Université de Montréal

Mechanisms underlying activation of neural stem cells in the adult central nervous system

par
Catherine-Alexandra Grégoire

Département de pathologie et biologie cellulaire
Faculté de médecine

Thèse présentée à la Faculté de médecine
en vue de l'obtention du grade de Philosophiae Doctor (Ph.D)
en Pathologie et biologie cellulaire
option système nerveux

Avril, 2016

Université de Montréal

Cette thèse intitulée:

Mechanisms underlying activation of neural stem cells in the adult central nervous system

Présentée par

Catherine-Alexandra Grégoire

a été évaluée par un jury composé des personnes suivantes :

Dre. Nicole Leclerc, Présidente-rapporteure

Dr. Karl Fernandes, Directeur de recherche

Dr. Michel Cayouette, Membre du jury

Dre. Diane Lagace, Examinatrice externe

Dre. Naglaa Shoukry, Représentante du doyen

Résumé

À la fin du 19e siècle, Dr. Ramón y Cajal, un pionnier scientifique, a découvert les éléments cellulaires individuels, appelés neurones, composant le système nerveux. Il a également remarqué la complexité de ce système et a mentionné l'impossibilité de ces nouveaux neurones à être intégrés dans le système nerveux adulte. Une de ses citations reconnues : "Dans les centres adultes, les chemins nerveux sont fixes, terminés, immuables. Tout doit mourir, rien ne peut être régénérer" est représentative du dogme de l'époque (Ramón y Cajal 1928). D'importantes études effectuées dans les années 1960-1970 suggèrent un point de vue différent. Il a été démontré que les nouveaux neurones peuvent être générés à l'âge adulte, mais cette découverte a créé un scepticisme omniprésent au sein de la communauté scientifique. Il a fallu 30 ans pour que le concept de neurogenèse adulte soit largement accepté. Cette découverte, en plus de nombreuses avancées techniques, a ouvert la porte à de nouvelles cibles thérapeutiques potentielles pour les maladies neurodégénératives. Les cellules souches neurales (CSNs) adultes résident principalement dans deux niches du cerveau : la zone sous-ventriculaire des ventricules latéraux et le gyrus dentelé de l'hippocampe. En condition physiologique, le niveau de neurogenèse est relativement élevé dans la zone sous-ventriculaire contrairement à l'hippocampe où certaines étapes sont limitantes. En revanche, la moelle épinière est plutôt définie comme un environnement en quiescence.

Une des principales questions qui a été soulevée suite à ces découvertes est : comment peut-on activer les CSNs adultes afin d'augmenter les niveaux de neurogenèse ? Dans l'hippocampe, la capacité de l'environnement enrichi (incluant la stimulation cognitive, l'exercice et les interactions sociales) à promouvoir la neurogenèse hippocampale a déjà été démontrée. La plasticité de cette région est importante, car elle peut jouer un rôle clé dans la récupération de déficits au niveau de la mémoire et l'apprentissage. Dans la moelle épinière, des études effectuées *in vitro* ont démontré que les cellules épendymaires situées autour du canal central ont des capacités d'auto-renouvellement et de multipotence (neurones, astrocytes, oligodendrocytes). Il est intéressant de noter qu'*in vivo*, suite à une lésion de la moelle épinière, les cellules épendymaires sont activées, peuvent s'auto-renouveller, mais peuvent seulement

donner naissance à des cellules de type gliale (astrocytes et oligodendrocytes). Cette nouvelle fonction post-lésion démontre que la plasticité est encore possible dans un environnement en quiescence et peut être exploité afin de développer des stratégies de réparation endogènes dans la moelle épinière.

Les CSNs adultes jouent un rôle important dans le maintien des fonctions physiologiques du cerveau sain et dans la réparation neuronale suite à une lésion. Cependant, il y a peu de données sur les mécanismes qui permettent l'activation des CSNs en quiescence permettant de maintenir ces fonctions. L'objectif général est d'élucider les mécanismes sous-jacents à l'activation des CSNs dans le système nerveux central adulte. Pour répondre à cet objectif, nous avons mis en place deux approches complémentaires chez les souris adultes : 1) L'activation des CSNs hippocampales par l'environnement enrichi (EE) et 2) l'activation des CSNs de la moelle épinière par la neuroinflammation suite à une lésion. De plus, 3) afin d'obtenir plus d'information sur les mécanismes moléculaires de ces modèles, nous utiliserons des approches transcriptomiques afin d'ouvrir de nouvelles perspectives.

Le premier projet consiste à établir de nouveaux mécanismes cellulaires et moléculaires à travers lesquels l'environnement enrichi module la plasticité du cerveau adulte. Nous avons tout d'abord évalué la contribution de chacune des composantes de l'environnement enrichi à la neurogenèse hippocampale (Chapitre II). L'exercice volontaire promeut la neurogenèse, tandis que le contexte social augmente l'activation neuronale. Par la suite, nous avons déterminé l'effet de ces composantes sur les performances comportementales et sur le transcriptome à l'aide d'un labyrinthe radial à huit bras afin d'évaluer la mémoire spatiale et un test de reconnaissante d'objets nouveaux ainsi qu'un RNA-Seq, respectivement (Chapitre III). Les coureurs ont démontré une mémoire spatiale de rappel à court-terme plus forte, tandis que les souris exposées aux interactions sociales ont eu une plus grande flexibilité cognitive à abandonner leurs anciens souvenirs. Étonnamment, l'analyse du RNA-Seq a permis d'identifier des différences claires dans l'expression des transcripts entre les coureurs de courte et longue distance, en plus des souris sociales (dans l'environnement complexe).

Le second projet consiste à découvrir comment les cellules épendymaires acquièrent les propriétés des CSNs *in vitro* ou la multipotence suite aux lésions *in vivo* (Chapitre IV). Une analyse du RNA-Seq a révélé que le transforming growth factor-β1 (TGF-β1) agit comme un régulateur, en amont des changements significatifs suite à une lésion de la moelle épinière. Nous avons alors confirmé la présence de cette cytokine suite à la lésion et caractérisé son rôle sur la prolifération, différentiation, et survie des cellules initiatrices de neurosphères de la moelle épinière. Nos résultats suggèrent que TGF-β1 régule l'acquisition et l'expression des propriétés de cellules souches sur les cellules épendymaires provenant de la moelle épinière.

Mots-clés : Cellules souches neurales, neurogenèse adulte, hippocampe, moelle épinière, environnement enrichi, exercice, comportement, neuroinflammation, transforming growth factor-β1, cellules initiatrices de neurosphères.

Abstract

At the end of the 19th century, Dr. Ramón y Cajal, a scientific pioneer, discovered that the nervous system was composed of individual cellular elements, later called neurons. He also noticed the complexity of this system and mentioned the impossibility of new neurons to be integrated into the adult nervous system. One of his famous quotes: "In adult centers the nerve paths are something fixed, ended, immutable. Everything may die, nothing may be regenerated" is representative of the prevalent dogma at the time (Ramón y Cajal 1928). Key studies conducted in the 1960-1970s suggested a different point of view. It was demonstrated that new neurons could be born during adulthood, but this discovery created an omnipresent skepticism in the scientific community. It took 30 years for the concept of adult neurogenesis to become widely accepted. This discovery, along with more advanced techniques, opened doors to potential therapeutic avenues for neurodegenerative diseases. Adult neural stem cells (NSCs) reside mainly in two niches in the brain: the subventricular zone of the lateral ventricles and the dentate gyrus of the hippocampus. Under normal conditions, neurogenesis level is relatively high in the SVZ whereas some steps are rate-limiting in the hippocampus. In contrast, the spinal cord is rather defined as a quiescent environment.

One of the main questions that arose from these discoveries is: how do you activate adult NSCs in order to increase neurogenesis levels? In the hippocampus, environmental enrichment (including cognitive stimulation, exercise and social interactions) has been shown to promote hippocampal neurogenesis. The plasticity potential of this region is important as it could play a crucial role in rescuing learning and memory deficits. In the spinal cord, studies conducted *in vitro* demonstrated that ependymal cells found around the central canal have self-renewal and multipotency capacities (neurons, astrocytes, oligodendrocytes). Interestingly, it turns out that *in vivo*, following a spinal cord lesion, ependymal cells become activated, can self-replicate, but can only give rise to glia cell fate (astrocytes and oligodendrocytes). This new post-injury function shows that plasticity can still occur in a quiescent environment and could be exploited to develop endogenous spinal cord repair strategies.

As mentioned above, NSCs play important roles in normal brain function and neural repair following injury. However, little information is known about how a quiescent NSC becomes activated in order to perform these functions. The general objective of this project was to investigate the mechanisms underlying activation of neural stem cells in the adult central nervous system. My specific aims were to address this question using adult mice in two complementary models: 1) activation of hippocampal NSCs by environmental enrichment, and 2) activation of spinal cord NSCs by injury-induced neuroinflammation. Moreover, 3) to gain new insights into the molecular mechanisms of these models, we will perform transcriptomics studies to open new lines of investigation.

The first project is expected to provide us with new insights into the basic cellular and molecular mechanisms through which environmental enrichment modulates adult brain plasticity. We first evaluated the contribution of individual environmental enrichment components to hippocampal neurogenesis (Chapter II). Voluntary exercise promotes neurogenesis, whereas a social context increases neuronal activation. We then determined the effect of these components on behavioural performances and transcriptome using an eight-arm radial maze to assess spatial memory, novel object recognition, and RNA-Seq, respectively (Chapter III). Runners show stronger spatial short-term memory recall, whereas mice exposed to social interactions had a better cognitive flexibility to abandon old memory. Surprisingly, RNA-Seq analysis indicated clear differences in the expression of modified transcripts between low runners and high runners, as well as for social interacting mice (within the complex environment).

The second project consists of discovering how ependymal cells acquire NSC properties *in vitro* or multipotentiality following lesions *in vivo*. A RNA-Seq analysis revealed that the transforming growth factor-β1 (TGF-β1) acts as an upstream regulator of significant changes following spinal cord injury (Chapter IV). We therefore confirmed the presence of this cytokine after lesion and investigated its role on proliferation, differentiation, and survival of neurosphere-initiating cells from the spinal cord. Our results suggest that TGF-β1 regulates the acquisition and expression of stem cell properties of spinal cord-derived ependymal cells.

Keywords: Neural stem cells, adult neurogenesis, hippocampus, spinal cord, environmental enrichment, exercise, behaviour, neuroinflammation, transforming growth factor-β1, neurosphere-initiating cells.

Table of contents

List of tables

List of figures

List of acronyms and abbreviations

AP: Alkaline phosphatase

APC: Antigen presenting cell

Ara-C: Cytosine-β-D-arabinofuranoside

BDNF: Brain-derived neurotrophic factor

BMP: Bone morphogenetic protein

BNST: Bed nucleus of the stria terminals

BrdU: Bromodeoxyuridine

CA: Cornu ammonis

CE: Complex environment

CNS: Central nervous system

CPP: Conditioned place preference

CSF: Cerebrospinal fluid

DCX: Doublecortin

DG: Dentate gyrus

DNMP: Delayed nonmatching to place

EE: Environmental enrichment

EGF: Epidermal growth factor

eNSC: Embryonic neural stem cell

EZ: Ependymal zone

FACS: Fluorescence-activated cell sorting

FGF-2: Fibroblast growth factor-2

GABA: γ-aminobutyric acid

GCL: Granular cell layer

GFAP: Glial fibrillary acidic protein

GLAST: Glutamate transporter

HPP: Hippocampus

ICM: Inner cell mass

IEG: Immediate early gene

IGF1: Insulin-like growth factor-1

IL-1β: Interleukin-1beta

IL-6: Interleukin-6

IPC: Intermediate progenitor cell

KCC2: K+-Cl- co-transporter

LIF: Leukemia inhibitor factor

LPS: Lipopolysaccharide

MAM: Methylazoxymethanol acetate

MAPK: Mitogen-activated protein kinase

ML: Molecular layer

mPFC: Medial prefrontal cortex

MTL: Medial temporal lobe

MWM: Morris water maze

NIC: Neurosphere-initiating cells

NKCC1: Na+-K+-2Cl- co-transporter

NOR: Novel object recognition

NPY: Neuropeptide Y

NSC: Neural stem cell

nACC: Nucleus accumbens

OB: Olfactory bulb

OCT4: Octamer-binding transcription factor 4

PV: Parvalbumin

RAM: Radial arm maze

RGC: Radial glial cell

RGL: Radial glia-like cell

RMS: Rostral migratory stream

RSP: Retrosplenial area¸

SCI: Spinal cord injury

SEZ: Subependymal zone

SGZ: Subgranular zone

SHH: Sonic hedgehog

SST: Somatostatin

SVZ: Subventricular zone

TGF-β1: Transforming growth factor-beta 1

TK: Thymidine kinase

TNF-α: Tumor necrosis factor- alpha

VEGF: Vascular endothelial growth factor

VTA: Ventral tegmental area

Dedication

I would like to dedicate my thesis dissertation

To my family,

in appreciation of their unconditional support throughout the years

To my friends,

without whom I wouldn't have gone this far

And to the love of my life,

who was there for me during the most challenging stretch.

Catherine-Alexandra Grégoire

Acknowledgments

This was quite a journey! Six year and a half went by since I have started as an intern in the Fernandes laboratory back in January 2010. I am taking the opportunity to thank my Ph.D supervisor, Dr. Karl Fernandes, for giving me the chance to be part of his laboratory and for providing useful advice throughout the years. I also want to say a special thanks to my committee members, Dr. Diane Lagace, Dr. Graziella DiCristo, Dr. Lionel Carmant, and Dr. Laurent Descarries for their insight and guidance, as well as my thesis committee members Dr. Nicole Leclerc, Dr. Michel Cayouette, and Dr. Naglaa Shoukry.

In May 2010, I decided to pursue my project to complete a two-year master's degree, which transformed into a six-year Ph.D degree by taking advantage of the fast-tracking process from M.Sc to Ph.D. I was glad to continue the environmental enrichment project that was started from scratch by a great scientist, Matthew Bednarczyk, who unfortunately past away in late 2014 (RIP). I keep wonderful memories from my first two years in the laboratory as I was surrounded by two amazing people who created a cheerful environment where it was a pleasure to come in to work: Stef Beaudoin and Greg Paliouras you will never be forgotten! I also had the chance to work with our wonderful technician, Anne Aumont, who is always there for the students and who created an organized lab easy to work in. I will always remember all the stories we shared when we commuted together on the train.

In late 2013, my motivation went down as I found myself at a status quo. I wanted to extend my knowledge and potential with new techniques, which was unfortunately not possible with my current project. I am grateful to my colleague, Laura Hamilton, for encouraging me at the time to continue and keep up the good work. In parallel, Karl was there to address my concerns and we finally agreed on a solution of adding the spinal cord project to my thesis, as no one was currently working on it at that time. In early 2014, I therefore had the chance to start performing *in vitro* experiments in our new lab at the CRCHUM. It was quite challenging to have 2 major branches for a total of 4 projects over the years, but I can say that I am proud of

my accomplishments considering all the challenges I had to face in both my professional and personal lives over these six years and a half.

I would also like to thank my colleagues Loïc Cochard (AKA Lolo), Chuck Levros (AKA Chuck maybe), and Brianna Goldenstein (AKA Bri) for their friendship and support, especially in the most challenging years that were these last two. Furthermore, the CRCHUM environment gave the opportunity to students to hang out more together and wonderful friendships emerged. Sacha (Lissouba), Vi, Arnaud, Maxime, Marc-André, Kessen, Camille (UdeM), Yoko, Nico, Jorge, Juliette, and Lamia, I will always remember our animated discussions and nights out having fun. To my friends outside the lab who kept me going by their admiration for my work and for always being honest and supportive, I will always be thankful to you for being my dearest friends for so many years: Sam, Cyn, Pré, the "Winnerslicious", Rémi, and Shirley. To those I forgot to mention, please be tolerant, I know that you are also important, but one side effect of writing this dissertation is amnesia (not to be taken seriously).

Finally, the last but not the least, my family and my boyfriend, for their unconditional support for everything I have undertaken. Maman, papa et Charles, je vous serai à jamais reconnaissante pour tout ce dont vous m'avez donné et appris. Ludo, tu es une source d'inspiration et de motivation. Je vous aime !

Chapter I – Introduction

I.1. General overview of nervous system development

The central nervous system (CNS) is composed of the brain and the spinal cord. Understanding how these structures developed is important in order to study their similarities and differences in how they regulate stem cells. The goal of this section is to briefly introduce a few concepts such as the role of embryonic cells forming the CNS, the stem cells and their importance in potential regeneration.

I.1.1. Common origin of the brain and spinal cord

Mammalian development starts from a fertilized egg, known as a zygote. This zygote goes through multiple cleavages: a 16-cell morula stage, a 32-cell stage, and a 64-cell blastocyst stage, composed of two clear layers (Gilbert 2006). The first layer of the blastocyst consists of external cells named the trophoblast that will form the embryonic portion of the placenta. The second layer is made of cells found within the trophoblast and is defined as the inner cell mass (ICM). The ICM is known to be pluripotent because it can create the entire embryo and related structures, except the trophoblast (Gage 2000; Gilbert 2006). The ICM gives rise to the hypoblast and the epiblast. These two tissues eventually develop into three well-defined layers: the endoderm, mesoderm and ectoderm in a well-defined structure called a gastrula following gastrulation (Gilbert 2006).

During later embryonic development, the ectoderm differentiates in three components: the epidermis, neural crest, and neural tube (Gilbert 2006). The neural tube will be described in greater detail as it forms the brain and spinal cord, the two regions of interest in this dissertation. Approximately 50% of the ectoderm is predetermined to become neural ectoderm, or the neural plate (Gilbert 2006). The remaining cells are thought to become the epidermis of the skin, mainly due to bone morphogenetic protein (BMP) signalling (Kandel et al. 2000). The neural plate will become the neural tube by a two-step process known as neurulation. Primary neurulation consists mainly of proliferating cells that form neural folds and invagination to create the neural groove (Gilbert 2006). Eventually, the folds will fuse to form the anterior

portion of the neural tube. Secondary neurulation is the creation of the posterior portion of the neural tube by merging mesenchyme cells, which are multipotent stromal cells (Gilbert 2006).

I.1.2. Regional specification of the neural tube

The anterior portion of the neural tube starts differentiating before the secondary neurulation finishes to form five main regions of the brain (Gilbert 2006; Kandel et al. 2000) (**Fig.I.1**). Three primary brain vesicles are created: the prosencephalon (forebrain (1)), mesencephalon (midbrain (2)), and rhomencephalon (hindbrain (3)). The prosencephalon is subdivided into the telencephalon ((1a) transforming into the cerebral hemispheres, including the olfactory bulb and hippocampal regions) and the diencephalon ((1b) transforming into the optic vesicles, thalamic, and hypothalamic regions). The mesencephalon will become the cerebral aqueduct, which contains the cerebrospinal fluid (CSF). Finally, the rhomencephalon subdivides into the metencephalon ((3a) transforming into the cerebellum and pons) and the myelencephalon ((3b) transforming into the medulla). It is from this caudal region of the neural tube, corresponding to the sixth major region of the CNS, which forms the spinal cord (Gilbert 2006; Kandel et al. 2000).

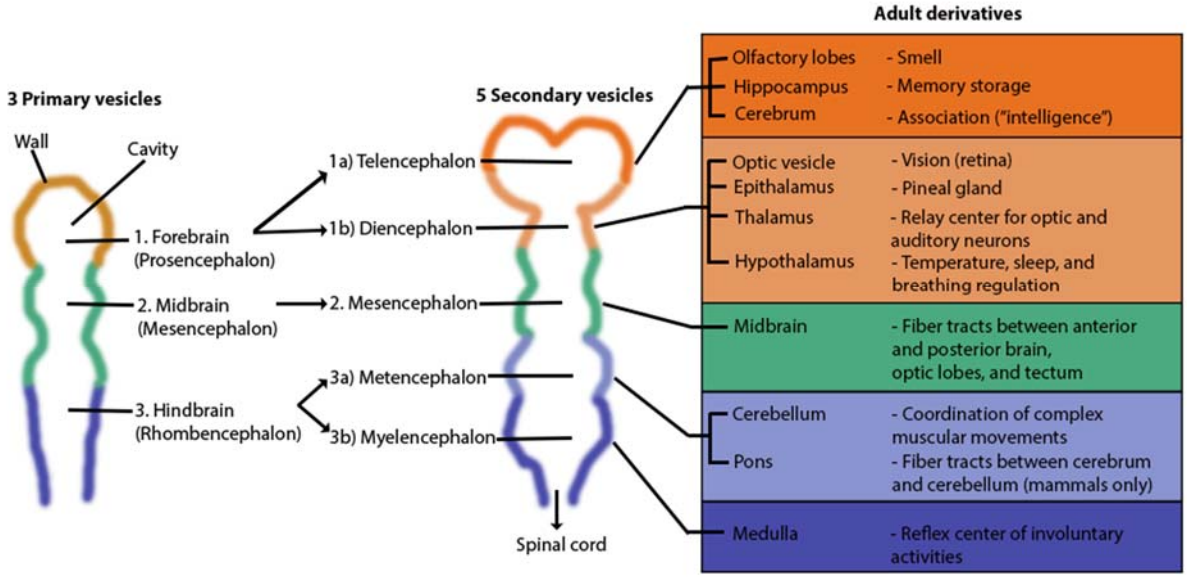


Figure I.1. Early human brain development

During development, the neural tube first divides into three primary vesicles (1. Prosencephalon (Forebrain), 2. Mesencephalon (Midbrain), and 3. Rhombencephalon (Hindbrain), then into five secondary vesicles (1a. Telencephalon, 1b. Diencephalon, 3a. Metencephalon, 3b. Myelencephalon, and 2. Mesencephalon stays the same). Each region has their adult derivative with specific functions. (Inspired from (Gilbert 2006), Fig. 12.9, and created by C-A. Grégoire)

A pattern is also established by a posterior-anterior gradient of retinoid acid that leads to the expression of *Hox* genes in the posterior region. These genes are responsible for the formation of the hindbrain and spinal cord (Gilbert 2006; Kempermann 2011). Other gradients are also present in the brain during development, including Pax6 (higher anterior pole concentrations) and Emx2 (higher posterior pole concentrations). These opposing gradients are important for positional identity (Bishop et al. 2002; Kempermann 2011).

I.1.3. Embryonic neural stem cells

The brain and spinal cord develop from differentiation of the neural tube. This process starts by the presence of embryonic neural stem cells (eNSCs) in the blastocyst.

I.1.3.1. Definition of neural stem cell

The eNSCs, present in the ICM have the capacity to differentiate into any types of tissues, including CNS tissue. They are therefore defined as pluripotent (Kempermann 2011). This pluripotency comes from the expression of several genes such as *Oct4*, *Nanog,* and *sox2*. The eNSCs located in the germinal neuroepithelium first become neuroepithelial precursor cells, then radial glia or neural stem cells (NSCs). NSCs can self-renew and divide asymmetrically to give rise to an intermediate progeny that can generate any tissue of the CNS (Gage 2000; Kempermann 2011).

I.1.3.2. Cell expansion

Cell expansion involves many cell divisions and thickening of the neural tube (Gilbert 2006; Kempermann 2011; Kriegstein and Alvarez-Buylla 2009). Nuclei of neuroepithelial cells are found at different heights in the lumen of the neural tube during development. During the S phase of the cell cycle, the nuclei move from the apical surface towards the pial surface of the neural tube, but return to the apical surface to perform mitosis. This phenomenon is called interkinetic nuclear migration. Neuroepithelial cells are first dividing symmetrically. Then, once the epithelium has thickened, neuroepithelial cells will elongate to become radial glial cells (RGCs, **Fig. I.2**). At this moment, RGCs start expressing astrocytic markers such as the glutamate transporter (GLAST), brain lipid-binding protein, Tenascin C, and in some species, glial fibrillary acidic protein (GFAP). RGCs divide asymmetrically to give rise to other RGCs or intermediate progenitor cells (IPCs). IPCs will then divide symmetrically to form identical daughter cells (Gilbert 2006; Kempermann 2011; Kriegstein and Alvarez-Buylla 2009).

I.1.3.3. Neurogenesis

Once cells are ready to form neurons, cell-division plane changes and one of the two daughter cells will detach, migrate, and differentiate (Gilbert 2006). IPCs migrate from the subventricular zone (SVZ) to the ventricular zone, a region next to the germinal neuroepithelium. There, they will form a second layer called the mantle or intermediate zone. In this layer, they differentiate into neurons and glia. This region is defined as the gray matter since it is where cell bodies reside. The neurons project axons away from the ventricular zone to create the marginal zone. This zone contains few cells but has many myelinated axons and therefore comprises the white matter (Gilbert 2006). Cajal-Retzius cells secreting reelin, a stop-and-go signal for migration, are found in this zone (Feng et al. 2007; Sanes et al. 2012). These three main regions: ventricular, intermediate, and marginal, are well retained in the spinal cord, whereas they are reorganized in the cerebral cortex. A new layer, called the neocortex, is formed at the outer side of the brain located between the marginal and intermediate zones. This cortical plate will eventually form the six layers of the cerebral cortex following an inside-out pattern.

Migrating neurons (i.e., neuroblasts) will use a radial glia cell process as a scaffold to migrate up the layers of cells (Gilbert 2006) (**Fig. I.2**).

I.1.3.4. Gliogenesis

At the end of cortical development, RGCs are no longer needed for scaffolding migration. Therefore, they detach from the ventricle, lose their radial process, and adapt an astrocytic morphology (Kriegstein and Alvarez-Buylla 2009). These astrocytes will keep their regenerative capacities in specific regions of the brain (**Fig. I.2**). For a long time, scientists believed regeneration was impossible in the adult brain. However, some of the adult mammalian brain and spinal cord cells maintain regenerative, stem cell properties (see section below).

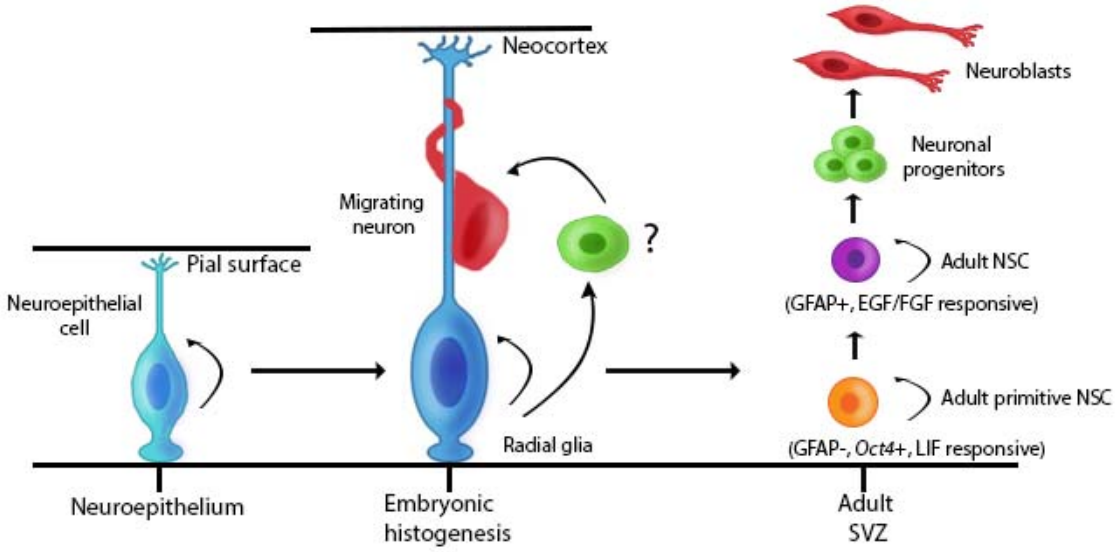


Figure I.2. Evolution of neuroepithelial cells

During development, neuroepithelial cells divide symmetrically and will elongate to become radial glia cells. The cells then behave as scaffolders for migrating neurons reaching the

neocortex, and divide asymmetrically. In adults, a rare population of primitive neural stem cells (GFAP- and leukemia inhibitor factor (LIF) responsive) give rise to GFAP+ neural stem cells. GFAP+ neural stem cells self-renew and give rise to neural progenitors that will mature into neuroblasts. (SVZ: Subventricular zone. Inspired from (Alvarez-Buylla et al. 2001) and (Sachewsky et al. 2014), and created by C-A. Grégoire and Loïc Cochard)

I.2. Adult neural stem cells

Adult NSCs are multipotent cells able to self-renew in the nervous system (Gage 2000). They are also referred to as primary progenitor cells or precursors. In specific regions of the brain, they are able to divide asymmetrically to give rise to three different types of cells in the following order: neurons (the functional component of the nervous system), astrocytes and oligodendrocytes (known as glia cells) (Kriegstein and Alvarez-Buylla 2009). *In vitro*, this multipotency is maintained in spinal cord-derived cultures (Weiss et al. 1996), but is lost *in vivo* as only glia-restricted progeny are produced (Horner et al. 2000; Martens et al. 2002). The regions of the hippocampus and spinal cord will be discussed in greater detail but first, we will review the origin and microenvironment of NSCs located within the hippocampus and spinal cord.

