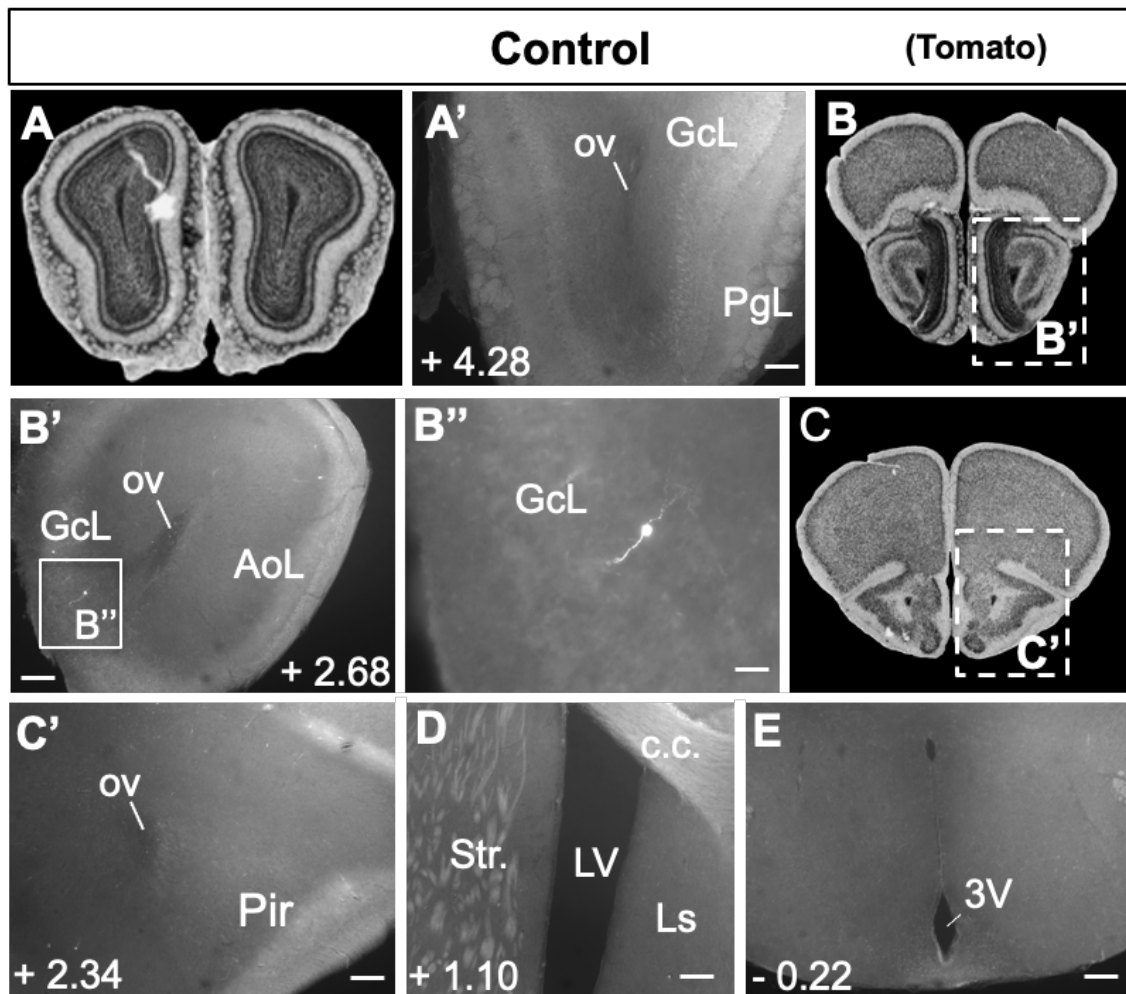


Figure S1. Tomato expression is not detected in untreated *Fgf18^{Lineage}* mice. Sections from non-tamoxifen pulsed *Fgf18^{Lineage}* mice ($n=2$) brains revealed the absence of Tomato expression in several regions of the adult brain: the anterior OB domain (A'); the posterior OB domain (B', B''); the RMS (C'); the V-SVZ (D); the hypothalamus (E). Isolated Tomato⁺ cells were rarely observed (B''). Atlas images are from the Mouse Brain Library (http://www.mbl.org/mbl_main/atlas.html). Approximate Bregma coordinates in the bottom left corners. Scale bars = 50 μ m. AoL, lateral anterior olfactory nucleus; c.c., corpus callosum; GL, periglomerular layer; Gr, granule layer; Ls, lateral septum; LV, lateral ventricle; ov., olfactory ventricle; Pir., piriform cortex; Str., striatum; 3V, Third ventricle

Table S2. Tomato⁺ cell number in the OBs of adult Short Chase mice.