I.2.1. Origin of adult NSCs

Recent studies have investigated the origin of the adult NSCs present in the SVZ, dentate gyrus (DG), and spinal cord (Fuentealba et al. 2015; Li et al. 2013; Yu et al. 2013). One study used *in utero* delivery of retroviruses and bromodeoxyuridine (BrdU, a thymidine analogue that incorporates into DNA during DNA synthesis) to show that the majority of pre-B1 cells present in the SVZ were produced by embryonic progenitors dividing between E13.5 and E15.5 (Fuentealba et al. 2015). A lineage-tracing method with a barcoded retroviral library was used to determine the clonal relationships among cells. This experiment showed that there is a link between progenitors producing olfactory bulb (OB) interneurons after birth and those producing other neurons in the forebrain. However, this relationship eventually disappears in mid-fetal development, suggesting a lineage divergence (Fuentealba et al. 2015). The adult NSCs of the DG were demonstrated to originate from the ventral hippocampus (HPP) (Li et al. 2013). This region is known to be a source of sonic hedgehog (SHH), which is important for the maintenance of the adult NSC population. A continuous stream consisting of Gli1-responsive cells (SHH effector) was observed from the ventricular zone of the ventral HPP to the ventral granular cell layer (GCL) of the DG. They concluded, using several genetic approaches, that embryonic NSCs from the ventral HPP contributed to create the postnatal subgranular zone (SGZ)

throughout the longitudinal axis of the HPP (Li et al. 2013). Another study, demonstrated the necessity of SHH for postnatal ependymal zone (EZ) cell formation (Yu et al. 2013). During late development, it was shown that adult ependymal cells, found around the central canal, are derived from two distinct progenitor domains: p2 and pMN from the ventral ventricular zone. They also observed a severe disruption in the formation of the EZ when embryonic SHH signaling was absent (Yu et al. 2013).

I.2.2. Adult neural stem cell niche

The NSC niche is a microenvironment composed of multiple cell types (such as astrocytes and ependymal cells), secreted molecules, extracellular matrix, and blood vessels (endothelial cells). The NSCs are therefore exposed to a variety of signals and cell-cell interactions (Riquelme et al. 2008). In adults, only specific regions maintain the capacity to produce new neurons, hence suggesting that adult NSC niches are environments supporting self-renewal and multipotency through signaling. However, the right balance between proliferation and differentiation signals is a priority to avoid tumor development or exhaustion of the NSC pool. Moreover, vasculature is an important factor as angiogenesis may have an impact on neurogenesis via hormones and cytokines (Riquelme et al. 2008). For example, vascular endothelial growth factor (VEGF) is responsible for angiogenesis and has been shown to influence neurogenesis, especially stimulating progenitor's proliferation (Jin et al. 2002). Furthermore, transplantation experiments showed the importance of external cues present in the microenvironment on stem cell fate. The spinal cord is defined as a non-neurogenic region, but fibroblast growth factor-2 (FGF-2)-responsive adult spinal cord-derived cells differentiated into neurons when transplanted and integrated into the GCL of the DG (Shihabuddin et al. 2000). Another experiment isolated neuronal precursors (predisposed to become interneurons) from the SVZ via magnetic activated cell sorting and transplanted them into the striatum (Seidenfaden et al. 2006). However, once implanted into the SVZ niche, these cells underwent glial differentiation. This example, once again, demonstrates the importance of the niche.

I.2.3. Brain

Adult neurogenesis is the postnatal formation by precursor cells of mature neurons (Kempermann et al. 2004; Ming and Song 2005). It was believed for a long time that neurogenesis in mammals was a development-restricted phenomenon. However, in the mammalian brain, adult neurogenesis occurs in a few areas including two main regions: the SVZ of the lateral ventricles and the DG of the HPP (Ihrie and Alvarez-Buylla 2011; Kempermann et al. 1998; Ming and Song 2005). Research conducted in 1965 shed light on the existence of postnatal neurogenesis, specifically in the DG of the HPP. Altman and Das first injected thymidine-H3 in rats to label cell nuclei where DNA was synthesized in order to identify proliferating cells (Altman and Das 1965). In this study, they demonstrated that the number of cells undergoing proliferation decreases with age (up to 8 month-old), while the number of differentiated cells (corresponding to granule cells) increases (Altman and Das 1965). In 1977, newly formed neurons were also labeled with thymidine-H3 and analyzed by electron microscopy, following a 30-day chase period in both the rodent DG and OB (Kaplan and Hinds 1977). This research showed the survival capacity of these newly-born neurons. Moreover, important research conducted in songbirds gave additional validity to the concept of adult neurogenesis in other vertebrates (Alvarez-Buylla and Kirn 1997; Alvarez-Buylla et al. 1988; Goldman and Nottebohm 1983). Unfortunately, widespread skepticism about adult neurogenesis delayed more scientific inquiries into its existence. Nonetheless, additional studies in most vertebrates and mammals confirmed the presence of stem cells in the SVZ and DG *in vivo,* and led to their isolation (in rodents) *in vitro* (Gage 2000; Lindsey and Tropepe 2006; Palmer et al. 1997; Reynolds and Weiss 1992). Finally, experiments using post-mortem brains from patients who received intravenous injections of BrdU showed that adult neurogenesis also occurs in the adult human brain (Curtis et al. 2007; Eriksson et al. 1998). Recent studies used stable ^{14}C present in genomic DNA and compared it to ^{14}C in the atmosphere (from nuclear bomb tests during the Cold War) to determine cell population date of birth (Ernst and Frisen 2015; Spalding et al. 2005). Using this method, they noticed that the age of human OB neurons was almost the same as the tested individuals, suggesting low turnover levels below 1% in this region. However, the role of OB between rodents and humans may differ (more details in section I.3.1.2) (Bergmann et al. 2012). Nonetheless, this technique allowed the discovery that

hippocampal neurogenesis occurs throughout life in humans at a rate similar to middle-aged mice (Spalding et al. 2013). These discoveries increased interest in adult neural stem cells as they could be perceived as potential therapeutic tools for neurodegenerative diseases and injuries.

I.2.4. Subventricular zone of the lateral ventricles

The SVZ, the main neurogenic niche, is comprised of several cell types. Ependymal cells, known as type E cells, are present along the lateral ventricle (next to the SVZ) forming a pinwheel (Mirzadeh et al. 2008). E cells are responsible for the production and circulation of a small amount of cerebrospinal fluid (Abrous et al. 2005; Doetsch et al. 1999a; Mirzadeh et al. 2008). Slowly dividing (quiescent) astrocytes, defined as type B1 cells, are present at the center of the ependymal pinwheels. B cells extend a basal process to the blood vessels and an apical cilium towards the cerebrospinal fluid (Kriegstein and Alvarez-Buylla 2009; Mirzadeh et al. 2008) (**Fig. I.3**). The B1 cells can be subdivided into activated and quiescent NSCs (Codega et al. 2014; Lim and Alvarez-Buylla 2016). Codega and colleagues isolated quiescent (GFAP+/CD133+) and activated (GFAP+/CD133+/EGFR+/Nestin+) NSC populations. After *in vivo* transplantation, both cells were neurogenic but behaved with different kinetics (quiescent NSCs (qNSCs) showing delayed kinetics). qNSCs rarely gave rise to neurospheres, in contrast to activated NSCs (aNSCs) that were enriched in colony-forming cells. Importantly, qNSCs could become activated in cultures with expression of Nestin and EGFR, suggesting that qNSCs and aNSCs can intervert between both states (Codega et al. 2014). Regeneration experiments conducted by Doetsch and colleagues in CD1 mice helped to first identify the NSCs in the SVZ niche. Cytosine-β-D-arabinofuranoside (Ara-C) was infused into the lateral ventricle via an osmotic pump for six days to eliminate dividing cells. At the end of the infusion, they observed the disappearance of all transit-amplifying cells (C cells) and proliferating neuroblasts (A cells, **Fig. I.3**), which are clustered migrating cells that go through the rostral migratory stream (RMS) to reach the OB and mature into interneurons. Only B cells and ependymal cells remained, suggesting they are potential NSCs (Doetsch et al. 1999b). This group further showed that only the astrocytes were labeled with a proliferating marker after Ara-C treatment, suggesting these cells, and not ependymal cells, repopulate the SVZ niche. After 36 hours of Ara-C treatment

cessation, C cells reappeared. Four days after cessation, A cells were also observed. Additionally, retrovirus and *in vitro* cultures experiments both confirmed that A and C cells were derived from B cells, and not from ependymal cells (Doetsch et al. 1999a). From these findings, B ependymal cells were defined as NSCs in the SVZ. Recently, Sachewsky and colleagues showed the existence of a rare population, called adult primitive NSCs (Sachewsky et al. 2014). These primitive cells are activated by the leukemia inhibitor factor (LIF) and demonstrated stem cell properties as they express octomer-binding transcription factor 4 (Oct4) and integrate into the ICM of blastocysts. In presence of LIF, these cells generated self-renewing and multipotent neurosphere colonies that gave rise to GFAP+ NSCs *in vitro*. Moreover, following *in vivo* depletion of GFAP+ NSCs, Oct4+ NSCs were able to repopulate the SVZ, suggesting that primitive NSCs are found upstream of GFAP+ NSCs (**Fig. I.2**) (Reeve et al. 2016; Sachewsky et al. 2014).

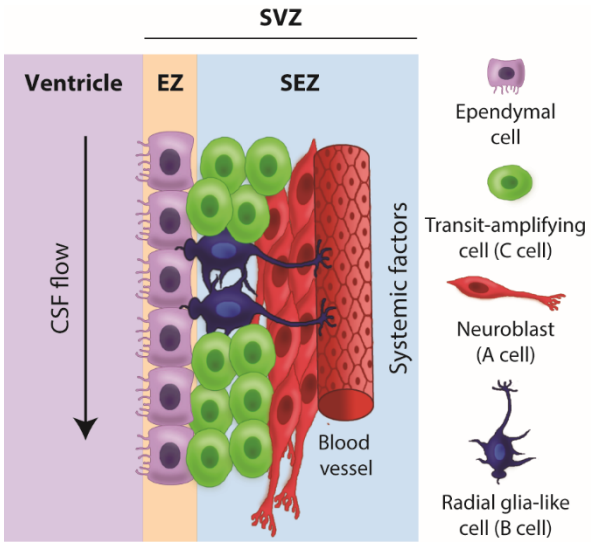


Figure I.3. Subventricular zone neurogenesis lineage

This figure represents the subventricular zone niche comprised of the ependymal and subependymal zones. Ependymal cells contact the cerebrospinal fluid while they line the ventricle. Activated B cells (radial glia-like cells), are in contact with blood vessels and divide asymmetrically to self-renew and give rise to C cells (transit-amplifying cells). C cells divide quickly, mature into neuroblasts, and migrate through the rostral migratory stream to the

olfactory bulbs. (CSF: Cerebrospinal fluid, EZ: ependymal zone, SEZ: Subependymal zone, SVZ: Subventricular zone. Inspired by (Bond et al. 2015), Created by C-A. Grégoire and Loïc Cochard).

I.2.5. Subgranular zone of the dentate gyrus

Adult neurogenesis also occurs in the SGZ of the DG, located in the HPP (Gage 2000). The DG is comprised of three layers: the molecular layer, the GCL, and the polymorphic cell also known as the hilus (**Fig. I.4**) (Anderson et al. 2007). In this niche, the cell lineage starts with slowly-dividing radial-glia like cells (RGL), known as type 1, whose endfeet touch the vasculature. These cells express GFAP, Nestin, and Sox2, a SRY transcription factor. They extend their radial process through the GCL to reach the molecular layer (Bonaguidi et al. 2012; Kempermann et al. 2015b; Seri et al. 2004). RGLs divide slowly in an asymmetric manner to produce intermediate progenitor cells (type 2a and 2b, or D cells). A proportion of these progenitors correspond to the Sox2+/Ki67+ proliferating cell population lacking radial processes, described as non-radial precursors (Suh et al. 2007). Most of these progenitors also express Tbr2 (a transcription factor) and are committed to a neuronal fate (Hodge et al. 2008). These progenitors divide quickly to give rise to neuroblasts (type 3), and express markers such as doublecortin (DCX, a microtubule-associated protein), and NeuroD (a basic helix-loop-helix transcription factor). Immature neurons will then become post-mitotic, mature into glutamatergic granule cells, and functionally integrate the circuitry (Bonaguidi et al. 2012; Kriegstein and Alvarez-Buylla 2009; van Praag et al. 2002) (**Fig. I.4**). These newly-born neurons form functional synapses with CA3 pyramidal cells at two weeks, which stabilize at four weeks (Gu et al. 2012). Only a small portion of these neurons become integrated as the majority of adult generated neurons die within the first four days after birth (Sierra et al. 2010). In order to identify the NSCs of the DG, Seri and colleagues used thymidine-H3 and GFAP labeling to look at the ultrastructure of proliferating cells (Seri et al. 2001). GFAP+ cells, described as B cells (or type 1 cells) in the SVZ, have an astrocytic morphology in contrast to GFAP-negative cells (rapidly dividing progenitors, SVZ D cells or type 2 cells) (Seri et al. 2001). Similarly to the Doetsch experiments (Doetsch et al. 1999a; Doetsch et al. 1999b), Seri and colleagues infused Ara-C and gave procarbazol (anti-mitotic drug) in drinking water for

seven days to eliminate proliferating cells. Two days following treatment, 91% of the cells left in the SGZ were type B cells. On day four, progenitor cells reappeared, and their numbers reached control levels by day 15 following treatment. They also introduced an avian leucosis virus including an alkaline phosphatase (AP) gene to label proliferating infecting cells and track their progeny. The maturation of GFAP+/AP+ SGZ astrocytes was studied. It was confirmed that these astrocytes behaved like NSCs and gave rise to neurons (Seri et al. 2001). For simplicity, the term RGL will be used throughout the dissertation to describe neural precursors behaving like stem cells in the DG.

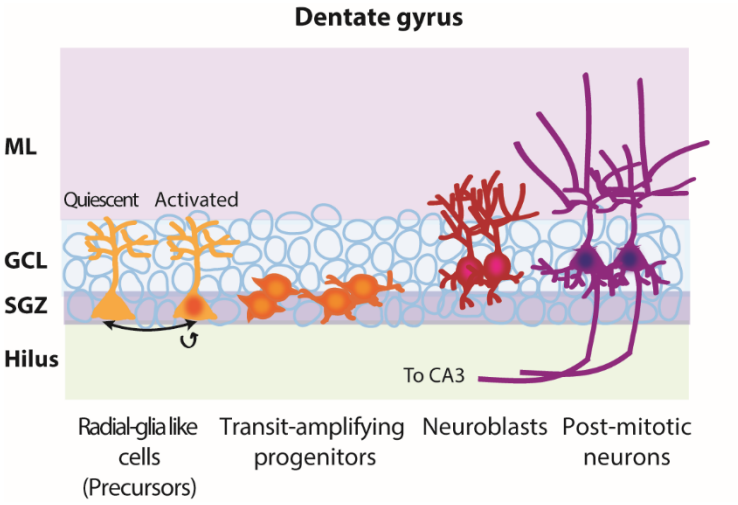


Figure I.4. Hippocampal neurogenesis lineage

The dentate gyrus niche is comprised of several layers: the molecular layer, the granular cell layer, the subgranular zone and the hilus. Hippocampal neurogenesis starts in the subgranular zone where quiescent precursors become activated and divide asymmetrically to give rise to another precursor and quickly dividing progenitors. These progenitors will then migrate a short distance to reach the granular cell layer and mature into neuroblasts. Neuroblasts will become post-mitotic and integrate the circuitry by receiving entorhinal cortex inputs in the molecular layer and projecting their axons towards the CA3 region of the hippocampus. (GCL: Granular cell layer, ML: Molecular layer, SGZ: Subgranular zone. Created by C-A. Grégoire and Laura Hamilton.).

Recently, different DG neurogenic lineage models were introduced to the field and caused controversy (Bond et al. 2015) (**Fig. I.5**). The first model is called the "disposable stem cell" model (Encinas et al. 2011). Quiescence is known to be one way to conserve the stem cell pool and limit their replication to avoid mutations in several tissues (Li and Clevers 2010). However, in this model, activated NSCs are believed to leave the stem cell pool, convert to the astrocytic fate, and not return to their quiescent state. This correlates with the observations made in aged mice where NSCs are lost and neurogenesis diminishes (Encinas et al. 2011). Encinas and colleagues confirmed progenitors were coming from RGLs following asymmetric division using a transgenic mouse model. They then used a BrdU paradigm in Nestin-CFPnuc reporter mice, which allowed them to label proliferating cells shortly after the pulse period, and long-retaining cells (RGLs) after a chase period. For a period of seven days, BrdU+ RGL numbers stayed constant, but dropped to zero after 10-15 days. In parallel, the number of BrdU+ astrocytes climbed and reached the initial number of RGLs by day 10, suggesting that these astrocytes could emerge from the activated RGLs. Moreover, they observed that activated RGLs were going through three rounds of division before exiting the cell cycle and becoming astrocytes (Encinas et al. 2011). Most tools, including the transgenic mice used in the previous study, label cell populations (Dhaliwal and Lagace 2011). However, an *in vivo* clonal analysis approach allowed tracing of individual nestin+GFAP+ RGL precursors (Bonaguidi et al. 2011). The inducible, sparse-labeling (low concentration of tamoxifen) model nestin-CreER^{T2}; Z/EG (lacZ and GFP) was used to understand RGLs' behaviour. The vast majority of RGLs are quiescent, but once they become activated they go through three modes of division: 1) symmetrical (shown for the first time) to expand the RGL pool (to a lower extent), 2) asymmetrical to give rise to a progenitor cell that will become a neuron and maintain the RGL pool, and 3) asymmetrical to give rise to an astroglia and maintain the RGL pool. After division, the activated RGL returns to quiescence. Bonaguidi and colleagues confirmed their clonal analysis results with the mosaic-analysis with double markers reporter that allows to label daughter cells and their progeny (Bonaguidi et al. 2011). Another model suggested RGL heterogeneity (DeCarolis et al. 2013). DeCarolis and colleagues used two inducible transgenic mouse lines to demonstrate the validity of this model: Nestin-CreER^{T2}/R26R:YFP and GLAST::CreER^{T2}/R26R:YFP. Currently, there is no unique markers for hippocampal RGLs, therefore they used Nestin (labels both RGLs and progenitors) and GLAST (labels both

astrocytes and RGLs). A week following Ara-C treatment, an increase in dividing GLAST-YFP+ cells, but not Nestin-YFP+ cells was observed. This suggests that GLAST-YFP+ cells contribute to the recovery of the RGL population. Consistent with this finding, following a 14-day exercise paradigm, only GLAST-YFP+ cells increased in numbers, even if proliferation was amplified in both lines (DeCarolis et al. 2013).

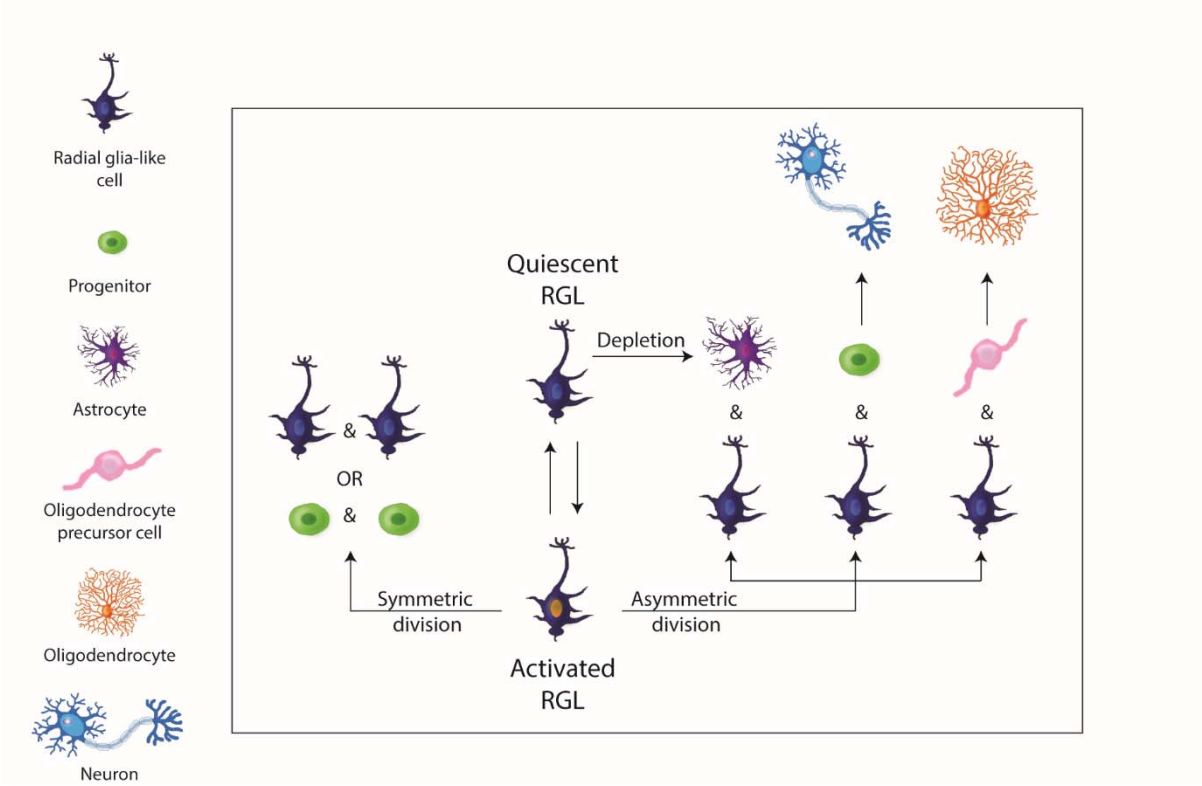


Figure I.5. Neural stem cell behaviour

Neural stem cells may have different behaviour based on different models. One model suggests that activated radial-glia like cells divide symmetrically. Another model suggests asymmetric division of activated radial-glia like cells that give rise to another radial-glia like cell and a progenitor that will differentiate into different cell types such as astrocytes, neurons and oligodendrocytes. Lastly, another model suggests a disposable model where a quiescent radial-glia like cell will leave the stem cell pool and convert into an astrocyte. (RGL: Radial-glia like cells. Inspired by (Bond et al. 2015). Created by C-A. Grégoire and Loïc Cochard).

I.2.6. Hypothalamus

Another brain region that gained popularity over the last decade as a potential neurogenic niche is the hypothalamus (Goodman and Hajihosseini 2015). The hypothalamus plays an important role in the secretion of essential hormones implicated in temperature regulation, food intake, sex drive, and sleep-wake cycle besides other physiologic functions (Kandel et al. 2000). The main focus of research on the hypothalamus has been on energy balance with several articles published on this topic. Two major neurons are found in the arcuate nucleus (adjacent to the third ventricle): neuropeptide Y (NPY) and pro-opiomelanocortin-expressing neurons, known as orexigenic and anorexigenic, respectively (Kokoeva et al. 2005; Pierce and Xu 2010). In 2004, progenitor cells from the hypothalamus were first isolated in cultures (Markakis et al. 2004). Then, central infusion of the ciliary neurotrophic factor into the lateral ventricle mouse brains, known to reduce body weight, induced proliferation of newborn BrdU+ cells. 42 days following infusion, 43% of the proliferating cells expressed neuronal markers (Kokoeva et al. 2005). Another study was conducted to confirm these previous results (Kokoeva et al. 2007). Three days following infusion, half of the BrdU+ cells also expressed Ki67, and infusion directly in the third ventricle led to increased proliferation throughout the hypothalamic parenchyma, but none around the lateral ventricle (Kokoeva et al. 2007). Moreover, a study shed light on the possibility that inhibition of *de novo* proliferation could lead to less food intake, therefore suggesting a link between hypothalamic new cell proliferation and energy balance (Pierce and Xu 2010).

In the past few years, researchers have been investigating the role of hypothalamic tanycytes that showed stem cell-like properties (Chaker et al. 2016; Goodman and Hajihosseini 2015; Lee et al. 2012; Robins et al. 2013). Four subtypes of tanycytes exist: α1, α2, both found dorsally, β1, and β2, found ventrically (Rodriguez et al. 2005). In 2012, β2-tanycytes were first identified as the neurogenic radial glia-like nonciliated ependymal cells in postnatal and pre-adult mice (Lee et al. 2012). Li and colleagues demonstrated that cells from adult hypothalami could give rise to self-renewing and multipotent neurospheres *in vitro*. Moreover, *in vivo*, they showed that the majority of sox2+ adult stem cells were present in the mediobasal region of the hypothalamus, and not in the lateral third-ventricle wall where tanycytes are found (Li et al.

2012). It was later specified, using a lineage-tracing approach in adult *GLAST::CreER^{T2}* mice, that α-tanycytes are the self-renewing and multipotent cells (astrocytes and neurons) *in vivo*, and give rise to neurospheres *in vitro* (Robins et al. 2013). Recently, Chaker and colleagues demonstrated for the first time that hypothalamic neurogenesis persists through aging (Chaker et al. 2016).

I.2.7. Spinal cord

In the spinal cord, ciliated cells involved in the propulsion of the cerebrospinal fluid, known as ependymal cells, seem to maintain a regenerative capacity. Located around the central canal of the rodent spinal cord, ependymal cells behave like stem cells as they demonstrate self-renewal and multipotency capacities *in vitro* (Johansson et al. 1999; Martens et al. 2002; Meletis et al. 2008; Weiss et al. 1996) (**Fig. I.6**). However, *in vivo*, BrdU-retaining ependymal cells show a glial-restricted differentiation into astrocytes and oligodendrocytes (Horner et al. 2000), hence lacking neurogenic capacity (Martens et al. 2002). In the field, this discrepancy between the *in vitro* and *in vivo* data contributes to define ependymal cells simply by their name instead of referring to them as stem cells. In the naive spinal cord, proliferating ependymal cell number is very limited and shows a dorso-ventral gradient with greater density at the dorsal part of the central canal. Proliferating cells are often found in doublets and associated with blood vessels. Vimentin+ ependymal cells might also express Nestin+ or GFAP+ processes elongating dorsally in the grey matter (Hamilton et al. 2009). In this niche, ependymal cells are mostly quiescent under normal conditions and divide symmetrically, suggesting a maintenance proliferation mechanism for this precise pool of ciliated cells (Barnabe-Heider et al. 2010; Hamilton et al. 2009; Meletis et al. 2008) (**Fig. I.6**). In the adult human spinal cord, the presence of NSC-like cells has been demonstrated for the first time in 2008 (Dromard et al. 2008), and their self-renewing capacity in an adherent monolayer culture was further shown in a more recent article (Mothe et al. 2011). The human spinal cord-derived neurospheres showed NSC and proliferation marker expression (Sox2, Nestin, and Ki67). They have the capacity to differentiate into astrocytes and neurons, but have limited self-renewal capacity. The ependymal layer organization in the human spinal cord is different than the one found in rodents. In humans, the central canal is often occluded and the ependymal layer is disorganized, frequently showing

rosettes or microcanals. The human central canal is surrounded by a hypocellular region containing high levels of GFAP+ and Nestin+ cells localized in the ventral part of the central canal. As in rodents, the human ependymal layer maintains immature features during adulthood. For instance, it expresses Nestin and Sox2, and proliferates at a low rate under naive conditions (Hugnot and Franzen 2011). In summary, the existence of self-replicating ependymal cells has been demonstrated in the adult human spinal cord, suggesting potential to develop endogenous spinal cord repair strategies that could target these cells (Dromard et al. 2008; Mothe et al. 2011).

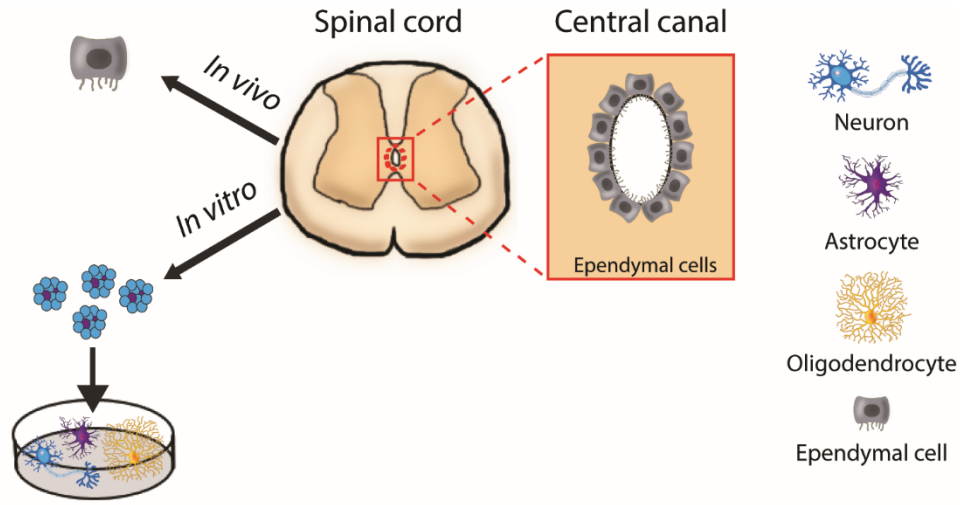


Figure I.6. Central canal ependymal cell behaviour under normal conditions

Under normal conditions, ependymal cells surrounding the central canal of the spinal cord will slowly self-renew as this niche is mostly quiescent *in vivo* giving rise to other ependymal cells. In contrast, *in vitro,* neurospheres can be grown from dissociated spinal cord-derived cells and these cells have multipotent capacities leading to neuron, astrocyte and oligodendrocyte differentiation (Created by C-A. Grégoire, Loïc Cochard, and Brianna Goldenstein).

I.2.8. Regenerative capacities throughout evolution

Throughout evolution, regenerative abilities have varied among species (Bonfanti and Peretto 2011; Ferreira et al. 2012). The distinction between regeneration and repair is important,

as repair is imperfect regeneration because it does not normally restore both the structure and function (Bonfanti 2011). Invertebrates conserve their ability to regenerate whole body parts, and vertebrates, such as fish, amphibians, and reptiles, maintain indeterminate growth throughout their lives (Lee-Liu et al. 2013; Tanaka and Ferretti 2009). In these species, embryonic radial glia cells are still present, and maintain the capacity of producing neurons and glial cells throughout adulthood. Moreover, in newts, proliferation and neurogenic regions (or hot spots) are not necessarily the ones demonstrating regenerative capacities. In response to injury, a *de novo* generation occurs by the reactivation of quiescent ependymoglial GFAP+ cells that will re-establish zones of regenerative proliferation and neurogenesis (Kirkham et al. 2014). In urodele amphibians, these ependymoglial cells transiently lose their GFAP expression and start expressing NSC markers such as Nestin. Then, they participate in the repair of the neuroepithelial tube to eventually rebuild the spinal cord (Walder et al. 2003). Newts and salamanders can regenerate their limbs throughout life, whereas frogs and toads can do so only during development (Bonfanti 2011). More recently, a group suggested that Sox2+ neural precursor cells in Xenopus tadpoles are involved in the regeneration process (Gaete et al. 2012). They observed a significant increase in Sox2 labeling four days after amputation of the tail, and 20% to 60% of them were proliferating (BrdU+). An increase in Sox2 mRNA levels was also observed as soon as one day following injury when compared to uncut tail controls. Moreover, they used a Sox2 dominant negative construct, mediating its translocation to the nucleus, and only 46% of tadpoles maintained their tail regeneration capacities (Gaete et al. 2012). These results show the importance of NSCs in the regenerative process. However, in mammals, these radial glial cells only exist during embryonic development and transform into astrocytes in the adult nervous system (Voigt 1989). Thus, mammals lose the ability to regenerate injured tissue. In adult birds, these radial cells are still present but restricted to the walls of the lateral ventricle (Alvarez-Buylla et al. 1990). In the SVZ of the lateral ventricles, NSCs are activated following stroke, and produce new cells that respond to the injury, eventually forming a glial scar (Gregoire et al. 2015; Zhang et al. 2008). A similar process is observed in the spinal cord following lesion, where ependymal cells produce astrocytes implicated in glial scar formation (Barnabe-Heider et al. 2010; Gregoire et al. 2015). One theory is that regenerative capacities over evolution would be negatively correlated with the development of the immune system as it gets more specialized, leading to a loss in regeneration (Bonfanti 2011).

I.3. Functional significance of adult NSCs

Adult neurogenesis is an interesting field due to therapeutic avenues that can emerge from understanding its functional importance. The physical location of NSCs can also determine their functions. The NSCs located in the SVZ and the DG will be discussed in further detail in this section.

I.3.1. Subventricular zone of the lateral ventricle and olfactory bulb

I.3.1.1. SVZ/OB functions

In the rodent brain, the OB plays an important role in olfaction as this sense is well developed to survive in their natural habitat (Lazarini and Lledo 2011). The SVZ of the lateral ventricle is the starting location of neurogenesis. The final location is achieved once neuroblasts migrate along the RMS to the OB and mature into either of two local interneuron types (periglomerular or granule cells). Only half of newly-born neurons will successfully integrate the OB circuitry. The OB acts as a relay station that processes and refines sensory inputs triggered by environmental cues. The information is then transmitted to the primary and accessory olfactory cortex via mitral and tufted principal neurons (Kempermann 2011; Lazarini and Lledo 2011; Sakamoto et al. 2014).

The functional significance in olfaction remains elusive even though several neurogenesis ablation studies demonstrated the importance of newly-born neurons in the maintenance of OB circuitry, olfactory memory formation, and odorant discrimination (Breton-Provencher et al. 2009; Moreno et al. 2009; Valley et al. 2009). Other functions are linked to the SVZ germinal niche. *In vivo*, Menn and colleagues showed that B cells are able to differentiate into migrating non-myelinating and myelinating oligodendrocytes through Olig2+ type C progenitors (Menn et al. 2006). Under normal conditions, the oligodendrocyte/neuron ratio is low, but following a demyelinating lesion in the corpus callosum, the number of oligodendrocytes increased by fourfold. This result suggests that B cells have the capacity to

participate in myelin repair (Menn et al. 2006). Another function is the capacity of SVZ astrocytes to repair the ependyma during aging in the SVZ (Luo et al. 2008). In elderly mice (22 to 24 month-old), a loss of ependymal cells in the ependyma is observed. BrdU+/GFAP+ cells with processes contacting the ventricle were found to integrate into the ependyma barrier. No BrdU+/S100β+ ependymal cells were found up to three weeks after the BrdU pulse period, suggesting an absence of proliferation. However, this population expanded up to 22% after 13 weeks. The authors suggested a conversion from SVZ astrocytes to ependymal-like cells as the BrdU+/GFAP+ cells started expressing S100β and cellular adherens. Over time, these cells adopted ependymal cell characteristics (losing GFAP expression). Nevertheless, their functional integration has yet to be shown (Luo et al. 2008). In an ischemic model, SVZ-generated astrocytes were found to be the ones to migrate to the injured cortex, and not the cortical astrocytes (Benner et al. 2013). This was discovered by assessing the level of thrombosponding 4 (a notch modulator) found in higher quantity in SVZ astrocytes (Benner et al. 2013). Faiz and colleagues further determined the origin of the cortical astrocytes found at the lesion site after stroke (Faiz et al. 2015). They confirmed that GFAP+ NSCs were the cells migrating to the injured cortex using several genetic tools, including a GFAP-TK mouse model and the more traditional Ara-C treatment. Finally, two months following injury, these astrocytes became reactive and contributed to the astroglial scar formation (Faiz et al. 2015).

I.3.1.2. Significance in human SVZ/OB

Human OB neurogenesis differs from rodents as it is almost inexistent (Bergmann et al. 2012). The human brain structure is more developed and shows smaller, differently organized OBs than rodents OBs. The existence of a human RMS similar to the one found in rodents is still unclear. Curtis and colleagues attempted to characterized the human ventriculo-olfactory neurogenic system, thought to include the SVZ, RMS, olfactory tract, and the OB (Curtis et al. 2007). They revealed PSA-NCAM+ neuroblasts through a small RMS that takes a caudal path before reaching the olfactory tract (Curtis et al. 2007). Another study detected a little neuronal turnover (less than 1%) using ^{14}C in the human OB, suggesting that the function of OB in humans is not as primordial as in rodents (Bergmann et al. 2012). Some may argue that during

evolution, the need for OB neurogenesis decreased, and therefore low turnover is observed in humans. However, Ernst and colleagues decided to investigate one step further to better understand this low OB neurogenesis turnover. They hypothesized that in the human brain, neuroblasts would migrate elsewhere besides the OB. They found that PSA-NCAM and DCX+ neuroblasts were also present in the adjacent striatum. They determined that the ^{14}C concentration in the subjects' striata corresponded to the atmosphere concentration after their birth, suggesting a cell turnover of approximately 2.7% per year. It was confirmed that striatal interneurons were continuously generated (Ernst et al. 2014). These results suggest the possibility that human SVZ-derived neurons exert a functional role that is different from other mammals. Their migration and differentiation in the striatum may suggest roles in cognition and motor coordination, but this theory remains to be proven (Ernst and Frisen 2015; Kandel et al. 2000).

I.3.2. Dentate gyrus of the hippocampus

I.3.2.1. Hippocampus anatomy

The HPP consists of one main unidirectional tri-synaptic circuit (Anderson et al. 2007; Lucassen et al. 2013). The HPP received its name from its sea horse shape, and includes three subdivisions: cornu ammonis (CA) 1, CA2, and CA3. The origin of the CA comes from Ammon's horn, the mythological Egyptian god, represented by a ram. The hippocampal formation consists of the DG, HPP, subiculum, presubiculum, parasubiculum, and entorhinal cortex. The entorhinal cortex is the first section of the circuit where the projections reach the DG through the unidirectional perforant path. In the DG, granule cells send their axons (called mossy fibers) to the CA3 field where they connect with pyramidal cells. These pyramidal cells provide the main input to the CA1 region, called the Schaffer collateral axons. The CA1 will project to both the subiculum and entorhinal cortex. The subiculum also projects axons to the presubiculum, parasubiculum, and entorhinal cortex, therefore closing the hippocampal formation loop (Anderson et al. 2007) (**Fig. I.7**).

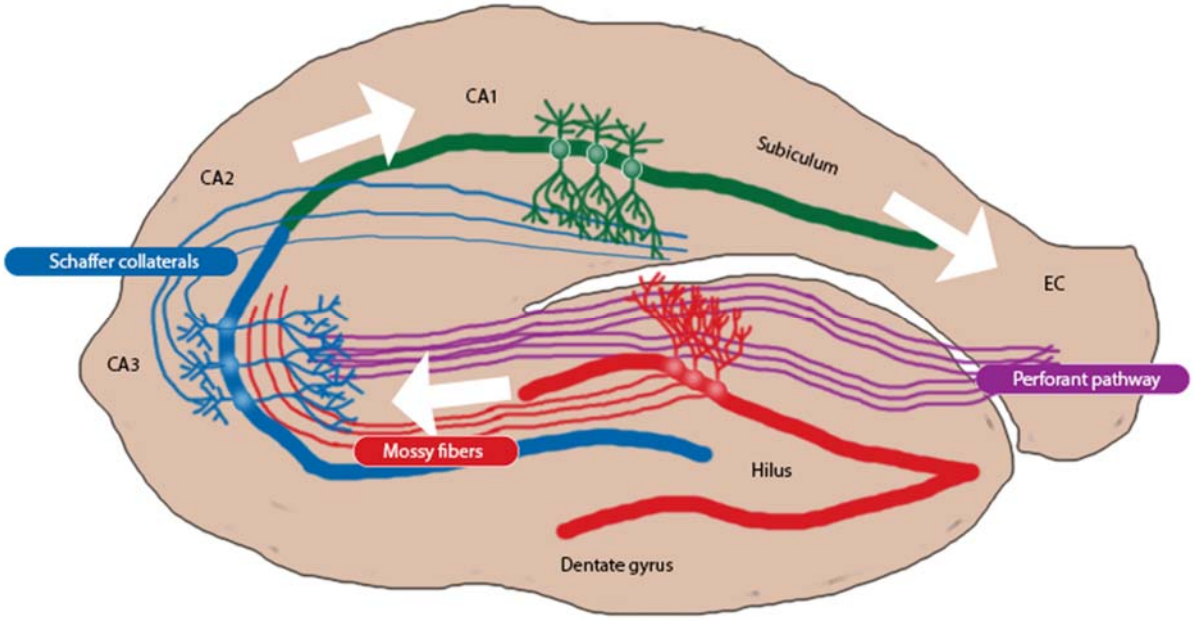


Figure I.7. Hippocampal formation circuitry

The hippocampal formation is comprised of the entorhinal cortex from which axons of the perforant pathway are projecting towards the dentate gyrus. The granule neurons of the dentate gyrus receive inputs from the entorhinal cortex and are sending ouputs to the CA3 region via their mossy fibers. Pyramidal neurons from the CA3 region projects axons, called the Schaffer collaterals, to the CA1. The CA1 neurons project to both the subiculum and entorhinal cortex to complete the loop. (EC: Entorhinal cortex, CA: Cornu ammonis. Inspired from (Lucassen et al. 2013); Created by C-A. Grégoire).

I.3.2.2. Functions of the hippocampus

The ventral and dorsal sections of the HPP exert different functional roles (Bannerman et al. 2003; Frankland et al. 1998; Kheirbek et al. 2013). The role of the HPP, part of the limbic system, seems to be based on the longitudinal axis. Some studies showed that the dorsal section of the HPP is implicated in learning and memory whereas the ventral section would have a role linked to anxiety (Bannerman et al. 2003; Frankland et al. 1998; Kheirbek et al. 2013). On one hand, the ventral HPP, involved in mood disorders, sends projections to regions implicated in

autonomic, neuroendocrine, and motivational responses to emotionally charged stimuli. These regions include the medial prefrontal cortex, amygdala, shell of nucleus accumbens, hypothalamus, bed nucleus of the stria terminalis, and medial entorhinal cortex (**Fig.I.8**). On the other hand, the dorsal HPP, associated with learning and memory, sends outputs to associational cortical regions such as the retrosplenial area (anterior cingulate cortex), ventral tegmental area (via the septum), lateral entorhinal cortex, and ventral HPP (Kheirbek et al. 2013; Sahay and Hen 2007; Tannenholz et al. 2014) (**Fig.I.8**). Moreover, the DG is exposed to several neurotransmitters such as acetylcholine (septum), dopamine (ventral tegmental area), GABA (septum), glutamate (entorhinal cortex), norepinephrine (locus coeruleus), and serotonin (median raphe nucleus) that influence HPP functions (Leranth and Hajszan 2007; Zhao et al. 2008). A pioneer study was conducted on H.M. patient by William Scoville and Brenda Milner (Scoville and Milner 1957). In 1953, H.M was an epileptic patient who went under surgery for a radical bilateral temporal-lobe resection. After surgery, his seizures were reduced but, unfortunately, he developed memory deficits (Scoville and Milner 1957). H.M died in 2008 and high-resolution *in situ* and *ex vivo* MRI allowed to determine what parts disappeared. Augustinack and colleagues (2014) noted that "substantial portions of the medial temporopolar, piriform, entorhinal, perirhinal, and parahippocampal cortices, as well as the subiculum, presubiculum, parasubiculum, amygdala, hippocampal fields CA1, CA2, CA3, and CA4 (in the hippocampal head and body), and DG (posterior head and body)" were removed (p.1281) (Augustinack et al. 2014). He experienced retrograde (before the operation), and anterograde amnesia, but his semantic memory was intact, showing the importance of the HPP in memory (episodic) for the first time (Anderson et al. 2007; Augustinack et al. 2014; Scoville and Milner 1957).

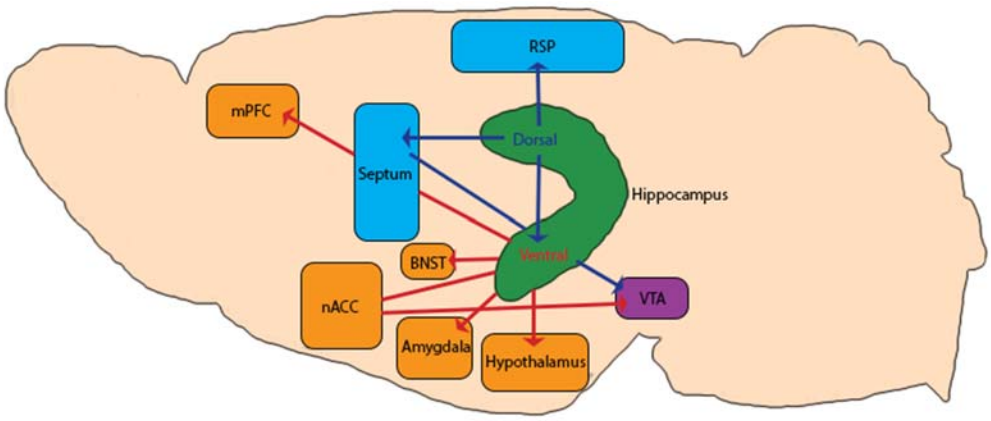


Figure I.8. Dorsoventral axis projections of the hippocampus

The hippocampus includes both a ventral and a dorsal region. Neurons from these regions project their axons to different areas of the brain. These outputs influence the functions of the ventral vs. dorsal hippocampal regions. The ventral hippocampus is associated with areas involved in autonomic and motivational responses function, whereas the dorsal hippocampus is linked to associational cortical regions. (BNST: Bed nucleus of the stria terminalis, mPFC: medial prefrontal cortex, nACC: nucleus accumbens, RSP: retrosplenial area, VTA: ventral tegmental area. Inspired by (Tannenholz et al. 2014); Created by C-A. Grégoire).

I.3.2.3. Hippocampal neurogenesis and memory

Studies, using a variety of techniques, showed that hippocampal neurogenesis improves learning and memory (Deng et al. 2009; Gu et al. 2012; Kheirbek et al. 2013; Snyder et al. 2005; Vukovic et al. 2013). A study conducted in birds was the first to suggest a link between neuronal replacement and new spatial memories (Barnea and Nottebohm 1994). Adult canaries have higher neurogenesis levels during March and October, correlating with the observation of additional new song syllables during these months (Alvarez-Buylla and Kirn 1997; Barnea and Nottebohm 1994; Barnea and Pravosudov 2011; Kirn et al. 1994). To study the function of hippocampal neurogenesis, several studies used non-cell specific ablation approaches (Deng et al. 2009; Snyder et al. 2005). A widespread ablation technique is brain irradiation (Monje et al. 2002). Snyder and colleagues detected that newly-born neurons, between four and 28 days old, are necessary for long-term spatial memory (Snyder et al. 2005). Retention tests were performed

one, two, and four weeks post-irradiation. Irradiated rats had a worse performance than control littermates when they were tested two or four weeks following the training period in the Morris water maze (MWM) (Morris et al. 1982). In contrast, rats that were irradiated only 4 days before training, instead of a month, did not show any long-term memory deficits (Snyder et al. 2005). Deng and colleagues further demonstrated that immature neurons are necessary for long-term spatial memory using an inducible Nestin-TK transgenic mouse line (Deng et al. 2009). This model showed a 50% reduction in BrdU+ cell number after a two-week ganciclovir antiviral treatment. They tested Nestin-TK mice one week after treatment in a MWM and performed probe trials one to three weeks later to assess long-term memory. No short-term memory deficits were observed, but mice demonstrated a poor performance when their long-term memory was assessed when compared to wild-type mice. These rodents had newly-born neurons aged from one to three weeks and two to four weeks during training, and three to five weeks at the end of the long-term memory test. Thus, these experiments assessed the role of immature neurons in both short- and long-term memory. Interestingly, when mice with more mature cells were tested (three to nine weeks following the end of the ganciclovir treatment), no memory deficits were observed (Deng et al. 2009). Vukovic and colleagues showed that DCX+ cells are required to learn a novel task, but not to remember the learned task (Vukovic et al. 2013). A novel knock-in mouse model, DCX^{DTR}, expressing the diphtheria toxin under the DCX promoter to specifically ablate immature DCX+ neurons was developed. The active place avoidance task (hippocampus-dependent learning task with a constant rotating platform) was performed and DCX^{DTR} mice entered the shock zone more frequently than wild-type counterparts, suggesting learning deficits for this task. Learning deficits could be reversed by the replenishment of immature neurons upon withdrawal of the toxin treatment. Absence of behavioural recall deficits was also observed when DCX^{DTR} mice were first exposed to the active place avoidance task without the toxic treatment and then put back into this familiar spatial task after treatment (Vukovic et al. 2013).

Optogenetics studies, using a cell specific approach, confirmed a role of dorsal hippocampal neurogenesis in learning and memory (Gu et al. 2012; Kheirbek et al. 2013). Optogenetics involves modifying neurons in such a way that they can express light-sensitive ion

channels. The neurons can therefore be silenced (yellow light-activated chloride pump halorhodopsin) or activated (cation channel channelrhodopsin 2 activated by blue light) by light exposure in a specific time manner. Gu and colleagues study, using optogenetics, confirmed results from (Deng et al. 2009) by showing that four-week-old neurons are required for memory retrieval (Gu et al. 2012). In an elegant study, Kheirbek and colleagues demonstrated a role of dorsal granule neuron activity in contextual learning, but not in the retrieval of memories from a learned task (Kheirbek et al. 2013). They excluded the role of ventral granule cells in these functions. Furthermore, mice were tested following optogenetic silencing of the dorsal DG for active place avoidance (which uses electric shock to induce avoidance behaviour) and found no difference. However, after switching the shock zone to the opposite side to create conflicting memories, mice showed deficits. Activation of the dorsal granule cells also led to an increase in exploration time in novel environments during the open-field test and elevated plus maze mediated through intact dopaminergic function (Kheirbek et al. 2013).

I.3.2.4. Hippocampal neurogenesis, mood and psychiatric disorders

Hippocampal neurogenesis mediates the behavioural effects of antidepressant effects and is implicated in anxious behaviour (Eisch and Petrik 2012; Kheirbek et al. 2013; Santarelli et al. 2003). The ventral HPP has been shown to be implicated in mood disorders such as depression and anxiety. Gould and colleagues discovered that corticosteroids inhibit cell division (Gould et al. 1992). They performed an adrenalectomy in rats, which blocks the body response to stress, and increased cell division. Moreover, this inhibition was reversed by corticosterone replacement (Gould et al. 1992). Results like these garnered interest in the effect of stress on neurogenesis since patients suffering from depression show increased corticosteroids level. One early hypothesis by Jacobs and colleagues linked depression and hippocampal neurogenesis (Jacobs et al. 2000). Other studies followed discussing how adult neurogenesis can be implicated in the antidepressant's mode of action (Eisch and Petrik 2012). In rats, chronic antidepressant treatment led to an increase in BrdU+ cells relative to their control littermates, 75% of which expressed neuronal markers (Malberg et al. 2000). Then, Hen and colleagues showed that hippocampal neurogenesis is required for the behavioural effects of

antidepressant effects (Santarelli et al. 2003). They performed focal irradiation on mice, eliminating approximately 85% of BrdU+ cells to investigate the role of newly-born neurons in antidepressant treatment. This reduction in newly-born BrdU+ neurons was maintained up to eight weeks after irradiation in the DG, whereas no effect was detected in the SVZ. The novelty-suppressed feeding test was used to measure rodent aversion to eating in novel environments. Irradiated and sham mice were treated with fluoxetine, a serotonin-selective reuptake inhibitor antidepressant. A reduction in latency to eat was detected in the sham animals but not in the irradiated mice. Another behavioural test, the chronic unpredictable stress paradigm (measured by impaired grooming) was also conducted with similar results. Fluoxetine treatment improved sham mice coat conditions, whereas irradiate mice coats remained unchanged. These results suggest hippocampal neurogenesis as one of the mechanisms in mediating the antidepressant effects (Sahay and Hen 2007; Santarelli et al. 2003). Finally, ventral hippocampal neurogenesis is required for anxiety-like behaviour (Kheirbek et al. 2013). Mice in which ventral granule cells were inhibited by optogenetics spent more time in the open arms in the elevated-plus maze, suggested less anxiety (Kheirbek et al. 2013).

I.3.2.5. Significance in human hippocampus

Hippocampal neurogenesis occurs in both adult rodent and human brains (Eriksson et al. 1998; Ernst and Frisen 2015; Kempermann et al. 2015a; Spalding et al. 2013). The presence of neurogenesis was first shown in humans in 1998 (Eriksson et al. 1998). Since then, more sophisticated studies came out studying human neurogenesis. In adult rats, it was suggested that 9,000 new cells are generated per day in the DG, corresponding to 6% of granule cells each month (Cameron and McKay 2001). In the adult human hippocampus however, Spalding and colleagues demonstrated with ^{14}C that neuronal turnover occurs at a rate of 1.75% per year, corresponding to approximately 700 newly-born neurons per day per hippocampus (Spalding et al. 2013). This turnover rate is not significantly different from the previously mentioned striatal interneuron turnover rate (Ernst et al. 2014). A study showed that CA3/DG are activated during a pattern separation task, whereas CA1 and other MTL regions would be activated during pattern completion tasks (Bakker et al. 2008). High-resolution functional magnetic resonance imaging

was used to measure brain activity (blood flow) in the medial temporal lobe (MTL). Here, a task was created where subjects were presented with different pictures of either a new, repetitive, or different version of an object. The patients had to mention if it corresponded to an indoor or outdoor object to assess their pattern separation or completion indirectly (Bakker et al. 2008). With regard to mood disorders and neurogenesis in humans, older studies using MRIs showed that patients with major depressive episodes demonstrated hippocampal atrophy (Sheline et al. 1996). A more recent study conducted on post-mortem brain tissues from patients treated for major depressive disorder showed that antidepressants increase progenitor proliferation in the DG (Boldrini et al. 2009). Patient DGs showed that antidepressant (sertraline and nortriptyline) treatment led to a three-fold increase in Ki67+ positive cells in comparison to non-treated patients. In addition, an increase was also observed in Nestin+ precursor cells. These results also coincide with other rodent studies (Boldrini et al. 2009).