Vibratome-generated sections of 60 µm thickness from adult short chase OBs (n=3) were used. To guarantee sata accuracy and reliability, the sections analysed (n=5 per brain) were chosen based on sections' availability and correspondence with specific Bregma coordinates. Average number of cells per brain is referred as mean± SD (3±3 per brain). A= anterior OB; P= posterior OB.

Adult Short Chase OBs					
Brain n°	Section n°	Tomato ⁺ cell number in the OB	Bregma Coordinates	Mean	Standard Deviation
804	1	0	Anterior OB (B. +4.28; +3.92; +3.56)	1	2
	2	1			
	3	0			
	4	1	Posterior OB (B. +3.20; +2.96)		
	5	3			
815	1	0	Anterior OB (B. +4.28; +3.92; +3.56)	5	1
	2	2			
	3	0			
	4	12	Posterior OB (B. +3.20; +2.96)		
	5	9			
816	1	1	Anterior OB (B. +4.28; +3.92; +3.56)	3	6
	2	2			
	3	3			
	4	1	Posterior OB (B. +3.20; +2.96)		
	5	6			

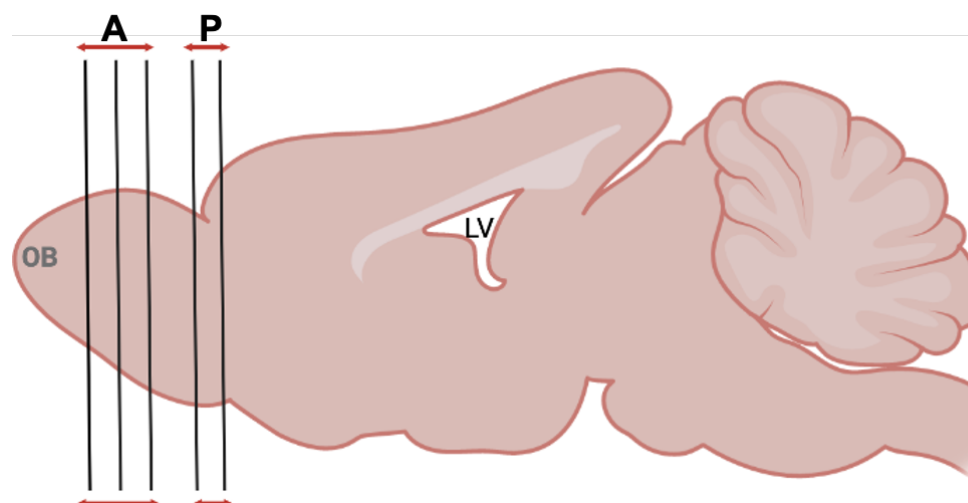


Table S3. Tomato⁺ cell number in the OBs of adult Long Chase mice.

Vibratome-generated sections of 60 μ m thickness from adult long chase OBs (n=3) were used. To guarantee data accuracy and reliability, the sections analysed (n=5 per brain) were chosen according to sections' availability and correspondence with specific Bregma coordinates. Average number of cells per brain is referred as mean $\pm$ SD (46 $\pm$ 20 per brain). A= anterior OB; P= posterior OB.

Adult Long Chase OBs					
Brain n°	Section n°	Tomato ⁺ cell number in the OB	Bregma Coordinates	Mean	Standard Deviation
924	1	38	Anterior OB (B. +4.28; +3.92; +3.56)	100	45
	2	100			
	3	86			
	4	118	Posterior OB (B. +3.20; +2.96)		
	5	162			
919	1	18	Anterior OB (B. +4.28; +3.92; +3.56)	24	8
	2	26			
	3	33			
	4	14	Posterior OB (B. +3.20; +2.96)		
	5	32			
800	1	10	Anterior OB (B. +4.28; +3.92; +3.56)	13	7
	2	10			
	3	6			
	4	22	Posterior OB (B. +3.20; +2.96)		
	5	21			