I.3.2.6. Neurogenesis and information encoding

Balance between glutamatergic granule cells and hilar mossy cells excitation, and GABAergic basket cells and somatostatin (SST)-expressing interneurons inhibition, is essential to maintain a healthy DG environment (Bannai et al. 2015). Neurons were shown to contribute in two different ways to the hippocampal function: 1) direct information processing via CA3 neurons, and 2) by modulating the local circuitry (Christian et al. 2014) (**Fig. I.9**). This environment and circuitry where DG adult neurogenesis occurs contributes to the different brain functions mentioned below.

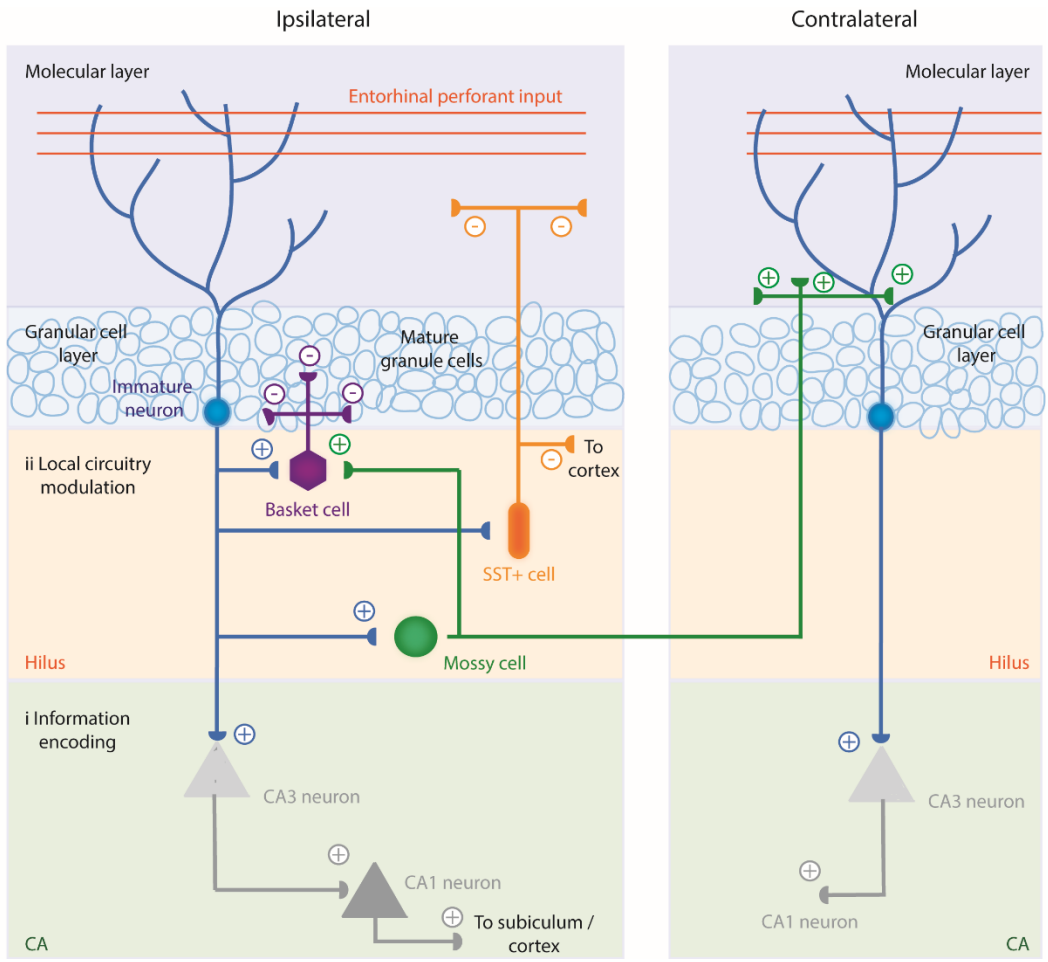


Figure I.9. Circuitry properties of newborn neurons

Newly-born neurons affect the hippocampal circuitry in different ways. First, it sends direct information via the CA3. Second, it modulates local circuitry by activating interneurons that can either activate glutamatergic hilar mossy cells that will then activate inhibitory interneurons. These GABAergic interneurons can inhibit mature granule cells or immature neurons. These modifications can have an impact on other brain region circuitries. (CA: Cornu Ammonis, SST: Somatostatin-expressing interneurons. Inspired by (Christian et al. 2014); Created by C-A. Grégoire).

I.3.2.6.1. Pattern separation

The role of DG in spatial pattern separation, defined as the encoding of "small or weak changes derived from increasingly similar or interfering inputs" (Clelland et al. 2009) to create discrete, non-overlapping representations has been studied for several decades (Treves and Rolls 1992) (**Fig.I.10**). There are five to ten times more neurons in the DG than in the entorhinal cortex from where it receives its inputs (Johnston et al. 2016). It suggests that the discrimination would be facilitated due to the larger space on which information is projected (Deng et al. 2010). The presence of a greater number of neurons allows less neurons to be used to represent the same information, therefore leading to a sparse signal in contrast to an interference signal (Aimone et al. 2014). Moreover, as shown in **Fig. I.9**, granule cell neurons are receiving feedback inhibition from local interneurons creating a low activity level from the neurons contributing to sparse coding (Aimone et al. 2014; Deng et al. 2010). Pattern separation is also evident in the OB (Sahay et al. 2011b). It was demonstrated that olfactory deprivation could impair pattern separation involved in odor acuity (Wilson and Sullivan 1995). However, this section will focus on the DG.

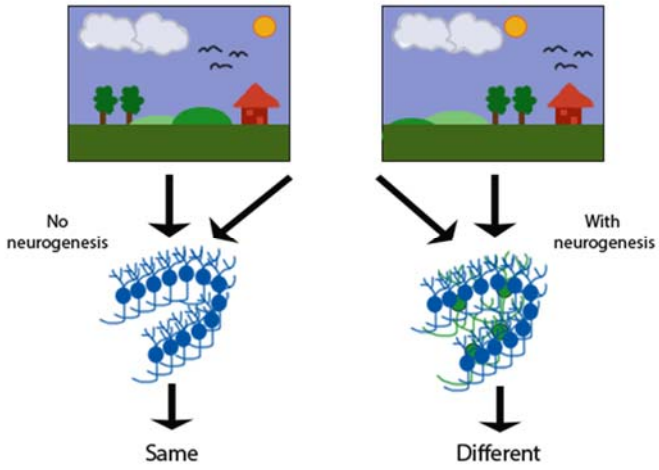


Figure I.10. Pattern separation in the dentate gyrus

In presence of neurogenesis, memories can be different (right), because similar inputs will lead to different representation. However, in absence of neurogenesis, similar images will be perceived as the same (left). (Inspired by (Aimone et al. 2014). Created by C-A. Grégoire).

The concept of determining how old inhibited DG neurons and more excitable newly-born neurons contribute to pattern separation has gained interest in the past few years (Aimone 2016; Johnston et al. 2016; McAvoy et al. 2015; Sahay et al. 2011a). Clelland and colleagues showed that hippocampal neurogenesis is required to discriminate between arms in close spatial proximity (Clelland et al. 2009). They irradiated at low-dose the hippocampus of adult mice and tested them two months later in a delayed nonmatching to place (DNMP) radial arm maze (RAM). This task is particularly challenging as it ensures the use of spatial cues. Correct arms (choice arms with the food pellet reward) were separated by two, three, or four arms from the sample arm (unrewarded). Pattern separation was analyzed based on how well the mice could differentiate between two arms that were close together (two-arm difference) versus those that had a larger distance between them (four-arm difference). Irradiated mice showed impaired behaviour in trials where arms were closer together when compared to sham mice, but no difference was observed for larger separation (Clelland et al. 2009). This paradigm was used in other studies to assess pattern separation (Zhang et al. 2014)(Grégoire *et al*. Unpublished). These results concur with another study showing that NMDA receptors in the DG are mediating pattern separation (McHugh et al. 2007). They genetically deleted an essential NMDA subunit, the NR1. These transgenic mice underwent contextual fear conditioning and showed impairments in the ability to discriminate between two similar contexts (McHugh et al. 2007). A gain-of-function study using *iBax*^{Nes} transgenic mice (where the pro-apoptotic gene *Bax* is deleted) demonstrated that hippocampal neurogenesis is not only necessary (required), as shown by Clelland and colleagues, but also sufficient to improve pattern separation (Sahay et al. 2011a). In this model of increased neurogenesis, transgenic mice could differentiate between two similar contexts better than controls during a contextual fear-discrimination learning task (Sahay et al. 2011a). Another study used running to increase DG neurogenesis and study the effect on pattern separation capacities using a touch-screen system (Creer et al. 2010). Both adult (3 months) and very aged mice (22 months) were exposed to stimuli with either small or big separations between them. However, only adult running mice showed a positive correlation between task performance and neurogenesis. Adult mice who ran were able to discriminate between close-proximity stimuli compared to controls, whereas no difference with controls was observed for more separated stimuli. In contrast, aged runners showed no effect on neurogenesis or improved task performance (Creer et al. 2010). Another study suggested that distinct DG cell

population are activated during learning and during retrieval of similar memories when tested in contextual fear conditioning (Deng et al. 2013). However, a recent study using GFAP-TK mice suggested that adult hippocampal neurogenesis is implicated in the separation of competing memories rather than to the discrimination of similar stimuli (Swan et al. 2014). Swan and colleagues used a touch-screen discrimination learning approach similar to (Clelland et al. 2009) and first showed that deficits were only present four to ten weeks after ablation of proliferating cells (delayed testing), not when tested immediately after ablation. Moreover, both control and transgenic mice could discriminate between small and large separations prior to the first reversal. However, mice with ablated neurogenesis showed deficits after correct and incorrect positions were reversed (Swan et al. 2014).

Another study specified that newly-born neurons are necessary for context discrimination, whereas old granule cells are implicated in rapid pattern completion-mediatd recall (Nakashiba et al. 2012). This research allowed to specify which DG cells contribute to the improved pattern separation. It could be either the granule cells generated during development (~95%) or other post-development neurons (~5%). To investigate this question, Nakashiba and colleagues used a transgenic model where old granule cell survival is inhibited by the tetanus toxin. These mice showed enhanced discrimination of similar contexts in the contextual fear discrimination test. However, when they irradiated these transgenic mice to target newly-born and proliferating neurons, they showed deficits. These results suggest that the newly-born and proliferating neurons are necessary for context discrimination. However, another feature of the hippocampus is pattern completion, defined as the ability to reactivate full representations of memories using partial information. Nakashiba and colleagues showed that old granule cells were implicated in rapid pattern completion-mediated recall, as the tetanus toxin transgenic mice displayed deficits in a contextual fear conditioning test and MWM with only partial cues available (Nakashiba et al. 2012).

I.3.2.6.2. Memory resolution

Some argues that while pattern separation is not unique to the DG, control of memory resolution is. Memory resolution is defined by Aimone and colleagues (Aimone et al. 2011) as

the "extent of information encoded by the DG, and thus the downstream hippocampal regions, during memory formation"(p.591) (Aimone et al. 2011). Mature, highly selective (tightly tuned) neurons provide better resolution than immature neurons following exposure to similar stimuli providing a sparse representation of cortical inputs. Mature neurons are sufficient to encode familiar features. However, immature neurons are less selective (broad tuning) and perform higher resolution encoding as their increased excitability leads to association within memories defined as a densely sampled representation of inputs. Therefore, immature neurons would have the capacity to represent any input (Aimone et al. 2011; Aimone et al. 2014; Aimone et al. 2009) (**Fig. 11**). Recently, it was suggested that adult-born DG cells would play a role in balancing between resolution and robustness (Johnston et al. 2016). On one hand, sparsity increases the number of no overlapping representations, therefore improving resolution. On the other hand, you have memory robustness that is obtained by avoiding memory interference, such as forgetting (described below) (Johnston et al. 2016).

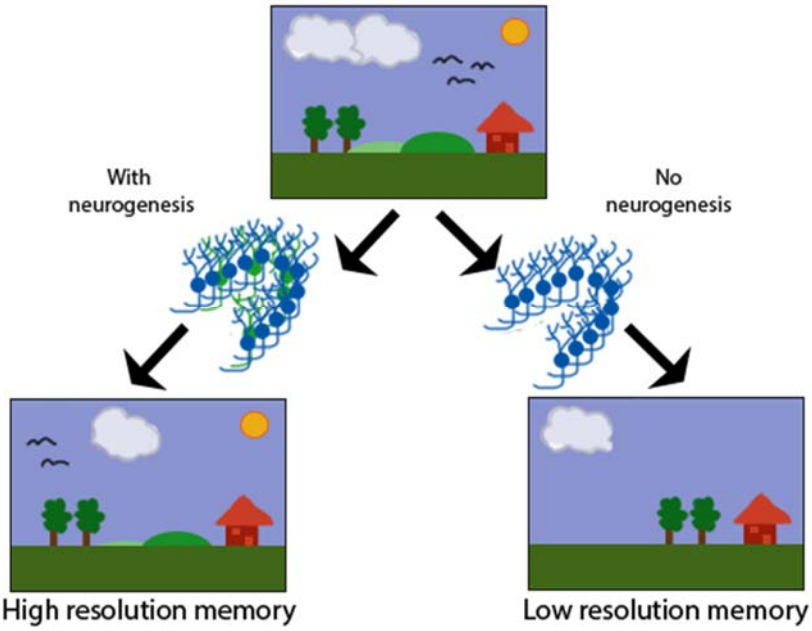


Figure I.11. Memory resolution

In high-resolution memory, more details of the event are remembered, whereas in low-resolution, less details (features) are. Mature and young neurons encode different types of information (past vs. novel events). In presence of neurogenesis, more features are remembered due to combined memories. (Inspired by (Aimone et al. 2014). Created by C-A. Grégoire).

I.3.2.6.3. Memory forgetting

How does the brain balance between incorporating new information and ensuring that this encoding process does not degrade pre-existing information? Newly-born neurons compete with mature neurons for inputs and outputs, but the memory-forgetting process is thought to refine DG axons (Yasuda et al. 2011). Forgetting can either be the loss of learned information, the inability to retrieve learned information, or a deterioration between the memories retrieved and acquired. One computational study suggested that high levels of neurogenesis can constitute a mechanism of interference with the retrieval of old memories described as forgetting (Weisz and Argibay 2012). Based on this theory, another study raised the hypothesis that forgetting should happen more frequently during infancy since hippocampal neurogenesis levels are high (Akers et al. 2014). Akers and colleagues first compared P17 (infants) to P60 (adults) mice and observed an age-dependent decrease in GFP+ DG granule cells four weeks following injection of a retrovirus. Using contextual fear conditioning, they discovered that one to 28 days following training, adult mice froze when exposed to the environment, whereas infant mice demonstrated high freezing behaviour only one day post-training, suggesting forgetting. They used several methods such as running and pro-neurogenic drugs (to increase neurogenesis), and forgetting was still observed in these mice. In contrast, when neurogenesis was decreased by treating TK mice with ganciclovir, these transgenic mice showed stronger freezing behaviour than their wild-type littermates. Further, they confirmed their results in infant guinea pigs and degu with reduced postnatal hippocampal neurogenesis, and no deficits were detected. Finally, they treated infant degu to similarly increase neurogenesis and it induced forgetting. These results demonstrate a link between neurogenesis levels and forgetting behaviour (Akers et al. 2014). A follow-up study suggested that forgetting caused by increased hippocampal neurogenesis minimizes proactive interference (Epp et al. 2016). Proactive interference is defined as the ability of neurogenesis to weaken existing memories and facilitating the encoding of new conflicting information in the same behavioural task. Epp and colleagues trained mice on the MWM, exposed them to either a sedentary or running environment for 28 days, and test them. Consistent with previous results, post-training running induced forgetting. However, when the platform was shifted to the opposite quadrant, runners performed better than sedentary mice,

suggesting weakening of old memories to better encode new information. Similar results were observed in a hippocampus-dependent odour-context paired associates task. The same paradigm was repeated in a new cohort of Nestin-TK- and Nestin-TK+ mice (sedentary or running). However, vanganciclovir was added during the month delay to ablate dividing cells, hence limiting neurogenesis levels. They could assess if other running-induced physiological changes play a role in the observed behavioural changes. This approach prevented forgetting and facilitated reversal learning, suggesting that neurogenesis is necessary to mediate these effects on memory. They finally conducted the odour task again, but this time the new paired associates were either in conflict or not with the original associates (one month post-training). Running-induced increases in hippocampal neurogenesis facilitated the acquisition of new memories only for highly conflicting odours (Epp et al. 2016).

I.4. Activation of adult neural stem cells by physiological events

Physiological events can be used to activate NSCs in different regions of the CNS. On one hand, a physiological stimulation, such as EE, is useful to better understand how NSCs improve cognitive function in a healthy brain. On the other hand, a pathological stimulation, such as spinal cord injury (SCI), can help us to better understand how NSCs modulate their activity to promote repair of the CNS after a lesion. The necessary background to better understand the rationale behind using these two complementary types of stimulation will be described in this section.

I.4.1. Hippocampal NSCs stimulated by environmental enrichment

I.4.1.1. Environmental enrichment

The importance of our surrounding environment has been studied for over 60 years (Hebb 1947). Experimenters used visual deprivation in kittens or exposure to enriched housing conditions to understand how this environment affects the anatomy, chemistry, and functional properties of the brain (Hubel and Wiesel 1970; Rosenzweig et al. 1962; Wiesel and Hubel 1965). Early studies showed that rodents exposed to EE, consisting of large cages, social enrichment, and diverse multisensory stimulation, displayed increased brain sizes, altered neurotransmitter levels and behavioral changes (Bennett et al. 1969; La Torre 1968; Manosevitz and Joel 1973; Rosenzweig et al. 1962) (**Fig. I.12**).

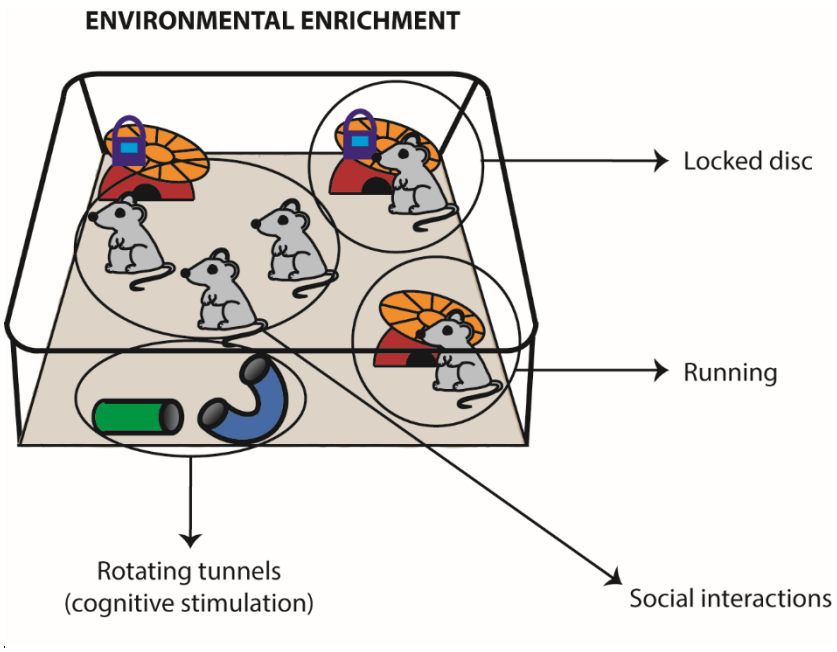


Figure I.12. Environmental enrichment

In an enriched environment, mice are exposed to a variety of stimuli, including rotating tunnels, social interactions, running discs, and locked discs. (Modified from (Gregoire et al. 2014). Created by C-A. Grégoire).

Adult neural stem cells continue to produce new neurons within the DG of the HPP, where these neurons are implicated in the processes of learning and memory (Deng et al. 2009; Kitamura et al. 2009; Leuner et al. 2006). Remarkably, this process of adult neurogenesis is highly stimulated by EE (e.g., providing rodents access to a voluntary running wheel) (van Praag et al. 1999b). Moreover, a recent study has shown that physical exercise increases the hippocampus volume and improves spatial memory in humans (Erickson et al. 2011). Early studies demonstrated that EE and exercise affected different stages of neurogenesis (Kronenberg et al. 2003; Olson et al. 2006). EE increases neurogenesis and survival (Kempermann et al. 1997b), while running has an effect on cell proliferation (Bednarczyk et al. 2009; van Praag et al. 1999b). It was also suggested that the combination of EE and exercise in a sequential manner could lead to additive effects on neurogenesis (Fabel et al. 2009). As most EE studies were conducted in C57BL/6 mice, we demonstrated in our laboratory that voluntary running also

increase hippocampal neurogenesis in the outbred CD1 mouse strain (Bednarczyk et al. 2009). Our group also performed a control experiment comparing neurogenesis levels in C57BL/6 and CD1 mice under normal and exercise conditions. It showed that the exercise-induced increases in neurogenesis were similar between both strains. However, CD1 mice showed a 47% lower baseline neurogenesis level, making the running-induced increases detectable with only four mice per group (Gregoire et al. 2014). This data is consistent with a study conducted in 12 mouse strains that showed that C57BL/6 mice were the least responsive to exercise (Clark et al. 2011b). Moreover, the presence of a locked wheel could be considered cognitive stimulation and should be used as a control, as it was suggested that the hippocampal neurogenic pathway could be activated by both exercise- dependent and independent processes (Bednarczyk et al. 2011). There is currently a tendency to exclude detailed information on EE components in publications, taking for granted that standards are well established in the field (Fabel et al. 2009; Garthe et al. 2016). Unfortunately, no standardization exists, and EE variables are not well isolated in each experimental condition. Some studies included running in their EE paradigm or include social interactions in their exercise paradigm that could bias results. The relative contribution of individual EE variables to hippocampal neurogenesis remains a longstanding and poorly understood issue. Additional information on the following EE components will be provided below: 1) moderate versus intense physical activity, 2) social interaction, 3) isolation, 4) stress, 5) environmental complexity, and 6) neuronal activation.

I.4.1.2. Physical activity

Van Praag and colleagues were the first ones who started dissecting out EE components, demonstrating that voluntary running is sufficient to increase hippocampal neurogenesis (van Praag et al. 1999b). It was later suggested by other studies that physical activity was the neurogenic component within an enriched environment (Kobilo et al. 2011; Mustroph et al. 2012a). Unfortunately, some issues arose from these studies. For example, even though social housing is considered a more natural environment for rodents, having multiple mice in a running environment leads to difficulties to accurately assess the running distances for each animal. Moreover, only a few studies used a locked disc as the control environment to correctly distinguish between the effects of running itself and those of a novel object.

I.4.1.3. Social context

Group housing is known as a beneficial and common component of EE (Lieberwirth and Wang 2012; Welch et al. 1974). In contrast, social isolation is thought to have a negative impact on brain function (Cacioppo et al. 2011). It was demonstrated in rats that social isolation could prevent exercise-induced proliferation and neurogenesis (Leasure and Decker 2009; Stranahan et al. 2006). However, Kannangara and colleagues demonstrated that voluntary exercise was neurogenic for both isolated and socially-housed mice (Kannangara et al. 2009). After exposing mice to one or three months of social isolation, another study observed a decrease in the DCX+ neuroblast population but saw a constant two-fold increase in their EYFP+ RGLs cell numbers when compared to their socially-housed controls (Dranovsky et al. 2011). It is worth mentioning that differences may be observed between studies conducted in rats or mice, as mice are known to prefer socially housed environments. Interestingly, a social environment can also buffer stress-induced inhibition of neurogenesis (Stranahan et al. 2006).

I.4.1.4. Stress

Stress can be detrimental or beneficial depending on what type of stressor you are exposed to: uncontrollable or predictable, respectively (de Quervain et al. 2000; Fitzsimons et al. 2016; Gould et al. 1997; Stranahan et al. 2006; Wosiski-Kuhn and Stranahan 2012). The stress response can be divided in two phases: 1) a rapid activation of the autonomic nervous system, releasing epinephrine and norepinephrine, and 2) a delayed response mediated by the activation of the hypothalamic-pituitary-adrenal axis resulting in the release of adrenal-derived corticosterone in rodents (Fitzsimons et al. 2016). Corticosterone can cross the blood brain barrier and activate glucocorticoid and mineralocorticoid receptors found within the hippocampus (Trivino-Paredes et al. 2016; Van Eekelen et al. 1988). Uncontrollable stressors, such as psychosocial and physical stressors, can increase neuronal vulnerability to neurodegenerative conditions (Fitzsimons et al. 2016; Gould et al. 1997; Wosiski-Kuhn and Stranahan 2012). It is well established that stress negatively affects DG neurogenesis in mammals and impairs retrieval of long-term memory in humans (de Quervain et al. 2000; Gould et al. 1997). Moreover, stress-associated corticosterone can suppress running-induced

neurogenesis after a short 12-day paradigm (Stranahan et al. 2006). However, predictable chronic mild stress, such as exercise, can be neuroprotective (Wosiski-Kuhn and Stranahan 2012). Exercise increases both corticosterone and neurogenesis levels, but it may be a concentration-dependent effect as long-term mild exercise and not intense exercise would increase neurogenesis (Inoue et al. 2015; Soya et al. 2007).

I.4.1.5. Environmental complexity

Components of a complex environment are not well defined. Environmental complexity has shown beneficial effects on neural parameters such as depression, and learning and memory functions (Pang and Hannan 2013; van Praag et al. 2000). Some studies suggested that a complex environment has stage-specific effects that differ from running (Bednarczyk et al. 2011; Fabel et al. 2009; Kannangara et al. 2009; Kronenberg et al. 2003; Olson et al. 2006), while other studies showed that it does not modulate neurogenesis to the same extent as running does (Kobilo et al. 2011; Mustroph et al. 2012a; van Praag et al. 2014; van Praag et al. 1999b). This discrepancy exists because the complex environment has not been well isolated as a EE variable. On one hand, the beneficial effects previously observed could be due to variables such as physical activity or social housing that were included in the complex environment. On the other hand, lack of effects could be caused by an increased basal neurogenesis in presence of components such as inanimate objects, enriched diets or behavioural testing.

I.4.1.6. Hippocampal neurogenesis lineage and markers

To study the effect of EE components on hippocampal neurogenesis, antigen-based identification methods are used to detect changes between experimental conditions. All stages of hippocampal neurogenesis can be identified by specific immunohistochemistry markers (Kempermann 2011; Kempermann et al. 2015b) (**Fig. I.13**). Quiescent and activated RGLs share an astrocytic morphology, therefore the marker for the calcium-binding protein β, S100β, expressed by mature astrocytes, is used to make the distinction (DeCarolis et al. 2013). Due to the glial nature of RGLs, astrocytic markers such as GFAP and GLAST are not sufficient on their own to identify RGLs (DeCarolis et al. 2013; Seri et al. 2004). Most studies combine

astrocytic markers with precursor markers such as Nestin, an intermediate filament marker, (Lagace et al. 2007) or Sox2, a member of the SRY-related HMG box family, to allow a more specific RGL identification (Bonaguidi et al. 2011; Kempermann 2011). Intermediate precursors can be identified using Tbr2, a T-domain transcription factor (Hodge et al. 2008). Late precursors and immature neurons (neuroblasts) can be labeled by markers for DCX, a microtubule-associated protein (Dhaliwal et al. 2015; Kim et al. 2009), and NeuroD, a basic helix loop helix transcription factor (Seki 2002). Mitotic cells mentioned previously can be labeled as proliferative by the cell cycle-associated protein, Ki67 (Scholzen and Gerdes 2000), and post-mitotic immature neurons can be identified by Calretinin, a calcium-binding protein (Brandt et al. 2003).

The final stage of neurogenesis is post-mitotic neurons, which can be identified using NeuN, a widely used neuronal marker (Kempermann 2011). Unfortunately, this marker is not specific to newly-born neurons, therefore it is often colabeled with BrdU (Kempermann 2011; Kuhn et al. 1996). Researchers are using systemic injections of BrdU that allows the identification of cells that underwent division at time of injection. It is then followed by a chase period long enough to assure that cells had time to differentiate and mature into a neuron. As neurons are post-mitotic, it suggests that BrdU-positive cells were dividing at time of injection. However, there are some pitfalls to this BrdU approach, the main argument being that it would pick up cell death or DNA repair (Taupin 2007). This was contradicted by studies suggesting that it occurs in extreme models of pathology (Cooper-Kuhn and Kuhn 2002; Kuan et al. 2004). Once newly-born neurons have been identified, the next question is: are they functionally responsive? This can be addressed using markers of immediate early genes (IEG), such as c-fos (Kempermann 2011; Sagar et al. 1988). The induction of IEG is rapid and transient upon cell stimulation, and known to encode transcription factors that will modify other genes expression, defined as target genes, which will, in turn, modify the cell phenotype (Herrera and Robertson 1996). Transcriptional activation of *C-fos* occurs minutes following stimulation and its mRNA accumulation reaching a peak 30-40 minutes later (Harris 1998). One of the early studies described c-fos as a neuronal activation marker following electrical stimulation of the motor/sensory cortex (Sagar et al. 1988). In fact, *C-fos* expression can be induced by several

types of stimuli such as brain injury, stroke, and epileptic seizures (Herrera and Robertson 1996). Exposure to voluntary running (Rhodes et al. 2003), to an enriched environment (Tashiro et al. 2007), and to a learning task such as the MWM (Jessberger and Kempermann 2003) were also positively associated to IEG expression in the DG (Clark et al. 2012).

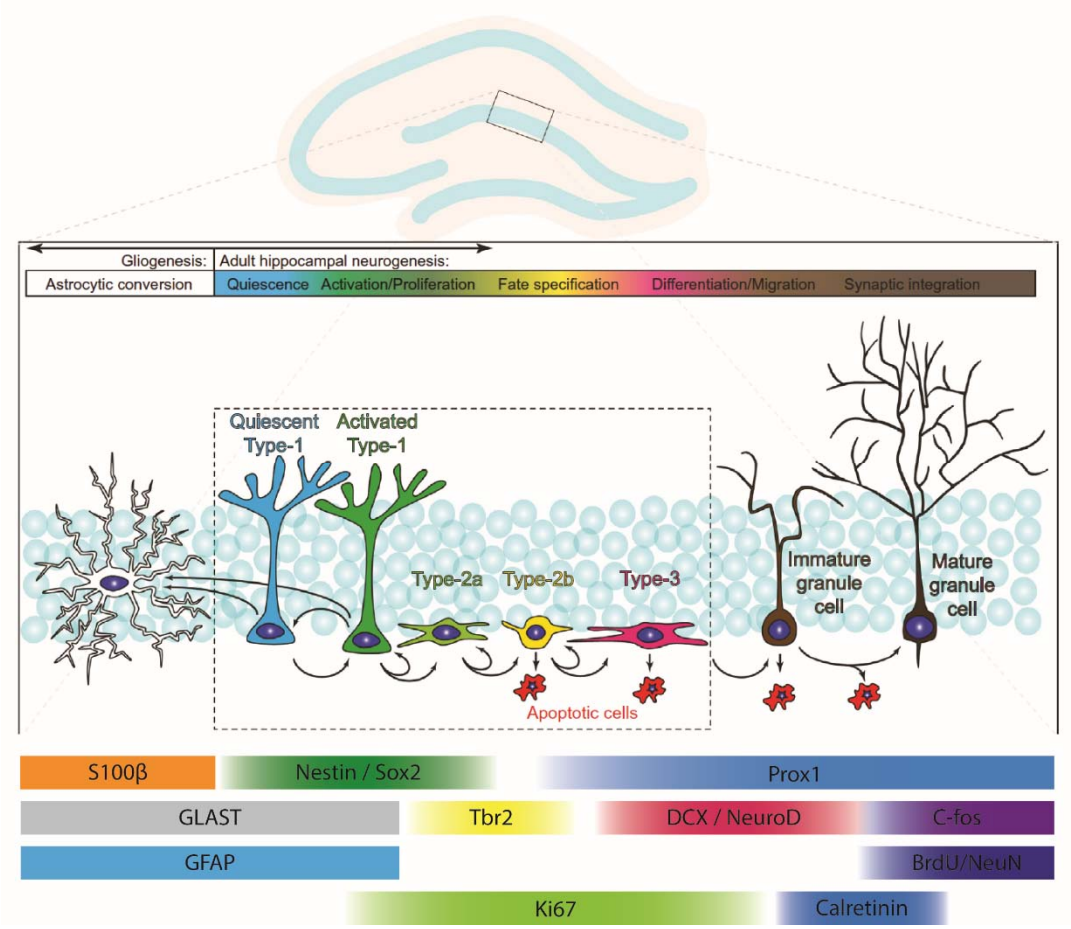


Figure I.13. Hippocampal neurogenesis lineage and markers

Each stage of hippocampal neurogenesis can be identified by specific immunohistochemistry markers. S100β is specific to mature astrocytes, whereas GLAST and GFAP also label quiescent and activated Type-1 cells (RGLs). Colabeling with Nestin or Sox2 allows a more specific identification of RGLs. Tbr2 labels intermediate precursors. Ki67-positive cells correspond to mitotic cells. DCX and NeuroD label immature neurons, whereas Calretinin identifies post-mitotic immature neurons. Prox1 labels a wide range of cells from intermediate precursors to mature neurons, whereas BrdU/NeuN-positive cells correspond to newly-born neurons that can be activated if positively labeled with c-fos (Modified from (Fitzsimons et al. 2016)).

I.4.2. Spinal cord ependymal cells (NSCs) stimulated by spinal cord injury

I.4.2.1. Impact of SCI on ependymal cells

SCI inevitably affects the central canal niche due to the small diameter of the spinal cord. Following SCI, ependymal cell proliferative capacity increases by four to five-fold when compared to their control littermates. These newly proliferating cells are mostly found around the central canal and at the site of injury (Barnabe-Heider et al. 2010; Johansson et al. 1999). However, this proliferating response is unique to SCI, as our group did not previously detect a similar reaction in demyelinating animal models (Lacroix et al. 2014). Although a proportion of the quiescent population found in the spinal cord becomes activated, limited multipotency (absence of neurogenesis) is now observed *in vivo* in the injury model (Horky et al. 2006). Fate-mapping experiments demonstrated that two weeks following SCI, dividing FoxJ1+ ependymal cells mainly give rise to sox9+ astrocytes located at the core of the glial scar. At four months post-injury, these cells also differentiate, but to a lesser extent, into oligodendrocytes in the surrounding uninjured white matter (Barnabe-Heider et al. 2010) (**Fig. I.14 and Fig. 3 of the appendix**).

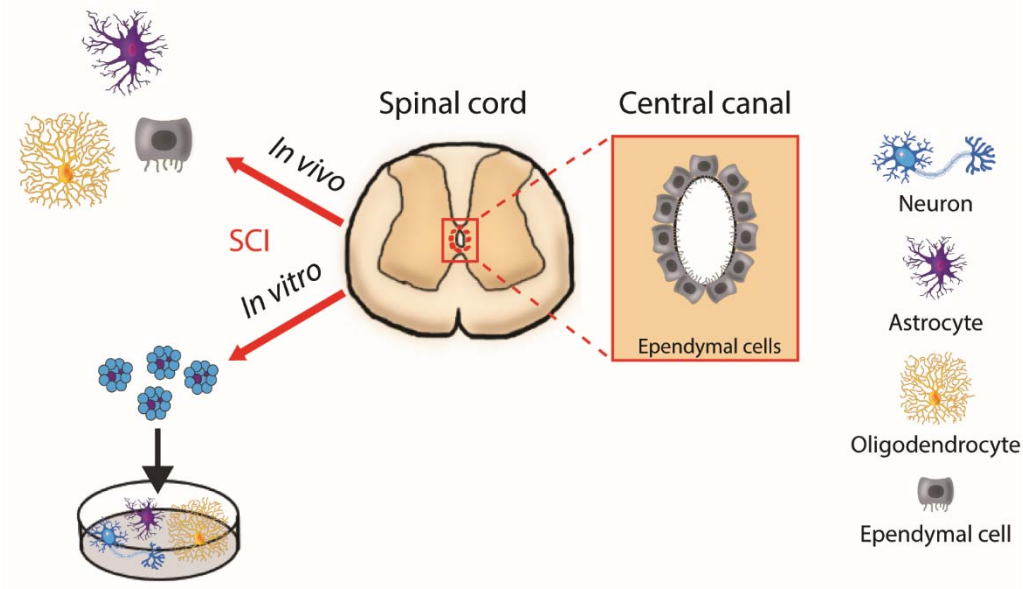


Figure I.14. Central canal ependymal cell behaviour under injury conditions

Under injury conditions, ependymal cells show a different behaviour *in vivo.* These cells still show self-renewal capacities, but also a glia-restricted multipotency. *In vitro*, cell behaviour remains the same as under normal conditions with self-renewal and multipotency characteristics (Created by C-A. Grégoire, Loïc Cochard, and Brianna Goldenstein).

I.4.2.2. SCI-induced changes in the spinal cord niche

The injury triggers acute (minutes) and chronic (years) events that could potentially change the spinal cord microenvironment and affect ependymal cells (**Fig. 3 of appendix**). First, at the site of lesion, damage to the blood-spinal cord barrier occurs within 5 minutes (Bartanusz et al. 2011). This immediate event leads to several other important ones: cell death of neural cells due to oxygen and glucose deprivation (Jullienne and Badaut 2013), axon degeneration and demyelination, and astrogliosis (Rolls et al. 2009; Silver and Miller 2004). These acute events lead to chronic events such as apoptotic death, evident by a 50% drop in the number of astrocytes and oligodendrocytes after 24 hours (Grossman et al. 2001). This cell death triggers the release of myelin debris containing axonal growth-inhibiting molecules (Bartsch et al. 1995; Bregman et al. 1995; Cafferty et al. 2010). Other key events include cyst formation (rats only), formation of a glial scar, and chronic inflammation (Jones et al. 2005; Rolls et al. 2009; Silver and Miller 2004). For additional details on the topic, please refer to our published review found in the appendix (Gregoire et al. 2015).

I.4.2.3. Potential role of inflammation in ependymal cell (NSC) activation

Following SCI, immune cells are activated and release cytokines, leading to a complex immune reaction that may affect ependymal cells (Pineau and Lacroix 2007). Microglia, resident phagocytes, react rapidly after injury (as part of the innate immunity system) and change from their resting branch-stellate state to their round phagocytic shape (Jones et al. 2005). Microglia also act as antigen presenting cells (APC) implicated in the adaptive immune response (Abbas and Lichtman 2006; Covacu and Brundin 2015). Naïve T-cells are activated when exposed to their specific antigen on an APC and their differentiation in T-helper cells (Th1 and Th17 in autoimmune responses, and Th2 in allergic responses) depends on the APC-

released cytokines. T-helper cells will then expand and activate B-cells and cytotoxic T-cells (Abbas and Lichtman 2006; Covacu and Brundin 2015). Other phagocytic cells, such as circulating neutrophils and macrophages, are also activated after injury and invade the lesion site (Abbas and Lichtman 2006; Popovich et al. 1997). Inflammatory cell response is complex as it can be polarized in different ways, secreting different molecules that can lead to either beneficial or detrimental effects (David and Kroner 2011). Microglia can undergo two forms of polarized activation: 1) M1 activation (classic) characterized by the release of pro-inflammatory factors such as interleukin (IL)-1β, tumor necrosis factor-α (TNF-α), and IL-6, and 2) M2 activation (alternative) characterized by anti-inflammatory cytokines release such as IL-4, IL-10 and transforming growth factor-β1 (TGF-β1) (Belarbi and Rosi 2013; Donnelly and Popovich 2008; Jones et al. 2005; Tyor et al. 2002).

There is currently a lack of studies addressing the effect of these released molecules on ependymal cell behaviour specifically (Gregoire et al. 2015). However, these cytokines have been extensively studied in the brain (Covacu and Brundin 2015; Ekdahl et al. 2009). Pro-inflammatory cytokines, as expected, negatively affect hippocampal neurogenesis (Gemma et al. 2007; Iosif et al. 2006; Monje et al. 2003; Vallieres et al. 2002). Vallières and colleagues demonstrated that hippocampal neurogenesis was significantly reduced in transgenic mice that chronically expressed IL-6 in astroglia (Vallieres et al. 2002). Another study showed that reduced hippocampal neurogenesis caused by a lipopolysaccharide (LPS) treatment could be restored *in vitro* following treatment with an anti-inflammatory drug, indomethacin, confirming the detrimental role of neuroinflammation on neurogenesis (Monje et al. 2003). Other *in vivo* studies showed that IL-1β and TNF-α inhibit neurogenesis and/or progenitor proliferation, respectively (Gemma et al. 2007; Iosif et al. 2006). One of the anti-inflammatory cytokines, TGF-β1, has been shown to have pleiotropic effects on cell proliferation, migration, differentiation, and immunological responses (Kleiter et al. 2007; McCartney-Francis and Wahl 1994; Taylor 2009). The Aigner laboratory demonstrated that TGF-β1 acts as a negative regulator of progenitor cell proliferation and as a promoter of stem cell quiescence and neuronal survival, confirming its pleiotropic role (Kandasamy et al. 2014; Wachs et al. 2006). They first showed in cultures that TGF-β1 inhibits proliferation of NSC and progenitor cells, shown by a decrease in sphere size and reduced BrdU incorporation. Moreover, TGF-β1 induced a shift of

cells found in the G2-M, and S phases of the cell cycle to the G0-1 quiescent phase. They then infused TGF-β1 into the lateral ventricle for seven days and observed decreases in numbers of proliferating cells and DCX+ cells in both the SVZ and DG. This effect was maintained for a period of at least four weeks, but differentiation was not affected (Wachs et al. 2006). In a follow-up study, they focused on TGF-β1 signaling (receptors and Smad2) (Kandasamy et al. 2014). They showed that pSmad2 is expressed only in quiescent and post-mitotic neurons. Furthermore, an increase in the number of newly-born neurons was detected despite an original reduction in cell proliferation using a transgenic model expressing TGF-β1 under the doxycycline-controlled Ca-Calmodulin kinase promoter. (Kandasamy et al. 2014). These results have to be interpreted with care as the spinal cord is a very different niche (non-neurogenic) and these factors could induce contrasting effects (Covacu and Brundin 2015; Horky et al. 2006).

I.4.3. Mediators of NSC activation

In the hippocampus, several mediators of NSC activation have been identified, whereas in the spinal cord, the complexity of the immune reaction following SCI makes the isolation of factors more difficult. Unraveling the mechanisms implicated in NSC activation is important as it can contribute to improve cognitive function in a healthy brain and promote repair after a lesion in both an active neurogenic niche and a quiescent niche, respectively. This section is an overview of key studied mediators and is not intended to be an exhaustive review of the literature on this topic.

Factors, such as growth factors and morphogens, were identified on a candidate-based approach as key mediators of hippocampal NSC activation using EE (Cao et al. 2004; Choe et al. 2016; Fabel et al. 2003; Fuentealba et al. 2012; Mu et al. 2010; Rossi et al. 2006; Suh et al. 2009). Following exercise, many growth factors are increased such as FGF-2, insulin-like growth factor-1 (IGF1), and VEGF and these were shown to stimulate neurogenesis in absence of exercise (Aberg et al. 2000; Fabel et al. 2003; Jin et al. 2002; Wagner et al. 1999). It was shown that VEGF is necessary for running-induced neurogenesis by acting directly on neural progenitors, but not for basal neurogenesis (Fabel et al. 2003). Similarly, Cao and colleagues

screened for several growth factors by quantitative real-time RT-PCR after rats were either exposed to four weeks of EE (including a running wheel) or MWM training and showed that VEGF was the only one to mediate both the environmental induction of neurogenesis and improved cognition in rats (Cao et al. 2004). Moreover, the brain-derived neurotrophic factor (BDNF) was also shown to be required for the neurogenesis increase following EE using a BDNF-/- transgenic mouse (Rossi et al. 2006). However, this EE including a running component and it was later confirmed that BDNF is increased by exercise, but not by EE (Kobilo et al. 2011). Noggin, which antagonizes BMP signaling, was shown to increase the number of proliferating GFAP+ putative stem cells *in vivo* (Bonaguidi et al. 2008). A follow-up study further demonstrated that BMP signaling mediates the exercise effect on hippocampal neurogenesis and hippocampus-dependent learning and memory (Choe et al. 2016; Gobeske et al. 2009). Gobeske and colleagues exposed C57Bl/6 mice to running discs for different durations (ranging from 0 to 14 days), and observed decreased BMP4 and increased noggin mRNA and protein levels. These effects were followed by improvements in cell proliferation (including GFAP+ and Sox2+ cells) Y-maze performance. Moreover, when BMP signaling was decreased *in vivo* by noggin intraventricular infusion, mice showed similar cellular and cognitive gains to running mice (Gobeske et al. 2009). A recent study also confirmed that inhibited BMP signaling by noggin leads to neural progenitor activation and maturation (Bond et al. 2014).

Under normal conditions, factors, such as neurotransmitters, were also identified as key mediators of hippocampal NSC activation (Choe et al. 2016; Song et al. 2016; Song et al. 2013; Song et al. 2012). The major inhibitory neurotransmitter in mature neurons, γ-aminobutyric acid (GABA), can be excitatory in NSCs and immature neurons (Ben-Ari 2002; Ge et al. 2006). In NSCs, the Na+-K+-2Cl- co-transporter (NKCC1) is highly expressed at early stages and is responsible for the higher intracellular $[Cl^-]_i$ concentration. The Cl- channels open upon GABA binding and Cl- flows out the cell leading to depolarization. In mature neurons, the K+-Cl- co-transporter (KCC2) now predominates, creating a reduction in $[Cl^-]_i$ and leading to an influx of Cl- upon GABA binding which hyperpolarizes the cell (Ben-Ari 2002; Ge et al. 2006). Song and colleagues discovered that parvalbumin (PV)+ interneurons could dictate the RGL choice between quiescence and activation (Song et al. 2012). Nestin+ RGLs responded tonically to

GABA via the γ2-containing $GABA_AR$ when treated with diazepam (specifically enhances this receptor response to GABA). Following diazepam treatment, a 45% decrease in proliferating RGLs was observed when compared to controls and an inhibition of symmetrical self-renewal, suggesting a quiescent fate choice. This effect could be blocked after a conditional deletion of this receptor. Using an optogenetic approach in PV-Cre mice (using a double-floxed adeno-associated virus to express different optogenetic channels), they demonstrated that adult RGLs respond to PV+ interneurons-released GABA, and that the activation of these interneurons led to a 53% decrease in RGL activation (Song et al. 2012). A follow-up study further showed that PV+ interneurons could also promote the survival of proliferating neuronal progenitors (Song et al. 2013).

SCI is known to activate endogenous ependymal cells, but studies looking specifically at mediators of this activation are almost inexistent (Barnabe-Heider et al. 2010; Lacroix et al. 2014). One group showed that activated spinal cord-derived neural precursor cells following injury had significantly reduced Connexin50 expression, a gap junction protein, compared to cells from uninjured tissue (Rodriguez-Jimenez et al. 2015). Moreover, over-expression of Connexin50 in differentiation conditions led to an astrocytic fate differentiation.

In global events, such as EE and SCI, changes in the environment are complex. Although some studies used a candidate-based approach to try to identify key mediators of NSC activation, it would be surprising that one factor would be responsible for all the observed effects in these situations. Fortunately, recent technological approaches, such as next generation sequencing, allow us to screen for unknown candidates (see section below).

I.5. Next generation sequencing

Next generation sequencing is an unbiased approach that can be used to detect transcriptome changes within a niche or a specific cell population between experimental conditions (Ozsolak and Milos 2011). Pioneer studies on DNA sequencing were conducted by Fredrick Sanger in the 1970s (Sanger et al. 1977). This discovery was followed by hybridization-based microarray technologies that were established at the end of the 20th century (Pinkel et al. 1998). However, this method is based on probes, hence limited to what is already known, and it does not have optimal resolution of the output (Wang et al. 2009; Wilhelm and Landry 2009). Recently, it is the next generation sequencing technologies that has revolutionized the molecular biology field, such as the RNA-Seq. Both microarrays and RNA-Seq look at transcriptomics, consisting in all the transcripts present in a cell and their respective quantities (Wang et al. 2009). As a hypothesis-free approach, RNA-Seq has some advantages. It is not limited to an existing genomic sequence and it can detect 30-bp short reads that give useful information, such as fusion transcripts, splice variants, and mutations (Ozsolak and Milos 2011; Wang et al. 2009). The main steps to create a RNA-Seq library are to 1) purify RNA (make sure to enrich for mRNA as rRNA is not informative), 2) fragment RNA, 3) prime for the reverse transcription reaction with the use of either random primers or oligo dT primers to create double-stranded cDNA for sequencing. 4) Then, you perform end repair, add A overhangs, and add sequence adapters to sequence your cDNAs (may use clonal amplification). Once your library is obtained, you can map it to the reference genome assembly (Ozsolak and Milos 2011; Wang et al. 2009). This method was used in chapters three and four of this dissertation to gain insights into the cellular and molecular changes behind NSC activation.

I.6. Rationale for studies

The importance of hippocampal neurogenesis in learning and memory processes and the potential therapeutic avenues of ependymal cells in the central canal of the spinal cord is of great interest. It shows that NSCs could play important roles in normal brain function and neural repair following injury. However, little information is known about how a quiescent neural stem cell becomes activated in order to perform these functions. The general objective of this dissertation was to investigate the mechanisms underlying activation of neural stem cells in the adult central nervous system. My specific aims were to address this question using adult mice in two complementary models: 1) activation of hippocampal NSCs by environmental enrichment (EE), and 2) activation of spinal cord NSCs by injury-induced neuroinflammation. Moreover, 3) to gain new insights into the molecular mechanisms of these models, we will perform transcriptomics studies to open new lines of investigation. This section summarizes the hypotheses, rationales, and specific objectives of each study to help transition to and better understand the results section.

I.6.1. Components of environmental enrichment (study 1)

Thoroughly understanding the impact of individual EE variables on hippocampal neurogenesis is crucial, as EE can improve some cognitive functions at the expense of others (Woollett and Maguire 2011), and can have unexpected effects under pathological conditions (Komitova et al. 2005; Risedal et al. 2002). *My hypothesis is that physical activity and complex environment act differently on hippocampal neurogenesis*.

Although some studies have concluded that physical activity is the main pro-neurogenic component of EE (Kobilo et al. 2011; Mustroph et al. 2012a), the challenges involved in experimentally separating EE variables has resulted in considerable disagreement concerning their relative influences (Bednarczyk et al. 2011; Fabel et al. 2009; Kannangara et al. 2009; Kronenberg et al. 2003; Mustroph et al. 2012b). As a result, the specific contribution of EE variables to hippocampal neurogenesis is still not well understood.

To address this hypothesis, our *specific objectives* were to: 1) establish a new EE paradigm that allows the stepwise addition/subtraction of individual EE variables, within the CD1 mouse model, and 2) to measure c-fos levels (a marker of neuronal activity), circulating corticosterone levels (a marker of stress levels), and the impact of each EE variable on each stage of adult neurogenesis.

I.6.2. Exercise and social context – Learning and memory (study 2)

From our first article (Gregoire et al. 2014), we demonstrated that exercise was responsible for increased neurogenesis, and we suggested that social interaction within a complex environment led to elevated c-fos expression (neuronal activation). Our *hypothesis is* that considering their different effects observed in the first manuscript, *exercise and social context exposure will lead to different behavioural and transcription responses.*

The factors mediating the hippocampal neurogenesis increase, neuronal activation and cognitive improvements following exposure to individual EE components remain unclear. Based on the findings of the previous study, mice were exposed to an eight-week paradigm including a four-week exposure to either a locked disc (control environment), running disc (continuously), or social environment followed by four weeks of behavioural testing while maintaining the enriched environments in home cages.

To address this hypothesis, our *objectives* were to: 1) evaluate the performance of CD1 mice on the 8-arm radial maze and novel object recognition test, 2) confirm the presence of hippocampal neurogenesis at the end of a longer paradigm, and 3) perform a RNA-Seq to understand the cellular and molecular changes occurring in the DG from mice exposed to either a locked disc, running disc, or a complex environment (including social interactions).

I.6.3. Impact of TGF-β1 on ependymal cells (NSCs) (study 3)

For this last project, we collaborated with Dr. Steve Lacroix's laboratory to isolate ependymal cells from FoxJ1-eGFP mice using fluorescence-activated cell sorting following sham treatment or SCI. An RNA-Seq of these purified cells before and after injury was performed to obtain the transcriptome of activated ependymal cells. One of the main factors found from this analysis was TGF-β1. Our *hypothesis is that chronically released TGF-β1 is the inflammatory cytokine involved in the transition from a quiescent to an activated state of the ependymal cells.*

TGF-β1 is another secreted factor following injury that plays a central role in wound healing in many tissues. TGF-β1 has been shown to have a pleiotropic role in cell growth, differentiation, organ development, migration, pro-inflammatory and anti-inflammatory processes, and wound repair (Kleiter et al. 2007; McCartney-Francis and Wahl 1994; Taylor 2009). Following SCI, TGF-β1 is highly expressed by inflammatory cells, with levels peaking at 48 hours post-SCI and maintaining for several days (Buss et al. 2008; McTigue et al. 2000). Previous studies have shown that this cytokine plays opposing roles after SCI, as TGF-β1-treated animals showed a 50% reduction in lesion volume by 48 hours post-SCI (Tyor et al. 2002), while TGF-β1-blocking antibodies inhibit glial scar formation and improve locomotor activity (Kohta et al. 2009). Therefore, TGF-β1 represents an excellent candidate for a potential role in the activation of ependymal cells following a lesion.

To address this hypothesis, our *specific objectives* are to: 1) confirm the presence of TGF-β1 mRNA around the central canal after SCI, 2) infuse TGF-β1 *in vivo* for six days to detect the effect on proliferation and cell fate and to put into culture the spinal cord to analyze the impact on neurosphere growth, 3) analyze proliferation, survival, and differentiation in high-density stem/progenitor cell cultures, 4) study the indirect effects of TGF-β1 using conditioned medium in cultures, and 5) block TGF-β1 signaling to analyze its endogenous effect, its effect

on neurosphere growth at different time points (acting on recruitment or expansion of neurosphere-initiating cells), and its effects *in vivo*.

Chapter II. Untangling the influences of voluntary running, environmental complexity, social housing and stress on adult hippocampal neurogenesis

II.1. Article context

Although some studies have concluded that physical activity is the main pro-neurogenic component of EE (Kobilo et al. 2011; Mustroph et al. 2012a), the challenging difficulties involved in experimentally separating EE variables has resulted in considerable disagreement concerning their relative influences (Bednarczyk et al. 2011; Fabel et al. 2009; Kannangara et al. 2009; Kronenberg et al. 2003; Mustroph et al. 2012b). The specific contribution of EE variables to hippocampal neurogenesis is still not well understood. We, therefore, established a novel paradigm that allows us to clearly discriminate between the impact of specific components of EE.

II.2. Authors' contributions

In this article, I designed, performed, and analyzed most of the experiments with the helpful advices of my research director, Dr. Karl Fernandes. David Bonenfant, a summer intern, helped me, under my supervision, with the experiments conducted for Figures II.5-6. Adalie Le Nguyen, another summer intern, also helped me setting up the paradigm described in Figure II.1 as well as for immunofluorescence of Figure II.2, under my supervision. Finally, our laboratory technician, Anne Aumont, helped with the intermittent EE paradigm described in Figure II.1 as well as the paradigms for Figure II.5-6. The manuscript was written by myself in collaboration with Dr. Karl Fernandes.