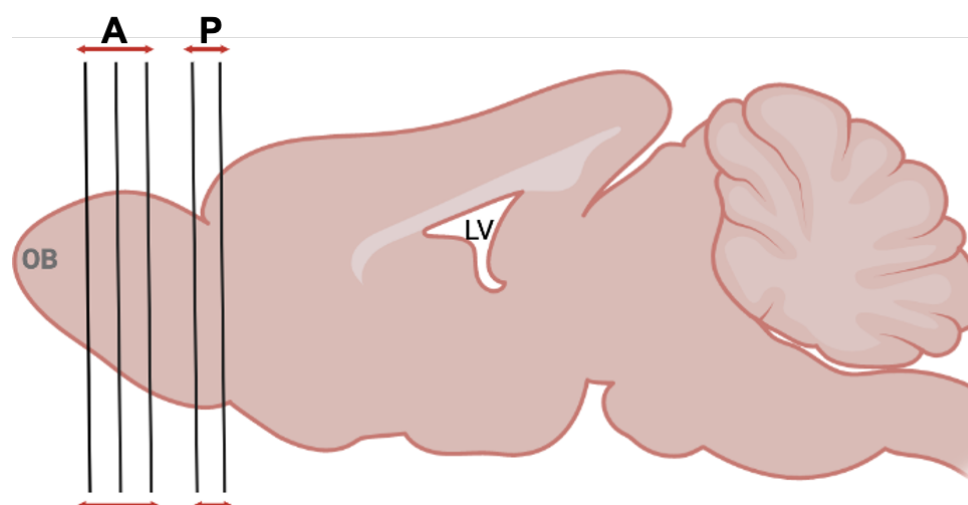
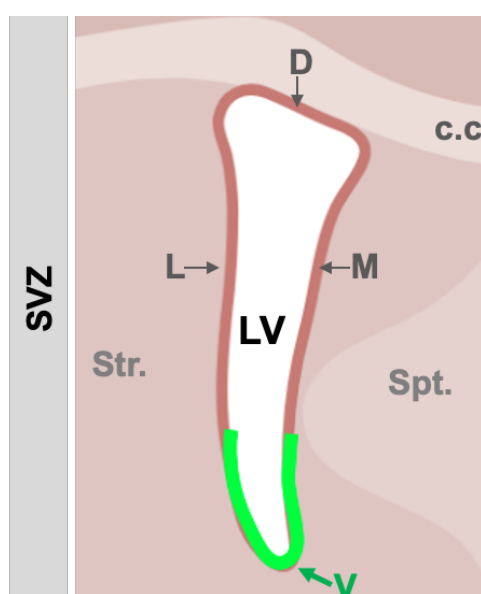


Table S4. Tomato⁺ radial glia-like cells in the ventral SVZ of adult long chase and young long chase mice. Vibratome-generated sections of 60 µm thickness from adult long chase SVZ(n=3) and young long chase SVZ (n=3) were used. To guarantee the accuracy and consistency of the dataset, the sections analysed (*n*=4 per brain) were chosen from each brain according to sections' availability and Bregma correspondence. Average number of cells per brain is referred as mean± SD (Adult = 3±3 per brain; Young = 11±7).SD= Standard Deviation; Ventral SVZ in green.

Adult Long Chase					Young Long Chase				
Brain n°	Section n°	Tom ⁺ cell number	Mean	SD	Brain n°	Section n°	Tom ⁺ cell number	Mean	SD
919	1	0	1	1	2	1	5	7	4
	2	1				2	8		
	3	1				3	11		
	4	1				4	3		
922	1	2	2	2	7	1	22	14	9
	2	0				2	6		
	3	4				3	22		
	4	0				4	6		
924	1	2	3	3	8	1	10	12	8
	2	4				2	10		
	3	0				3	5		
	4	6				4	24		



ACKNOWLEDGMENTS

I would like to express my gratitude to my supervisor Dr Mohammad Hajihosseini without whom this project would have not been possible. His support and guidance have been fundamental in every stage of this study while his encouragement and the opportunities he has offered me have certainly contributed towards my growth as a scientist.

I would also like to thank Dr Mette Mogensen for the insightful comments given and Dr Tianqi Li for her help with the transgenic mouse line treatment and maintenance. Moreover, thank you to the previous members of the lab for the open discussions and chats between breaks.

On a personal note, I would like to thank all my friends, particularly the ones I have met in Norwich, for always pushing me to do better and for being good listeners every time I start overthinking. Each one of you has made my time in Norwich an unforgettable experience. A special thank you goes to Mona and Clare for the many nights spent together but most importantly, for the support and reassurance they have given me during the many lockdowns we have faced and throughout this whole year away from home. Without even knowing, you have been fundamental in every step of this long journey and I am glad that I have met you.

I cannot conclude this thesis without thanking my family for their support throughout my studies, and for always believing in me and encouraging me. This achievement could have not been possible without you and I am grateful to always have you by my side.