Untangling the influences of voluntary running, environmental complexity, social housing and stress on adult hippocampal neurogenesis

Catherine-Alexandra Grégoire[1,2], David Bonenfant[1], Adalie Le Nguyen[1], Anne Aumont[1,2], and Karl J.L. Fernandes[1,2]

[1]Department of Pathology and Cell Biology, Groupe de recherche sur le système nerveux central (GRSNC), and Center of Excellence in Neuroscience of the Université de Montréal (CENUM), Université de Montréal, Montréal, Canada.

[2]Present address: Department of Neurosciences, Faculty of Medicine, and Centre de Recherche du Centre Hospitalier de l'Université de Montréal (CR-CHUM), Université de Montréal.

Corresponding author: [redacted]

Abbreviated Title: EE variables and hippocampal neurogenesis

Keywords: Alternating EE, neural stem cells, environmental complexity, stess, social interactions **Conflict of Interest:** None

Number of Pages: 38
Number of Figures: 7
Number of Words: Abstract - 248

Acknowledgements: The authors are grateful to members of the Fernandes laboratory, as well as the thesis advisors, Dr. Laurent Descarries, Dr. Graziella Di Cristo and Dr. Lionel Carmant, for helpful discussions and technical advice. Vertical running wheels were kindly provided by Dr. Raynald Bergeron (Université de Montréal). CAG is funded by a studentship from the Université de Montréal Faculté des Études Supérieures. KF is supported by a Canada Research Chair in Neural Stem Cell Biology. Operating funds were provided by the Canadian Natural Sciences and Engineering Research Council (NSERC).

II.3. Abstract

Environmental enrichment (EE) exerts powerful effects on brain physiology, and is widely used as an experimental and therapeutic tool. Typical EE paradigms are multifactorial, incorporating elements of physical exercise, environmental complexity, social interactions and stress, however the specific contributions of these variables have not been separable using conventional housing paradigms. Here, we evaluated the impacts of these individual variables on adult hippocampal neurogenesis by using a novel “Alternating EE” paradigm. For 4 weeks, adult male CD1 mice were alternated daily between two enriched environments; by comparing groups that differed in one of their two environments, the individual and combinatorial effects of EE variables could be resolved. The Alternating EE paradigm revealed that (1) voluntary running for 3 days/week was sufficient to increase both mitotic and post-mitotic stages of hippocampal neurogenesis, confirming the central importance of exercise; (2) a complex environment (comprised of both social interactions and rotated inanimate objects) had no effect on neurogenesis itself, but enhanced depolarization-induced c-Fos expression (attributable to social interactions) and buffered stress-induced plasma corticosterone levels (attributable to inanimate objects); and (3) neither social isolation, group housing, nor chronically increased levels of plasma corticosterone had a prolonged impact on neurogenesis. Mouse strain, handling and type of running apparatus were tested and excluded as potential confounding factors. These findings provide valuable insights into the relative effects of key EE variables on adult neurogenesis, and this "Alternating EE" paradigm represents a useful tool for exploring the contributions of individual EE variables to mechanisms of neural plasticity.

II.4. Introduction

It has been known for over half a century that the surrounding environment affects the anatomy, chemistry, and functional properties of the brain (Hebb 1947; Hubel and Wiesel 1970; Rosenzweig et al. 1962; Wiesel and Hubel 1965). Early studies showed that rodents exposed to environmental enrichment (EE), consisting of large cages, social enrichment and diverse multisensory stimulation, displayed increased brain sizes, altered neurotransmitter levels and behavioral changes (Bennett et al. 1969; La Torre 1968; Manosevitz and Joel 1973; Rosenzweig et al. 1962). Likewise, in humans, life experiences such as spatial memory training (Proulx et al. 2014; Schwabe and Wolf 2012; Woollett and Maguire 2011), cardiovascular exercise (Aberg et al. 2009; Herting and Nagel 2012; Woollett and Maguire 2011), sensory deprivation (Proulx et al. 2014), and stress (de Quervain et al. 2000; Schwabe and Wolf 2012) can affect learning and memory. The profound effect of environmental parameters on brain function has led to the widespread use of EE paradigms as tools for both research and rehabilitation. However, our basic understanding of this phenomenon remains remarkably nebulous. Diverse EE paradigms are now used, with little understanding of how differences in individual EE variables (such as physical exercise, cognitive stimulation, stress, and social interactions) might impact on specific downstream biological mechanisms, such as changes in neurotrophic factor synthesis (Ickes et al. 2000), dendritic growth (Fiala et al. 1978; Leggio et al. 2005; Volkmar and Greenough 1972), synaptic plasticity (Liu et al. 2012), electrophysiological properties (Green and Greenough 1986) and adult neurogenesis (Kempermann et al. 1997b).

The hippocampal dentate gyrus (DG) is a rare niche where neurogenesis is preserved throughout life (Eriksson et al. 1998; Kempermann et al. 1998; Ming and Song 2005; Spalding et al. 2013). DG neurogenesis is a multi-step process in which radial glia-like precursors generate proliferating progenitors and neuroblasts that mature into DG granule neurons implicated in learning, memory and mood regulation (Deng et al. 2009; Kempermann 2002; Kitamura et al. 2009; Saxe et al. 2006; Snyder et al. 2005). It is now well established that EE modulates adult hippocampal neurogenesis (Kempermann 2002; Kempermann et al. 1997b). Recent studies have identified physical activity as an important proneurogenic stimulus within EE (Kobilo et al. 2011; Mustroph et al. 2012a). However, previously used housing paradigms could not unambiguously separate the effects of running from other EE variables, such as environmental complexity, social context and

stress, which are also reported to influence neurogenesis (Bednarczyk et al. 2011; Fabel et al. 2009; Kannangara et al. 2009; Kronenberg et al. 2003; Leasure and Decker 2009). Clearly defining the relative and/or combinatorial effects of such variables is essential for the rational design of EE paradigms, as EE can improve some cognitive functions at the expense of others (Woollett and Maguire 2011), and can have unexpected consequences under pathological conditions (Komitova et al. 2005; Risedal et al. 2002).

Here, we developed a novel "Alternating EE" paradigm to experimentally isolate EE variables and to gain insights into their specific contributions to adult hippocampal neurogenesis.

II.5. Materials and methods

A total of 129 two-month-old male CD1 mice (Charles River, Senneville, QC, Canada) and eight 2-month-old male C57BL/6 mice (Charles River, Senneville, QC, Canada) were used in these studies. All experiments were conducted in accordance with the guidelines of the Canadian Council of Animal Care and were approved by the Animal Care committee of the Université de Montréal.

II.5.1. Housing conditions and Experimental groups: Alternating EE paradigm

All animals were provided with food and water *ad libitum,* and all environments contained nesting material and a basic litter (PRO-CHIP 8-16, PWI brand). Four types of housing environments were used in the Alternating EE paradigm, as shown in **Fig. II.1B** and detailed here:

Empty environment: Animals were housed in empty 24.0 cm x 44.0 cm x 20.0 cm rat cages. Mice were housed either individually or in groups of 3, depending on whether they were in the Impoverished or Social housing experimental groups (below).

Locked disc environment: Animals were housed in 24.0 cm x 44.0 cm x 20.0 cm rat cages containing a locked or unlocked running disc (Red mouse igloo, K3327, and amber fast- trac running disc, 7.5 cm in diameter, K3250, Bio-Serv, Frenchtown, NJ, USA). Running cages were outfitted with odometers (Sigma BC509) to measure the running distance. Mice were housed individually.

Running disc environment: Identical to the Locked disc environment except that the running disc was unlocked to permit voluntary running.

Complex environment: Animals were housed in 24.0 cm x 44.0 cm x 20.0 cm rat cages that contained an igloo, locked running disc and colored tunnels for hamsters (Habitrail, 8 inches green trail, yellow curve, blue U-turn, transparent tee and blue elbow). Tunnels were re-oriented and their conformations re-arranged at each cage alternation (4 times/week). Mice were housed in groups of 3. This *Complex* environment thus provides social interactions, inanimate objects, and frequent conformational novelty.

***Experimental groups used in the Alternating EE paradigm**:* The Alternating EE paradigm was repeated using two separate cohorts of mice (n=36-42/cohort) in order to obtain sufficient tissues for analysis of all markers. One cohort received two intraperitoneal injections of 5-bromo-2-deoxyuridine (BrdU, Sigma-Aldrich, Oakville, ON, Canada, 100mg/kg) at 9am and 4pm on the first experimental day to assess cell survival. Mice were randomized and separated into one of 7 different groups. Each group alternated between 2 housing conditions, 6 times per week for 4 weeks, as shown in Fig. 1A-D and described here: 1) Maximal enrichment ("MAX", n=6) mice were alternated between the *Running disc* and the *Complex* environments, 2) Intermittent Running ("I-RUN", n=6) mice were alternated between the *Running disc* and the *Locked disc* environments, 3) Intermittent Complex Environment ("I-CPX", n=6) mice were alternated between the *Locked disc* and the *Complex* environments, 4) Minimal enrichment ("MIN", n=6) mice were alternated between identical *Locked disc* environments, 5) Impoverished ("IMP", n=6) mice were alternated between identical *Empty* environments and were housed individually, 6) Continuous Running ("C-RUN", n=6) mice were alternated between identical *Running disc* environments, 7) Social housing ("SOC", n=6) mice were alternated between identical *Empty* environments and were housed in groups of 3. Mice used for analysis of c-fos immediate early gene expression remained within their enriched environments until time of anesthetic overdose.

II.5.2. Housing conditions: Strain comparison, Handling effect, and Wheel versus Disc paradigms

All animals were provided with food and water *ad libitum,* and all environments contained nesting material and a basic litter (PRO-CHIP 8-16, PWI brand).

***Strain Comparison**:* 2-month-old adult male C57BL/6 mice (n=8) and 2-month-old adult male CD1 mice (n=8) were individually housed in 24.0 cm x 44.0 cm x 20.0 cm rat cages. Mice were exposed to either *Empty* (n=4/strain) or *Running disc* environments (n=4/strain) for 4 weeks.

***Handling Effect**:* 2-month-old adult male CD1 mice (n=12) were individually housed in 24.0 cm x 44.0 cm x 20.0 cm rat cages. Mice were separated into No Handling and Handling groups (n=6/group). The No Handling group was exposed to the *Empty* environment for the entire period and was only handled only twice (cage changing), while the Handling group was treated as for the Intermittent EE paradigm, i.e., holding the mouse by the tail and transferring it to another

cage 6 days a week for a total of 24 times during the 4-week experimental period.

***Wheel vs. Disc**:* 2-month-old adult male CD1 mice (n=25) were randomized and separated into one of the five environments (n=5/group): 1) *Empty*, 2) *Locked wheel*, 3) *Locked disc* 4) *Running wheel* and 5) *Running disc*. Mice were individually housed in either 17.0 cm x 28.0 cm x 12.5 cm mouse cages (*Empty*), 12.7 cm x 20.3 cm x 35.6 cm cages (*Locked* and *running wheel*, 22.9 cm diameter wheel) or 24.0 cm x 44.0 cm x 20.0 cm rat cages (*Locked* and *running disc*).

II.5.3. Tissue Preparation

Mice received a lethal dose of chloral hydrate (7%), followed by a dose of Xylazine (0.1%) and were then perfused trans-cardially with 30 mL of 1X phosphate-buffered saline (PBS, pH 7.4) (PBS 10X, Wisent, 311-012-CL), followed by 40 mL of 4% formaldehyde fixative solution (freshly hydrolyzed from 4% paraformaldehyde, pH 7.4, Fisher, T353-500). The brains were removed and post-fixed in 4% formaldehyde overnight and then kept in PBS at 4□C until sectioning. The entire brain of each animal was cut into 40µm coronal sections using a vibrating microtome (Leica VT1000S, Leica Microsystems, Richmond Hill, ON, Canada), and the tissue sections were stored at -20ºC in an antifreeze solution (glycerol:ethylene glycol:PBS 1X, 3:3:4).

II.5.4. Immunohistochemistry

Primary antibodies used in this study were mouse anti-human Ki67 (1:200, BD Biosciences, Mississauga, ON, Canada, 556003), goat anti-rabbit NeuroD (1:500, Santa Cruz Biotechnology, Santa Cruz, CA, SC-1084), goat anti-human Doublecortin (DCX; 1:500, Santa Cruz Biotechnology, SC-8066), rabbit anti-human Calretinin (1:2500, Swant, Bellinzona, Switzerland, CR7699/3H), rabbit anti-human c-fos (1:5000 for fluorescence, 1:20000 for DAB, Calbiochem, San Diego, CA, PC38), mouse anti-cow S100β (1:1000, Sigma-Aldrich, Oakville, ON, Canada, S2532), rat anti-BrdU (1:800, AbD Serotec, Oxford, UK, MCA2060) and mouse anti-mouse Neuronal nuclei (1:100, NeuN, Milllipore, Temecula, CA, MAB377).

For Calretinin and c-fos immunohistochemistry and BrdU/NeuN/c-fos triple

immunofluorescence, the labeling procedure was performed as previously described (Bednarczyk et al., 2011). For Ki67 and NeuroD immunohistochemistry, the protocol was modified to include an antigen retrieval step. Free-floating 40-µm sections were washed in PBS, mounted onto glass slides, post-fixed with 4% formaldehyde solution for 10 minutes, washed with PBS, and then incubated for 40 minutes in a Citrate-EDTA (10mM Citric Acid, 2mM EDTA, 0.05% Tween 20, pH 6.2) antigen retrieval solution. They were then blocked for 2 hours in 4% bovine serum albumin (BSA)/0.1% Triton-X/PBS (for Ki67) or 10% normal donkey serum (NDS)/0.1% Triton-X/PBS (for NeuroD). Sections were incubated overnight at room temperature in primary antibodies diluted in either 2% BSA or 5% NDS in PBS.

II.5.5. Corticosterone assay

Blood samples were collected from the anaesthetized animals, prior to cardiac perfusion, using a 23-gauge needle inserted into the posterior *vena cava*. Interval between anaethesia injection and blood collection was approximately 5 minutes. Blood samples were transferred to a microtainer containing K2EDTA (BD Biosciences, Mississauga, ON, 365974), inverted 20 times, and the plasma extracted after centrifuging at 1000g for 15 minutes at 4°C. Samples were stored at -80ºC. Plasma was diluted 1:200 and plasma corticosterone concentrations were assayed in duplicate using an enzyme-linked immunoassay kit, according to the manufacturer instructions (Cayman Chemical, Ann Arbor, MI; #500655).

II.5.6. Cell quantifications

For the Alternating EE paradigm, the number of SGZ/GZ cells positive for Ki67, NeuroD, Calretinin, and BrdU was quantified on every 6th section between Bregma -1.06mm and -2.98mm of the hippocampus (8 sections total/marker/animal). The raw cell counts were corrected for oversampling due to split cells by multiplying by (1 - object diameter/section thickness), where the object diameter refers to the average diameter of the marker in question. Mean object diameters were determining by measuring the diameter of 100 positive cells for each marker (NIH ImageJ, 64-bit Java software for Mac), and yielded correction factors of 0.77 (Ki67, NeuroD), 0.75 (Calretinin) and 0.79 (BrdU, c-fos). The corresponding SGZ/GZ reference volumes of the sections

were determined using the Cavalieri principle (grid size of 10 microns, 20x objective) in StereoInvestigator (MBF Bioscience, VT). The mean cell density was then obtained by dividing the corrected total number of marker-positive cells on the sampled sections by the sum of the section SGZ/GZ reference volumes. Results are expressed as density of marker-positive cells per mm^3 of SGZ/GZ. Cell counts were performed manually by a blinded observer using a 40X objective, and slide codes were only broken after all quantifications had been completed for any given marker.

For the control experiments (Strain comparison, Handling, Wheel vs. Disc), raw cell counts were corrected for oversampling as above and then multiplied by 6 to obtain an estimate of the total number of marker-positive cells between the Bregma coordinates.

Triple immunofluorescence stainings for BrdU/NeuN/c-fos and for BrdU/S100beta/DCX were performed to determine i) the proportion of c-fos-positive cells that co-express the mature neuronal marker NeuN, and ii) the proportions of BrdU-positive cells that co-express NeuN, DCX or S100β. To do so, each c-fos or BrdU-positive cell in a complete 1-in-6 series of sections was brought into focus in turn using a 40x objective (400x total magnification) and scored for absence or presence of coexpression of the co-labels. In the case of BrdU/NeuN/c-fos staining, the NeuN antibody penetration through the tissue was observed to be incomplete; to avoid obtaining false NeuN-negative cells, NeuN double-labelling analyses were therefore restricted to the z-levels of NeuN antibody penetration.

II.5.7. Statistical analyses

Statistical analyses were performed using SAS 9.3 statistical analysis software (SAS Institute). All experimental groups were first analysed together by One-way ANOVA. Rejection of the null hypothesis was followed by the application of specific contrasts (linear combination of the means) that tested 7 pre-defined hypotheses summarized in Fig. 1D. Only these 7 specific comparisons between experimental groups were made, in order to restrict statistical

analyses to groups that differ in only one experimental variable. Significance level was set at $p=0.05$.

II.6. Results

II.6.1. Design of the Alternating EE paradigm

To effectively isolate the effects of running, environmental complexity, social interactions and stress, we devised an "Alternating EE" paradigm that would: 1) allow for the stepwise addition/subtraction of EE variables between conditions of maximal and minimal enrichment, 2) preserve as much uniformity as possible across groups, enabling statistical comparisons to be focused on groups for which only a single independent variable had been altered, and 3) in the case of running environments, permit individualized measurement of running distances. The Alternating EE paradigm is based on intermittent exposures to EE (**Fig. II.1A**). Over a 4-week period, adult male mice were alternated daily between two types of basic environments (6 times per week); by comparing experimental groups in which only one of the two environments differed, individual EE variables could be effectively isolated.

Four basic environment types were used to construct the Alternating EE experimental groups (detailed in the Methods and shown in **Fig. II.1B**): an *Empty* environment that was devoid of all objects (1 mouse or 3 mice per cage); a *Locked disc* environment containing a non-functional running disc apparatus (1 mouse per cage); a *Running disc* environment providing voluntary access to a functional running disc (1 mouse per cage); and a *Complex* environment that consisted of inanimate objects (a locked disc and colored tunnels), social enrichment (3 mice per cage), and conformational novelty (change of object organization).

Four Alternating EE experimental groups (**Fig. II.1C**) were used to test for primary influences of the *Running disc* and the *Complex* environments. The Maximal Enrichment ("MAX") group was sequentially exposed to both the *Running disc* and the *Complex* environments. The Intermittent Running ("I-RUN") and Intermittent Complex ("I-CPX") groups alternated between a *Locked disc* environment and either a *Running disc* or *Complex* environment, respectively. A Minimal Enrichment ("MIN") group served as the baseline control group and alternated between identical *Locked disc* environments. This approach allows statistical comparisons to be focused on groups that spend about half their time in identical environments, and enabled us to test for individual and combinatorial effects of the running and complex environment variables. Besides these four main Alternating EE groups, three additional groups were also used (**Fig. II.1C**): an

Impoverished ("IMP") group that alternated between *Empty* environments and was individually housed (allowing assessment of the effect of the Locked disc by comparison to the MIN group), a Continuous Running ("C-RUN") group that alternated between *Running disc* environments (allowing assessment of differences between 3 and 7 days of running by comparison to the I-RUN group), and a Social Housing ("SOC") group that alternated between *Empty* environments but that was housed 3 mice/cage (allowing assessment of differences between individual and social housing by comparison to the IMP group). A summary of the 7 pre-determined groupwise comparisons and hypotheses is presented in **Fig. II.1D**.

Several additional features of the experimental design should be noted. First, we used a 4-week experimental paradigm in order to focus on the effects of longer term EE, as previous studies have shown transient effects of some forms of EE on certain aspects of neurogenesis (Kronenberg et al. 2006; Stranahan et al. 2006). Second, animals were individually housed during their exposure to running discs, ensuring that running data could be collected for each animal. Lastly, we used outbred CD1 mice rather than the more commonly used C57BL/6 strain, in order to ensure that weaker neurogenic effects of EE are not masked by the high baseline neurogenesis reported in C57BL/6 mice (Clark et al. 2011b). Indeed, in our own control experiments, we found that male CD1 mice exhibited a 47% lower baseline proliferation rate compared to age-matched C57BL/6 mice (C57BL/6: 2672 ± 166.9 vs. CD1: 1427 ± 129.2 Ki67+ cells/DG), permitting a running-induced increase to be detectable with as little as 4 mice/group in CD1 but not C57BL/6 mice (C57BL/6 RUN: 2696 ± 281.8 vs. CD1 RUN: 2639 ± 240.2 Ki67+ cells/DG) (not shown).

II.6.2. Running, but not the Complex environment, stimulates hippocampal neurogenesis

We began by examining whether the Alternating EE groups exhibit differences in key parameters of adult neurogenesis (proliferation, neuroblast, immature neuron, and survival). Immunohistochemistry was performed for Ki67+ proliferating cells (**Fig. II.2A**), NeuroD+ neuroblasts (**Fig. II.2B**), Calretinin+ maturing post-mitotic neurons (Brandt et al. 2003; Todkar et al. 2012) (**Fig.II.2C**), and BrdU+ surviving cells (**Fig.II.2D**). Since one-way ANOVA showed differences for all four markers, statistical contrasts were applied to test our 7 pre-determined hypotheses. As shown in **Fig.II.2A-D**, running had a significant effect on all four neurogenic

markers compared to the locked disc (I-RUN vs. MIN groups). Conversely, the Complex environment had no significant effect compared to the locked disc (I-CPX vs. MIN). While running increased the density of NeuroD+ and BrdU+ cells when combined with a complex environment (MAX vs. I-CPX), complex environment failed to potentiate the effects of Running at any stage of neurogenesis (MAX vs. I-RUN). Since the locked disc itself did not have an elevated baseline neurogenesis (MIN vs. IMP), it is not responsible for masking possible neurogenic effects of the Complex environment. Besides running, the only variable impacting neurogenesis was social housing: socially housed mice in the impoverished environment exhibited a small but significant increase in NeuroD+ neuroblasts compared to individually housed impoverished mice (IMP vs. SOC). Co-labelling of the surviving BrdU+ cells showed that there was no primary effect of running or the complex environment on the proportions of BrdU+ cells that had acquired phenotypes of NeuN+ mature neurons, DCX+ neuroblasts or S100β+ astrocytes. I-RUN mice had a small but significant increase in NeuN-labelled BrdU+ cells compared to C-RUN mice.

Interestingly, Ki67, NeuroD, Calretinin and BrdU+ cells were all increased to a similar extent in mice that ran 3d/week versus 7d/week (I-RUN vs C-RUN groups). In fact, comparison of the three groups containing running mice (MAX, I-RUN and C-RUN groups) showed that despite the fact that C-RUN mice ran 2-3x greater total distances on average (MAX=130.5 ±16.92 km; I-RUN=153.8 ± 15.09 km; C-RUN=329.5 ± 27.26 km), they did not achieve higher levels of any neurogenic marker than intermittently running I-RUN or MAX mice (**Fig. II.2A-D**).

These results demonstrate that intermittent exposure to running discs for 4 weeks significantly increases the proliferative, neuroblast, post-mitotic and cell survival stages of DG neurogenesis, while comparable exposure to a complex environment comprised of inanimate objects, social interactions and conformational novelty has no effects on these stages of neurogenesis.

II.6.3. The Complex environment, but not running, increases depolarization-associated c-fos expression

To determine whether the Alternating EE groups exhibited differences in the pattern of DG activation, we analyzed the numbers of dentate granule cells expressing the depolarization- induced immediate early gene, c-fos (**Fig. II.3**). Since dentate granule neurons are generated in an outside-in layering pattern during development, with older DG neurons being found in the outer granule cell layer and more recently born neurons in the inner region (Mathews et al. 2010), we quantified inner and outer c-fos-expressing DG neurons separately (**Fig. II.3A**). In contrast to its lack of effects on neurogenesis, the complex environment significantly increased the density of c-fos-expressing cells in both the inner and outer GCL compared to the locked disc (I-CPX vs. MIN)(**Fig.II.3B,C**). Conversely, running did not alter c-fos expression in the outer GCL and reduced its expression in the inner GCL (I-RUN vs. MIN) (**Fig. II.3B,C**). Running did not modify the ability of the complex environment to increase c-fos expression (MAX vs. I-CPX), while the complex environment increased c-fos expression in intermittently running mice (MAX vs. I-RUN). Mice running for 3 or 7 days per week exhibited low levels of c-fos expression (I-RUN vs. C-RUN), with C-RUN mice tending towards lower levels ($p=0.0967$ in outer GCL, $p=0.1044$ in inner GCL). The locked disc had no difference in c-fos compared to isolated impoverished mice (MIN vs. IMP). Interestingly, social housing tended to increase c-fos expression compared to isolated mice (IMP vs. SOC) ($p=0.0548$ in outer GCL, $p=0.0754$ in inner GCL), suggesting that the social interaction component of a complex environment is responsible for its stimulation of c-fos expression.

Since the dorsal and ventral DG play roles in distinct hippocampal-dependent functions (Kheirbek et al. 2013; Snyder et al. 2009), we also separated the c-fos quantifications according

to rostral (dorsal) and caudal (ventral) sections and found that the effects of the *Complex* environment on c-fos expression were not regionally specific: c-fos-expressing cells were distributed across both rostral and caudal dentate gyrus, and the *Complex* environment stimulated c-fos expression to a similar extent in both regions (**Fig. II.3D**).

These results indicate that the complex environment increases depolarization-associated c-fos expression in the DG, likely mediated by the social interaction component, while running decreases basal c-fos expression.

II.6.4. The Complex environment reduces plasma corticosterone

Stress has been shown to negatively regulate hippocampal neurogenesis (Mitra et al. 2006; Schoenfeld and Gould 2012), but paradoxically, increased levels of circulating stress hormones have also been positively associated with EE-induced neurogenesis (Stranahan et al. 2006; Wosiski-Kuhn and Stranahan 2012). In order to test whether chronic changes in stress- induced hormones are observed and potentially involved in the persistent effects of the *Running disc* and/or the lack of effects of *Complex* environment on adult neurogenesis, we assessed blood plasma levels of the stress-induced hormone, corticosterone, at the end of the 4-week Alternating EE paradigm (**Fig. II.4**).

Compared to the locked disc environment, plasma corticosterone concentrations were diminished by the complex environment (I-CPX vs. MIN) and unchanged by the running environment (I-RUN vs. MIN). There was no difference in corticosterone concentrations in mice running for 3 or 7 days per week (I-RUN vs. C-RUN). The suppressive effect of the complex environment still occurred when used in alternation with running (MAX vs. I-RUN), while running did not alter the effect of the complex environment (MAX vs. I-CPX). Corticosterone levels were not affected by the presence of the locked disc (MIN vs. IMP) or by individual vs. social housing of mice in the impoverished environment (IMP vs. SOC).

These data yield several informative observations. First, since groups exposed to the complex environment (I-CE and MAX) have low plasma corticosterone, their lack of neurogenic effects cannot be attributed to elevated stress. Second, since running groups with both high corticosterone (I-RUN and C-RUN) and low corticosterone (MAX) displayed comparable running-induced increases in neurogenesis (**Fig. II.2**), chronically increased corticosterone is neither required for nor adversely affects running-induced neurogenesis. Third, since two of the elements in the complex environment (social interactions and the locked disc) had no effect on corticosterone levels by themselves, it is likely that the regularly rotated tunnels are essential for the corticosterone suppression observed.

II.6.5. Absence of neurogenic effects of the Complex environment is not due to daily handling or type of running apparatus

Our preceding data using the Alternating EE paradigm indicated that the *Complex* environment enhances DG neuronal activity but does *not* enhance adult neurogenesis. We next sought to eliminate the possibility that neurogenic effects of the *Complex* environment might be masked by intrinsic features of our Alternating EE paradigm: specifically, by an increased basal level of neurogenesis due to i) the daily handling of mice associated with alternation between environments or ii) the use of horizontal running discs versus vertical running wheels.

To evaluate whether four weeks of daily handling was capable of altering the basal levels of hippocampal neurogenesis (**Fig. II.5**), we repeated the handling that was executed between two *Empty* cages (i.e., IMP group) during the main experimental paradigm and compared the results with a group of mice that was maintained for 4 weeks without any daily handling (**Fig. II.5A**). The Handling group was handled 6 times per week while the No Handling group was only manipulated only once every 2 weeks (cage changing). Quantification of immunohistochemical results showed no changes in the estimated numbers of Ki67+ cells (**Fig. II.5B**), Calretinin+ cells (**Fig. II.5C**), and c-fos+ cells (**Fig. II.5D,E**). These data indicate that the absence of effects of the *Complex* environment on neurogenesis is not due to a handling-induced increase in basal neurogenesis.

Since several types of running equipment are used in the EE paradigms in the literature, we also tested the possibility that the horizontal running discs and igloos used in the present study might serve as greater baseline enrichment than commonly used vertical running wheels (Clark et al. 2011b; Fabel and Kempermann 2008; Kronenberg et al. 2006; van Praag et al. 1999b). We therefore compared neurogenesis in mice following 4 weeks of exposure to an Empty cage, to Locked or Running wheels, or to the Locked or Running discs used throughout this study (**Fig. II.6A**). Interestingly, mice running on discs ran 84 % more than mice on running wheels (Running disc: 13.80 km/day, Running wheel: 7.49 km/day; $p<0.0001$, **Fig. II.6B**). Immunohistochemical analysis and quantification revealed that mice exposed to Locked wheels and Locked discs had equal estimated numbers of Ki67+ cells (**Fig. II.6C**), NeuroD+ cells (**Fig. II.6D**), and Calretinin+ cells (**Fig. II.6E**), and no difference compared to the control mice, confirming that the locked running apparatus did not have any persistent effects on neurogenesis. Mice housed with Running wheels or Running discs likewise achieved equal levels of running-induced Ki67 cells, NeuroD+ cells and Calretinin+ cells (**Fig. II.6C-E**), despite their significantly greater running distances on discs than on wheels.

These findings reveal that the absence of neurogenic effects of the *Complex* environment in the Alternating EE paradigm is not due to masking by an elevated basal rate of neurogenesis caused by the mouse handling or the type of running apparatus

II.7. Discussion

EE has gained widespread use as a means for enhancing brain function, both as an experimental tool and for rehabilitative therapy. However, a lack of understanding of this important phenomenon has led to the use of widely varying EE paradigms, with little comprehension of the potential consequences. It has therefore become imperative to better define the contributions of individual EE variables to specific underlying neural mechanisms (Pang and Hannan 2013), including adult neurogenesis. Here, we developed a novel "Alternating EE" paradigm that facilitates experimental isolation of key EE variables, and we used this paradigm to assess the impact of voluntary exercise, environmental complexity, stress and social interactions on hippocampal neurogenesis (summarized in **Figure II.7**).

II.7.1. Running

It is well established that exposure to an enriched environment containing a running wheel enhances adult hippocampal neurogenesis (Kobilo et al. 2011; Mustroph et al. 2012a; van Praag et al. 1999b). The picture that has emerged from such studies is that running itself is likely to be the prime positive regulator of neurogenesis within such an environment (Kobilo et al. 2011; Mustroph et al. 2012a). Unfortunately, direct comparison of data across previously published studies is hampered by differences among uncontrolled variables in the diverse EE paradigms that have been used. For instance: group housing is often considered a more naturalized environment, but it introduces significant social variables and prevents acquisition of individualized running data; few previous studies have used a locked running apparatus to control for its running-independent influences; and conclusions regarding the effects on adult neurogenesis have often been based on only a single stage analysis of the neurogenic pathway (typically BrdU-NeuN to label the total number of newly generated neurons).

Our data confirm and extend upon previous conclusions concerning the role of running. As expected, findings obtained using our Alternating EE paradigm are consistent with recent studies showing that running is indeed the principle neurogenic stimulus within an enriched environment (Kobilo et al. 2011; Mustroph et al. 2012a). We were able to rule out any

significant environmental complexity component of the running apparatus, as we controlled for the presence of the running disc itself. Our data revealed that the neurogenic effects of a 4-week running paradigm were not improved by a *Complex* environment, were not dependent on social context, and were not affected by stress-associated corticosterone levels (discussed individually below). Moreover, we were able to show that continuous voluntary running (7d/week) did not elicit greater increases in neurogenesis than intermittent running (3d/week), and mice exposed to running discs did not have greater neurogenesis than those exposed to running wheels (despite the fact that discs yielded about 80% greater running distances); these data suggest a running-induced plateau in neurogenesis. Interestingly, at the end of our 4 week paradigm, the neurogenic effects of running on CD1 mice remained significant at all 4 stages of the neurogenic process (proliferation, neuroblasts, immature post-mitotic neurons, and cell survival); this contrasts previous findings using group-housed C57BL/6 mice, which exhibited a proliferative peak after 3-10 days that returned to baseline after 32 days (Kronenberg et al. 2006).

Our results also showed that, despite significantly increasing expression of all markers of neurogenesis, 4 weeks of running unexpectedly decreased the total number of DG cells expressing c-fos (a surrogate marker of neuronal depolarization). Since 90-94% of c-fos-expressing cells were NeuN-negative, we speculate that this reduction in depolarized neurons may be a by-product of the increased proliferation by transit-amplifying progenitors and neuroblasts, temporarily delaying the production of mature neurons. Consistent with this scenario, recent studies have shown that running increases immediate-early gene expression at longer 5-7 week timepoints (Clark et al. 2009; Clark et al. 2011b).

II.7.2. Environmental complexity

Environmental complexity has been shown to positively affect a variety of neural parameters, including electrophysiological characteristics, depression, and learning and memory functions (Pang and Hannan 2013; van Praag et al. 2000). However, mixed conclusions have been reached regarding whether a complex environment acts on adult neurogenesis, with some studies suggesting that it may have stage-specific effects on adult neurogenesis that are dissociable from

the effects of exercise (Bednarczyk et al. 2011; Fabel et al. 2009; Kannangara et al. 2009; Kempermann et al. 2010; Kronenberg et al. 2003; Olson et al. 2006) and other studies indicating that it does not appreciably modulate neurogenesis in comparison to running (Kobilo et al. 2011; Mustroph et al. 2012a). These conflicting views have likely arisen because environmental complexity has generally not been well isolated as a variable. Previous experimental designs could not exclude the possibility that observed effects of a complex environment were actually due to EE variables such as physical activity and social housing/isolation, or conversely, that a lack of observed effects was because basal neurogenesis levels had been raised by factors such as inanimate cage constituents, enriched feeding paradigms, handling or behavioral testing

The *Complex* environment used in the present study was multi-factorial in nature, consisting of social housing (3 mice per cage), tunnels whose orientations were rotated 4 times per week, and a locked running apparatus (igloo and running disc). Thus, this environment possesses inanimate objects, social enrichment and conformational novelty. However, to our surprise, exposure to this environment did not affect any of the 4 stages of the neurogenic pathway examined. When combined with intermittent exposure to a running disc, it also did not potentiate running-induced neurogenesis. Importantly, control experiments allowed us to exclude a variety of factors that could possibly have masked the effects of the complex environment: by using CD1 mice that have low baseline neurogenesis, by testing for any effects of the locked disc environment itself, and by assessing possible neurogenic influences of daily handling or the use of vertical running wheels vs. horizontal running discs.

While the *Complex* environment did not alter any parameters of neurogenesis that we tested, it increased depolarization-associated c-fos expression within the GCL by 2-3 fold. The upregulation of c-fos expression was detectable in both the inner and outer portions of the granule cell layer (generated during adulthood and early postnatally, respectively) and occurred to a similar extent in both the rostral (dorsal) and caudal (ventral) hippocampus. Interestingly, 90-94% of c-fos+ cells within the GCL were immature (NeuN negative) in all experimental groups, and this proportion remained the same in the *Complex* environment, indicating that while

the *Complex* environment activates greater total numbers of cells, it does not have a preferential effect on immature versus mature neurons.

We conclude from these data that environmental complexity as it is typically used in rodent models is not a significant or persistent regulator of hippocampal neurogenesis, and that its previously reported effects on neural function may involve improved electrophysiological incorporation of newly generated neurons rather than increases in neuronal production. Because the *Complex* environment used here was multi-factorial, consisting of inanimate objects, social interactions and conformational novelty, further experiments will be required to identify the specific sub-component responsible for stimulating c-fos expression. A possible caveat to these conclusions is that despite being multi-factorial, the *Complex* environment might still be considered a mild or restricted form of cognitive stimulation. For example, previous work has indicated that learning-associated stimuli can enhance hippocampal neurogenesis via increased cell survival (Gould and Tanapat 1999; Leuner et al. 2004). In future experiments using this Alternating EE paradigm, it will be of interest to determine whether more focused or intensive types of cognitive stimulation can affect neurogenesis (i.e., maze training, controlled appropriately for exercise levels).

II.7.3. Stress

Stress is generally associated with strong negative effects on neural functions, including learning and memory (de Quervain et al. 2000; Roozendaal et al. 2006; Schwabe and Wolf 2012). Activation of the hypothalamic-pituitary-adrenal (HPA) axis represents one of the key mechanisms underlying stress-associated effects, triggering release of adrenal-derived corticosterone into the circulation, from where it can enter the brain and activate the high levels of glucocorticoid and mineralocorticoid receptors found within the hippocampus (Van Eekelen et al. 1988). Adult neurogenesis is among the hippocampal parameters regulated by stress-induced corticosterone. Considerable data support a negative correlation between corticosterone levels and dentate gyrus proliferation and neurogenesis (Ambrogini et al. 2002; Cameron and McKay 1999; Gould et al. 1992), and stress-associated corticosterone can suppress running-induced neurogenesis in the short term (Stranahan et al. 2006). However, the relationship between stress

and adult neurogenesis is complex, as running is itself often considered a type of mild stressor, and has been associated with HPA activation and corticosterone release (Schoenfeld and Gould 2012; Stranahan et al. 2006). Furthermore, adult neurogenesis may play a feedback role in regulating stress responses, as hippocampal neurogenesis has been implicated in the mood-stabilizing effects of antidepressant treatments (Eisch and Petrik 2012) and inhibition of neurogenesis alters the responses to stress (Lagace et al. 2010; Schloesser et al. 2009; Snyder et al. 2011).

Plasma corticosterone levels were significantly altered across our experimental groups, and the only EE variable that correlated with increased corticosterone was social isolation. Remarkably, neurogenesis markers (proliferation, neuroblast, immature neurons, survival) were comparably increased in all three running groups (I-RUN, C-RUN, MAX), despite the fact that the socially isolated I-RUN and C-RUN groups exhibited chronic 300% higher plasma corticosterone levels than the MAX group; thus, while an increase in corticosterone can negate running-induced neurogenesis in the short-term (Stranahan et al. 2006), it does not appear to have any significant long-term neurogenic impact. Exposure to an intermittent complex environment, either alone (I-CE) or in combination with intermittent running (MAX), was sufficient to maintain low baseline plasma corticosterone, demonstrating that the lack of neurogenic effects of CE observed in our study cannot be explained by high stress levels. While these observations may not apply to all types of stressors, the maintenance of high neurogenesis in the presence of chronically increased plasma corticosterone indicates that corticosterone-based mechanisms are unlikely to mediate long-term stressor effects on hippocampal neurogenesis.

II.7.4. Social context

Group housing has long been used as a key component of EE (Kobilo et al. 2011; La Torre 1968) and contributes to adaptive behavioral and anatomical changes in both the developing and adult brain (Lieberwirth and Wang 2012; Welch et al. 1974). Conversely, social isolation has a significant negative impact on brain development and function (Cacioppo et al. 2011). In terms of hippocampal neurogenesis, many aspects of social context have been shown to

affect neurogenesis, including social isolation (Leasure and Decker 2009; Stranahan et al. 2006), psychosocial stress (Czeh et al. 2001; Czeh et al. 2007; Czeh et al. 2002; Gould et al. 1997; Gould et al. 1998; Mitra et al. 2006; Simon et al. 2005; Thomas et al. 2006; Van Bokhoven et al. 2011), dominance hierarchy (Kozorovitskiy and Gould 2004), social instability (McCormick et al. 2012), reproductive behavior (Leuner et al. 2010b) and parenthood (Glasper et al. 2012; Leuner et al. 2010a; Leuner et al. 2007; Mak and Weiss 2010). Of particular relevance here, previous work has shown that a social environment is capable of buffering stress-induced inhibition of neurogenesis, and at least in the short term, can be essential for allowing a running-induced up-regulation of adult neurogenesis (Stranahan et al. 2006).

The data obtained in the present study indicates that social context does not have a long- term influence on running-induced neurogenesis. After 4 weeks, I-RUN and C-RUN mice displayed robust running-induced increases in neurogenesis, despite being socially isolated. Intermittent exposure to a complex environment that included social housing (i.e., the MAX group) completely prevented the corticosterone increases in the isolated running groups, supporting the idea that social housing can act to buffer the effects of stress. In spite of this, groups with intermittent social housing (i.e., I-CE and MAX) did not exhibit increases in either basal (I-CE) or running-induced (MAX) neurogenesis. Thus, the negative neurogenic impact of social isolation is likely to only exert a temporary inhibitory influence on running-induced neurogenesis (Stranahan et al. 2006).

II.7.5. A novel Alternating EE paradigm for isolating individual EE variables

Few studies have attempted to dissect the impact of multiple EE variables within the same set of experiments. This is likely owing to the technical challenge associated with effectively isolating each variable, because "single" variables are typically multifactorial (a running wheel can serve as a source of both environmental complexity and physical activity, for example). We described here the development of a novel Alternating EE paradigm that enabled us to obtain a clearer idea of how each EE component impacts neurogenesis.

A major strength of this paradigm is its alternating nature. Mice are exposed to each

environment for a pre-determined period of time (in the present case, alternating each day between one of two environments); consequently, statistical comparisons are made between groups that have been exposed to *identical conditions for about 50% of their time*. This approach also allowed the use of a 2-way analysis of variance (*Running disc* versus *Complex* environments), decreasing the required animal use per group and allowing assessment of possible interaction effects between the two primary variables. The intermittency of the approach also allows the possibility of a graded (intermittent vs. continuous) exposure to individual EE variables.

A range of additional technical considerations was also incorporated into the experimental design. While C57BL/6 mice have been the strain of choice in most mouse studies, a recent strain comparison revealed that C57BL/6 mice have the highest baseline neurogenesis level of 12 inbred strains examined, and thus exhibit the smallest EE-induced increase in adult neurogenesis (Clark et al. 2011b). To ensure that high baseline neurogenesis does not mask more subtle neurogenic effects of EE variables, we used male CD1 mice, a commonly used outbred mouse strain that we found exhibits low baseline neurogenesis and significant running- induced increases that are detectable even with low numbers of mice per group. Animals were individually housed during their exposure to running discs, ensuring that running data could be collected for each animal. The use of a locked disc group allowed controlling for the environmental complexity component of the running disc. Calretinin, a late marker that is expressed transiently in postmitotic, newly generated DG neurons (Brandt et al. 2003; Todkar et al. 2012), as well as a more traditional BrdU-incorporation strategy, were used as independent measures of the total number of surviving newly born neurons. Finally, our control experiments revealed that inherent aspects of this Alternating EE paradigm, such as daily handling of the mice or the use of horizontal running discs versus vertical running wheels, had no detectable effects on the measured outcomes.

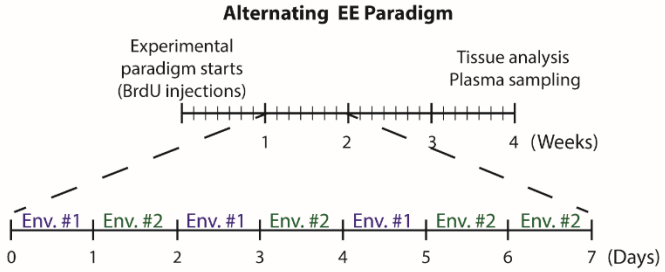
II.8. Summary

The present study investigated the contributions of individual EE variables to adult hippocampal neurogenesis. A novel Alternating EE paradigm was developed that enabled us to

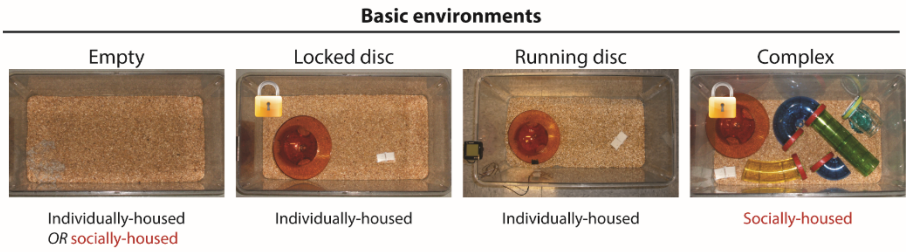
effectively isolate single EE variables, that yielded highly consistent data, and whose design allows for wide flexibility in experimental parameters. Our data confirm and extend the understanding of the central role of voluntary running in the pro-neurogenic effects of EE. In contrast to some previous reports, we found that environmental complexity did not directly regulate or enhance running-induced neurogenesis; however, it did produce a significant stimulation of c-fos expression in both mature neurons and newly-generated cells of the GCL, suggesting that it acts by enhancing the activity or integration of newly generated neurons into hippocampal circuitry. Social context and chronically elevated circulating stress hormones (EE variables that can have significant effects on overall brain function) did not have long-term effects on basal or running-induced neurogenesis. The Alternating EE paradigm represents a useful experimental system for optimizing EE-based therapies in contexts such as developmental disorders, depression, aging, neurodegenerative diseases and rehabilitation following CNS lesions.

II.9. Figures and figure legends

A



B



C

Experimental Groups

Groups	Environment #1	Environment #2
Maximal Enrichment **(MAX)**	Running disc	Complex
Intermittent Running **(I-RUN)**	Running disc	Locked disc
Intermittent Complex **(I-CPX)**	Locked disc	Complex
Minimal Enrichment **(MIN)**	Locked disc	Locked disc
Impoverished **(IMP)**	Empty - Individual	Empty - Individual
Continuous Running **(C-RUN)**	Running disc	Running disc
Social Housing **(SOC)**	Empty - Social	Empty - Social

D

Statistical contrasts

Group #1	Group #2	Hypothesis tested
I-RUN	MIN	Effect of running
I-CPX	MIN	Effect of the complex environment
MAX	I-RUN	Effect of adding a complex environment to running
MAX	I-CPX	Effect of adding running to a complex environment
MIN	IMP	Effect of the locked disc
I-RUN	C-RUN	Effect of 3 days vs. 7 days / week of voluntary running
IMP	SOC	Effect of individual vs. social housing

Figure II.1. The Alternating EE paradigm

Timeline of the Alternating EE paradigm. Each experimental group was alternated between two types of housing environments 6 times/week for a period of 4 weeks. Each mouse received two

injections of BrdU on the first day of the paradigm (**A**). Basic environments used in the Alternating EE paradigm: *Empty* (devoid of all items, in which mice are either isolated or socially housed in groups of 3), *Locked disc* (containing a running disc that has been locked to prevent running exercise), *Running disc* (containing a normal running disc), and *Complex* (a *Locked disc* cage to which multi-colored tunnels have been added (rotated 4 times/week) and 3 mice are socially housed) (see Methods for additional details) (**B**). Alternating EE experimental groups: Each experimental group was built using 2 of the basic environments in B. Mice in each group alternated daily between these two basic environments for the entire 4 week period (**C**). Statistical contrasts: For each parameter measured in this study, a difference detected by one- way ANOVA was followed by the testing of 7 specific and pre-determined hypotheses. Each of these 7 statistical contrasts is made between two experimental groups that differ in only one EE variable. No statistical analyses were made between groups in which more than one variable differed (**D**).

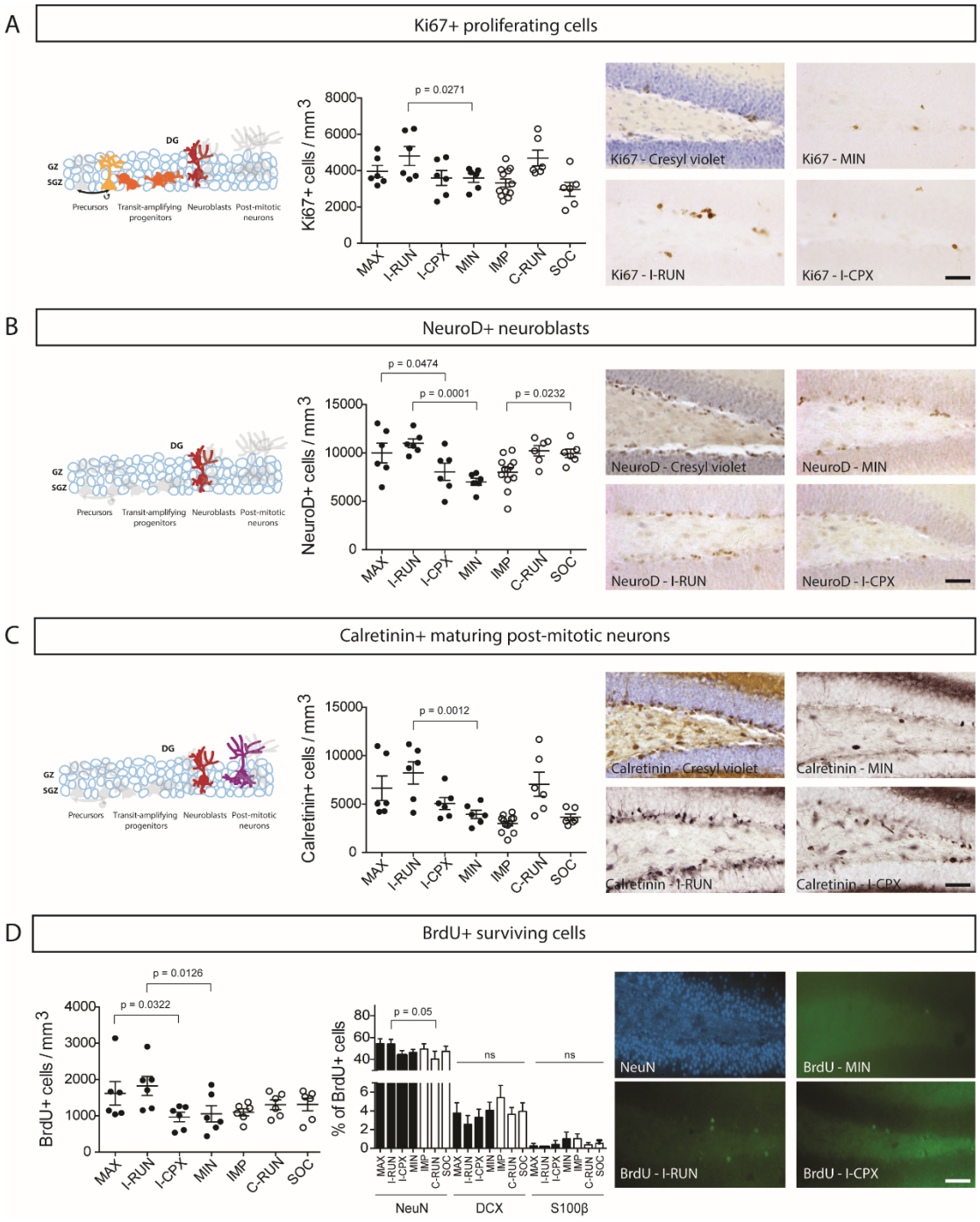


Figure II.2. Effects of Alternating EE on the main stages of dentate gyrus neurogenesis

Quantification of the density of Ki67+ proliferating cells (A), NeuroD+ neuroblasts (B), Calretinin+ immature post-mitotic neurons (C), and BrdU+ surviving cells (D). At the right of each panel is a sample cresyl-violet counterstaining (A-C) or NeuN-labeling (D) (upper left

image), and representative non-counterstained sections from MIN (upper right), I-RUN (lower left) and I-CPX (lower right) experimental groups. Note that a main significant effect of the Running disc environment (I-RUN) was detected for all four neurogenesis markers, while the Complex environment (I-CPX) did not have a main effect on any marker and did not potentiate the effects of running. See Results for further details. Scale bar = 50 μm. DG=Dentate Gyri.

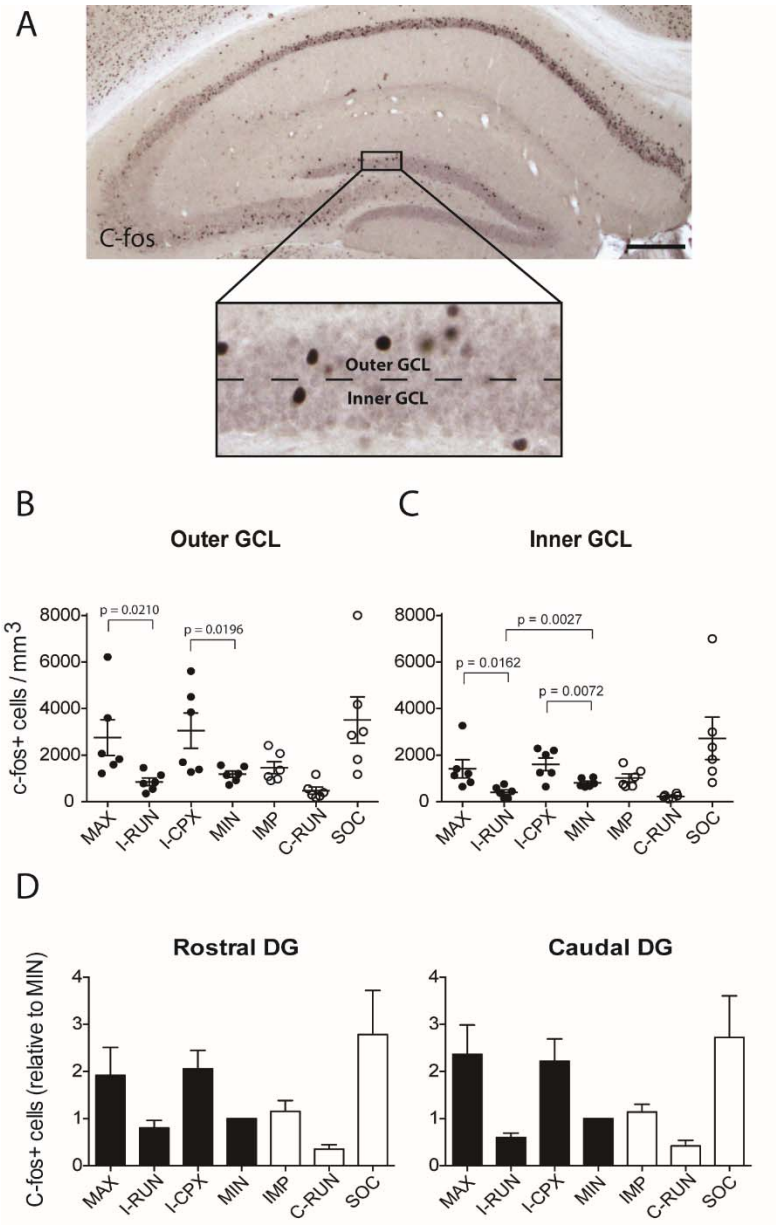


Figure II.3. Effects of Alternating EE on depolarization-associated c-fos expression

Low magnification image of c-fos immunohistochemistry on a coronal section of the hippocampus. Dotted line in the enlarged box illustrates how the granule cell layer (GCL) was divided into outer GCL and inner GCL for quantification purposes (**A**). Quantifications of c-fos expression in the Inner GCL (**B**) and Outer GCL (**C**). A main effect of the *Complex* environment (I-CPX) was detectable in both the Inner GCL and Outer GCL, while the *Running disc*

environment (I-RUN) had no significant effect. Socially-housed mice in empty cages (SOC) also exhibited increased c-fos expression compared to isolated mice (IMP) (**B-C**). See Results for further details. Rostral-caudal distribution of c-fos-expressing cells in the DG. The rostral and caudal tissue sections correspond to the dorsal and ventral hippocampus regions, respectively (**D-E**) Note that the pattern of c-fos expression across groups is virtually identical in both the rostral and caudal DG.

Scale bars: 250 µm (A) and 10 µm (B). DG = Dentate Gyri. GCL = Granule Cell Layer.

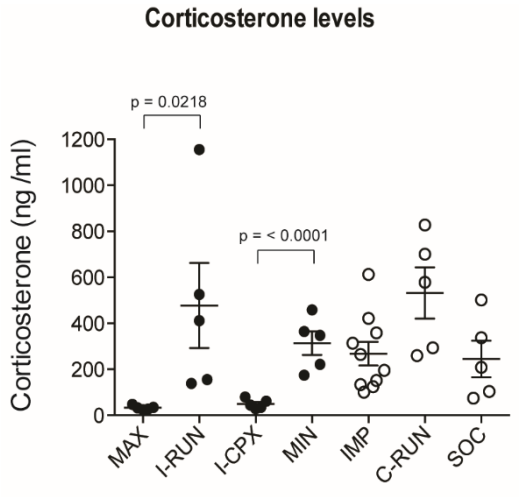


Figure II.4. Plasma corticosterone concentrations are reduced in the Complex environment

Plasma corticosterone concentration was measured by ELISA. Note that corticosterone levels are high in all experimental groups except those that include the Complex environment (I-CPX, MAX) See Results for further details.

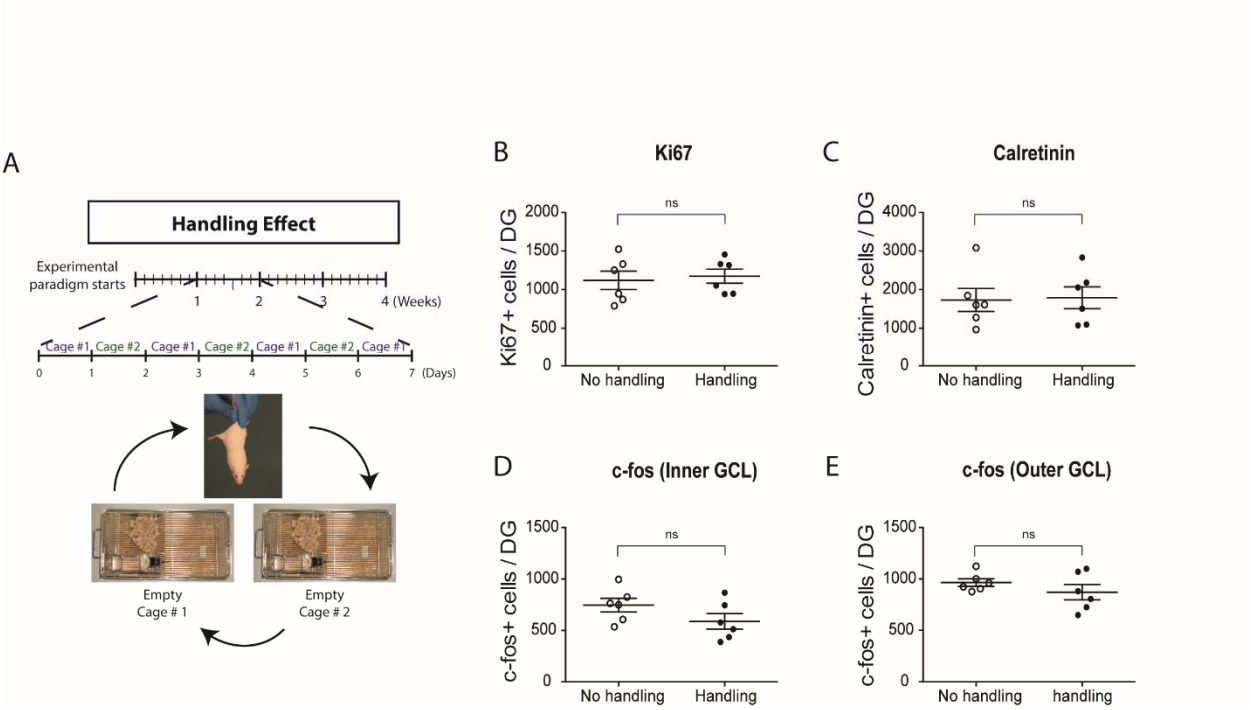


Figure II.5. Daily handling does not affect basal neurogenesis in the Alternating EE paradigm

Timeline of the Handling Effect experiment (**A**). Mice were either housed as for the IMP group of the Intermittent EE paradigm (i.e., *Empty* environment with daily handling) or in an *Empty* housing condition without daily handling for 4 weeks. Quantifications of the number of Ki67+ proliferating cells (**B**), Calretinin+ maturing post- mitotic neurons (**C**), Inner GCL c-fos+ cells (**D**) or outer GCL c-fos+ cells (**E**). No significant effects of daily handling on these markers were detected (t-tests). See Results for statistical details. DG = Dentate Gyri.

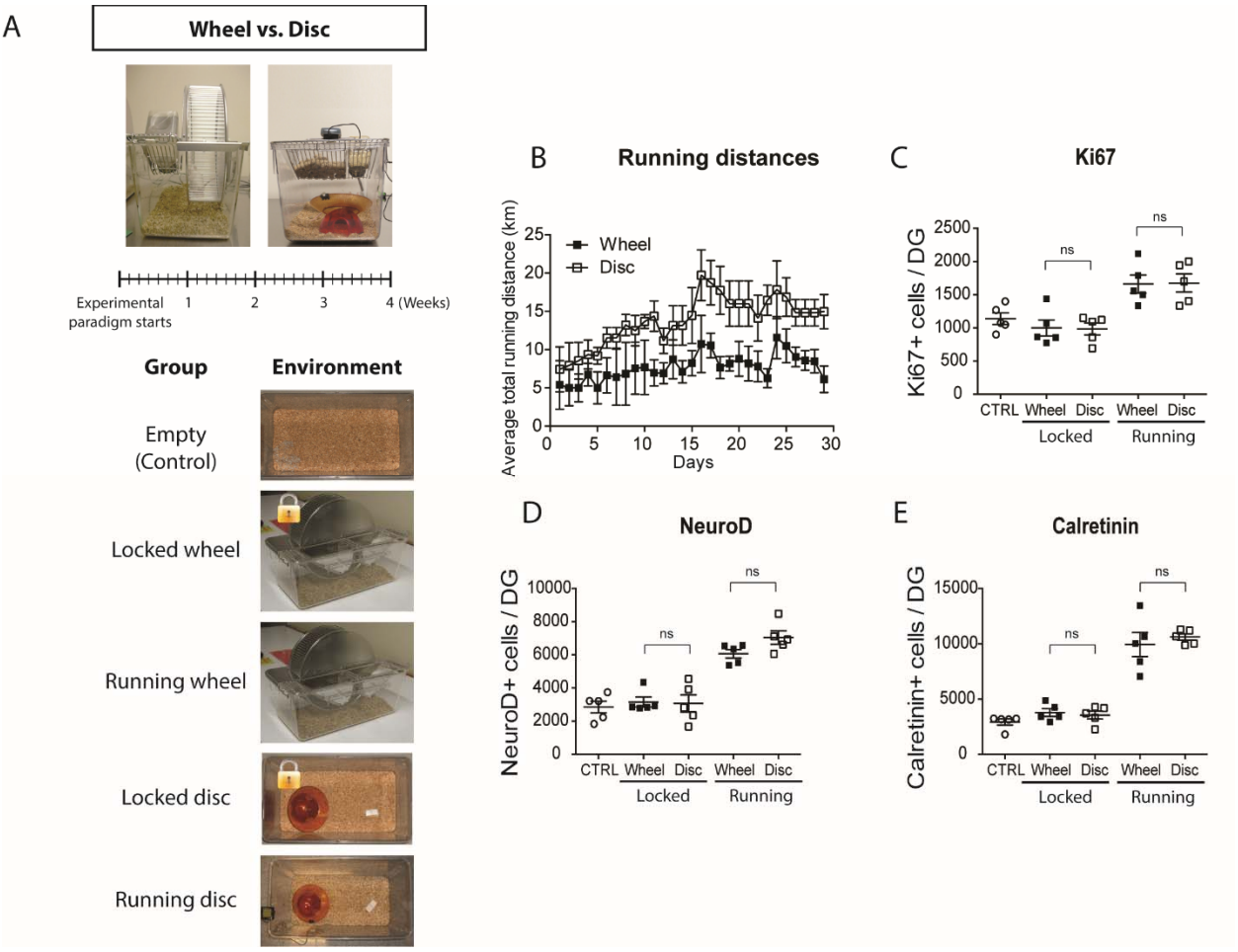


Figure II.6. Comparison of the effects of running wheels and running discs on adult neurogenesis

The Wheel versus Disc experiment. Mice were maintained for 4 weeks in one of five housing conditions: Control, Locked running wheel, Running wheel, Locked running disc, and Running disc (**A**). Comparison of average daily running distances on Running Wheels and Running Discs (**B**). Note that average running distances were about 80% higher on Discs than on Wheels. Quantifications of the numbers of Ki67+ proliferating cells (**C**), NeuroD+ neuroblasts (**D**) and Calretinin+ maturing post-mitotic neurons (**E**). In all cases, there was no significant difference between the Control, Locked wheel and Locked disc groups, or between the Running wheel and Running disc groups (t-tests). See Results for statistical details. DG = Dentate Gyri.

A

Groups	Proliferating cells (Ki67)	Neuroblasts (NeuroD)	Post-mitotic (Cal / BrdU)	Activation (C-fos)	Corticosterone
Maximal Enrichment (**MAX**)					
Intermittent Running (**I-RUN**)					
Intermittent Complex Environment (**I-CPX**)					
Minimal Enrichment (**MIN**)					
Impoverished (**IMP**)					
Continuous Running (**C-RUN**)					
Social housing (**SOC**)					

B

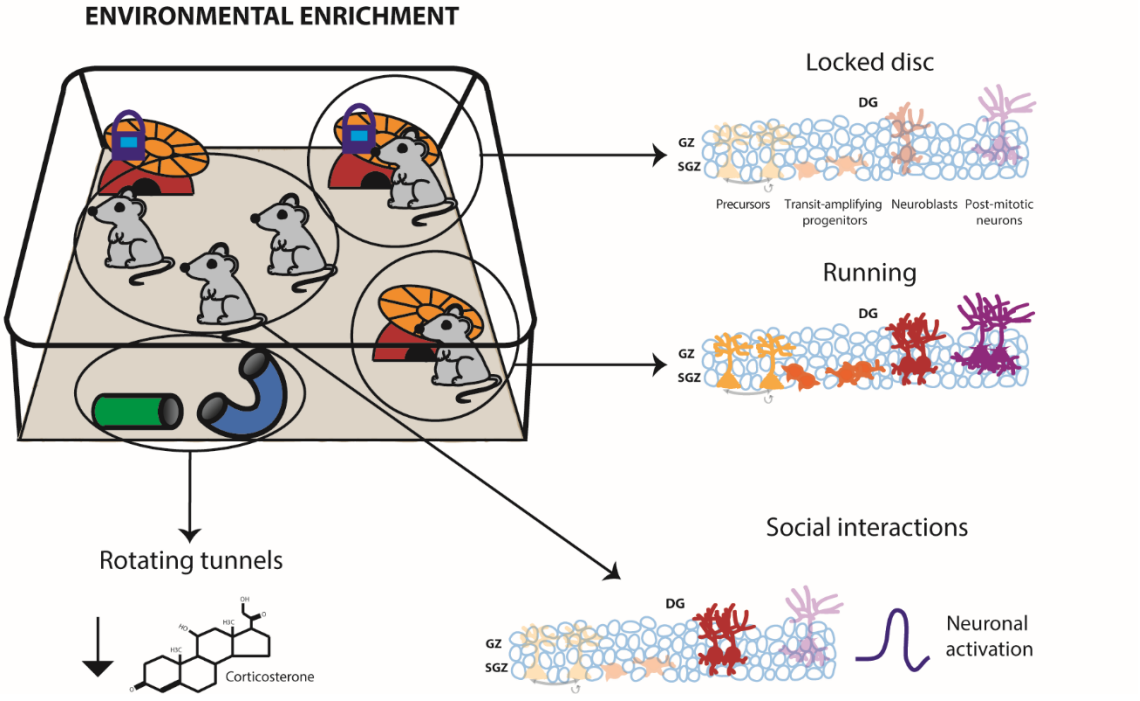


Figure II.7. Summary of long-term effects of individual EE variables on hippocampal neurogenesis, c-fos expression and corticosterone levels

Table summarizing relative changes to DG neurogenesis, depolarization-associated c-fos expression within the granule cell layer, and plasma corticosterone levels in the 4-week Alternating EE paradigm (**A**). The EE variables examined in the present study included running, environmental complexity, social context (isolation and social enrichment) and stress-

associated plasma corticosterone. **Voluntary running** strongly increased all stages of neurogenesis compared to exposure to a locked disc. Continuous voluntary running did not have a greater neurogenic effect than intermittent running. **Environmental complexity** (involving a combination of inanimate objects, social interactions and conformational novelty) did not affect basal or running-induced neurogenesis, but enhanced depolarization-associated c-fos expression within the granule cell layer (likely due to **Social interactions**) and decreased plasma corticosterone concentrations (likely due to physical **Complexity** associated with tunnels and toys). Chronic differences in the levels of **stress-associated corticosterone** had no detectable positive or negative effects on running-induced neurogenesis and was not responsible for preventing effects of environmental complexity on neurogenesis. The baseline environment, a Locked disc, had no detectable impact on any stage of neurogenesis, neuronal activation or corticosterone levels when compared to a completely impoverished environment. Thus, the Alternating EE paradigms enables dissociation of the impacts of distinct elements of environmental enrichment (**B**).

Chapter III. Behavioural studies and transcriptomics following exposure to running and social interactions

III.1. Chapter context

The factors mediating the hippocampal neurogenesis increase, neuronal activation and cognitive improvements following exposure to individual EE components remain unclear. Based on previous findings, we exposed mice to a locked disc environment, a social environment, and to continuous exercise. Behavioural studies and a transcriptomics analysis were done to detect major changes following exposure to either a complex environment or exercise.

III.2. Author's contributions

In this study, I designed, performed, and analyzed most of the experiments with the helpful advices of my research director, Dr. Karl Fernandes. Dr. Stephanie Tobin, a post-doctoral fellow from Dr. Stephanie Fulton's laboratory, helped to design, perform, and analyze the behavioural experiments from Figure III.1. Dr. Brianna Goldenstein, a former post-doctoral fellow from our laboratory, helped with the immunohistochemistry and NOR experiments from Figures III.1-2. Andréanne Leclerc, a summer intern, helped with the paradigm set-up as well as for CPP and RAM experiments under my supervision for Figures III.1. Dr. Stephanie Fulton provided the equipment to perform the behavioural studies, and helped to analyze experiments from Figures III.1. Patrick Gendron, a biostatistician from the IRIC genomics platform, performed the main analyses of bioinformatics from Figure III.4. The manuscript was written by myself in collaboration with Dr. Karl Fernandes.

Transcriptomic analyses reveal distinct cellular and genetic impacts of voluntary running and socially enriched complex environments on the hippocampal dentate gyrus

Catherine-Alexandra Grégoire[1,2,3], Stephanie Tobin[1,5], Brianna L. Goldenstein[1,2,4], Éric Samarut[1,2,4], Andréanne Leclerc[4], Anne Aumont [1], Pierre Drapeau[1,2,4], Stephanie Fulton[1,5], Karl J. L. Fernandes[1,2,4]*

[1]Research Center of the University of Montreal Hospital (CRCHUM),
[2]CNS Research Group (GRSNC), University of Montreal
[3]Department of Pathology and Cell Biology, Faculty of Medicine, Université de Montréal, QC, Canada, H3T1J4
[4]Department of Neurosciences, Faculty of Medicine, Université de Montréal, QC, Canada, H2X0A9
[5]Department of Nutrition, Faculty of Medecine, Université de Montréal, QC, Canada, H3T1A8

III.3. Introduction

The hippocampus is a component of the brain's limbic system that has been implicated in higher cognitive processes such as learning and memory, spatial navigation, and emotional regulation (Bannerman et al. 2003; Frankland et al. 1998; Kheirbek et al. 2013). Its cellular organization includes a prominent tri-synaptic circuit that spans across its two main sub-structures, the DG and Ammon's horn (CA), with the neocortex serving as the principal source of inputs and outputs (Anderson et al. 2007). The precise mechanisms by which the hippocampal tri-synaptic circuit mediates higher cognitive processes remains incompletely understood, but it is likely that the DG plays a particularly prominent role in hippocampal-dependent functions (Arruda-Carvalho et al. 2011; Kheirbek et al. 2013; Vukovic et al. 2013). Anatomically, synapses between axons of entorhinal cortex neurons and DG granule cells are the first synapses of the tri-synaptic circuit and represent the gateway to hippocampal function (Anderson et al. 2007). By virtue of its dense granule cell layer, which vastly outnumbers inputs from the cortex, the DG performs a pattern separation function that enables closely related inputs to be encoded distinctly within the CA3 layer (Aimone et al. 2014; Deng et al. 2010). The DG is also the sole region of the hippocampus in which NSCs remain active throughout life (Bond et al. 2015; Gage 2000; Kriegstein and Alvarez-Buylla 2009). NSCs continuously produce new, highly plastic granule neurons through the process of adult neurogenesis; ablation of these newly generated granule cells in rodents compromises normal learning and memory as well as regulation of stress and emotion (Aimone 2016; Kheirbek et al. 2013).

Many brain functions, including hippocampus-mediated processes, are enhanced by EE (Bennett et al. 1969; Hebb 1947; Kempermann et al. 1997b; La Torre 1968; Rosenzweig et al. 1962). EE typically involves exposure to multisensory stimuli such as spatial complexity, social enrichment, and physical activity (Nithianantharajah and Hannan 2006). Complex cellular and molecular changes are triggered by an enriched environment, including growth factor synthesis, dendrite growth, synaptic plasticity, and increased neurogenesis, and these are thought to underlie EE-induced improvements in brain function. We recently devised an "Alternating EE" paradigm, which enabled us to demonstrate that individual components of EE can have distinct and separable effects on the DG: notably, while physical activity mediated EE-induced increases in neurogenesis, a socially enriched complex environment (comprised of inanimate objects and social interactions)

decreased circulating levels of the stress hormone corticosterone and increased expression of the depolarization-associated immediate-early gene c-fos (Gregoire et al. 2014).

Here, we build on this previous work, investigating the cellular and molecular mechanisms potentially mediating the distinct consequences of individual EE components on the DG. Previous studies on the mediators of EE effects have primarily been candidate-based, and have shown the involvement of specific molecules, such as BDNF, FGF2, IGF1, and VEGF, in the running-induced neurogenesis process (Aberg et al. 2000; Fabel et al. 2003; Jin et al. 2002; Rossi et al. 2006; Wagner et al. 1999). To investigate this question in a more unbiased manner, we used an RNA sequencing (RNA-Seq) transcriptomic strategy to study genome-wide EE-induced genetic changes, focusing specifically on the DG. Our data provide a first description and comparison of the overall cellular processes and genetic pathways activated within the DG by physical activity and a socially enriched complex environment.

III.4. Materials and methods

III.4.1. Mice

Experiments were conducted in accordance with the guidelines of the Canadian Council of Animal Care and were approved by the institutional animal care committee of the University of Montreal and the CRCHUM. Experiments were performed using three-month-old male CD1 mice from Charles River Laboratories (St-Constant, QC, Canada). Mice were housed in a reversed 12-hour light/dark cycle (lights on at 22:00h and off at 10:00h). The animals had free access to food and water, unless specified otherwise.

III.4.2. Housing conditions and Experimental groups

The experimental paradigm was repeated using two separate cohorts of mice (n=18, then n=27 per cohort) in order to obtain enough animals per group for behavioural tests. Mice were randomized and separated into one of the three different groups. All environments contained nesting material and basic litter (Beta chip, Nepco).

Locked disc environment (n=15). Animals were housed in 39.3cm x 28.5cm x 19.4cm rat cages containing a locked running disc (Red mouse igloo, K3327, and amber fastrac running disc, 7.5cm in diameter, K3250, Bio-Serv, Frenchtown, NJ, USA). Mice were housed individually.

Social environment (n=15). Animals were housed in 39.3cm x 28.5cm x 19.4cm rat cages containing a locked running disc and mice were socially-housed in groups of three.

Running disc environment (n=14). Identical to the locked disc environment except with a free-moving running disc was to permit voluntary running. Running cages were outfitted with odometers (Sigma BC509) to measure the running distance. One mouse died due to excessive running during the paradigm. Mice were housed individually.

III.4.3. Tissue fixation and processing

Mice received a lethal dose of ketamine (Bimeda-MTC), xylazine (Bayer Healthcare), and

acepromazine (Boehringer Ingelheim Canada Ltd) and perfused transcardially with phosphate-buffered saline (PBS), followed by 4% formaldehyde (pH 7.4), freshly prepared from paraformaldehyde (Fisher). Brains were removed and post-fixed in 4% formaldehyde overnight. Brains were sectioned coronally (40µm) using a vibrating microtome (Leica VT1000S, Leica Microsystems, Richmond Hill, ON, Canada) and sections were stored at -20°C in an antifreeze solution (glycerol:ethylene glycol:PBS 1X, 3:3:4) until further use.

III.4.4. Immunohistochemistry

Immunohistochemical procedures were performed as detailed previously (Gregoire et al. 2014). Mouse anti-human Ki67 was used at 1:200 (BD Biosciences), and goat anti-rabbit NeuroD at 1:500 (Santa Cruz Biotechnology). Microscopy was performed using a motorized Olympus IX81 microscope (40X objective). All quantifications were performed on coded slides by a blinded experimenter.

III.4.5. Cell quantifications

As described previously (Gregoire et al. 2014), the number of subgranular zone (SGZ)/granular zone (GZ) cells positive for Ki67 and NeuroD was quantified on every 6th section between Bregma -1.06mm and -2.98mm of the hippocampus (8 sections total/marker/animal). The raw cell counts were corrected for oversampling due to split cells by multiplying by (1 - object height/section height), where the object height refers to the average diameter of the marker in question. To calculate the mean density of marker-positive cells, the corresponding SGZ/GZ reference volumes of the sections were determined using the Cavalieri principle (grid size of 10 microns, 20x objective) in StereoInvestigator (MBF Bioscience, VT); mean cell density was then obtained by dividing the corrected total number of marker-positive cells on the sampled sections by the sum of the section SGZ/GZ reference volumes. Results are expressed as mean number of marker-positive cells per mm3 of SGZ/GZ. Cell counts were performed manually by a blinded observer using a 40X objective, and slide codes were only broken after all quantifications were completed for any given marker.

III.4.6. Conditioned place preference

During the conditioned place preference (CPP) paradigm, mice were food restricted two hours before testing (8:00h) and the discs removed. Food and discs were returned following testing. CPP was conducted in Med Associates chambers consisting of three compartments: a center compartment (11.7 cm) with a neutral gray finish and a smooth PVC floor; and two choice compartments (17.4 cm each). One black compartment with a stainless steel grid rod floor, the other a white compartment with a stainless steel mesh floor. Thus, each chamber contains a distinct set of spatial and tactile cues. These cues were paired in either the presence or absence of sugar pellets (20mg, BioServ) and place preference were assessed following multiple conditioning sessions. On the first day, mice were confined to one of the two chambers for 20 minutes, followed by another 20 minutes in the opposite chamber. The following day, a pre-conditioning session of 20 minutes was conducted, where a single mouse was placed in the center chamber and allowed to explore freely. Time spent in each chamber was recorded and used to assess any initial chamber preferences. On days 3 to 12 of behavioural testing, each animal was given two daily 20-minute conditioning sessions during which they were confined to one of the two choice compartments. About half of the animals were confined in one of the two choice compartments with access to 20 sugar pellets, for the other animals, they were confined in the other choice compartment which was paired with the absence of sugar pellets. On day 13, a post-conditioning test was conducted, where each mouse was placed in the center chamber of the CPP apparatus and allowed to explore the center and choice compartments. Time spent in each compartment was calculated for the conditioned place preference score.

III.4.7. Delayed non-matching to place radial arm maze

Mice received a small amount of palatable food (sugar pellets, BioServ) in their home cages several days before training and testing to familiarize the reward. Mice were also food restricted two hours before testing (8:00h) and discs were removed. Food and discs were given back following testing. The radial arm maze (RAM) from Med Associates consisted of eight (37 cm long) equidistantly spaced arms radiating from a small octagonal platform. The delayed non-matching to place (DNMP) test is designed to assess the ability of a mouse to discriminate the sample arm (familiar-first sugar bated arm) from the new arm (opposite arm baited on choice test)

on a choice test (Clelland et al. 2009). For training and testing, animals were only able to access the center and one or two radial arms. For training, mice were given two pre-exposures to the RAM. The first pre-exposure consisted of an exposure of five minutes to the RAM with a few sugar pellets spread randomly throughout the maze. This promotes exploration, which helps with training and reduces the potential for anxiety associated with the maze. The next day, during the second pre-exposure to the RAM, sugar pellets were added only at the end of the arms to encourage head entries. The mice were taken out of the RAM when one of the three possibilities was fulfilled: 1) the mouse's head entered all eight arms in less than five minutes, 2) the mouse's head entered in six out of eight arms in five to ten minutes, or 3) 10 minutes passed without six out of eight head entries. During the 10-day testing period, each mouse received two trials per day (one low-arm and one high-arm separation trial) of pseudo-randomly presented combinations of the start, sample, and correct arms. A trial consisted of a sample phase (pellet in the sample arm), where all arms are blocked except for the start arm and the sample arm. This was followed by a one-minute delay (time necessary to clean the maze) and a choice phase where arms in the start and sample (unrewarded) locations and a new arm (rewarded with a sugar pellet) were open (Fig. 2A). The distance between arms was varied during testing as this technique targets hippocampal neurogenesis and ablation of neurogenesis (Clelland et al. 2009). Spatial cues were present on the four walls surrounding the RAM for orientation during the testing phase. Errors were defined as entries into the sample or unrewarded arms, but mice were allowed to self-correct.

III.4.8. Novel object recognition

Novel object recognition (NOR) test capitalizes on an animal's innate preference for novelty and assesses object recognition (Antunes and Biala 2012; Hammond et al. 2004; Reger et al. 2009). The delayed version of NOR (more than 10 minutes after familiarization) is known to be hippocampus-dependent (Cohen and Stackman 2015). NOR was conducted in a 46cm by 46 cm by 52cm grey opaque box (i.e., arena). Mouse behaviour was recorded with a video camera above the arena. This camera was interfaced with a video tracking system (EthoVision XT8, Noldus). The habituation phase was conducted first, where each mouse was placed in the test box (without objects) for 10 minutes. This gave the mouse time to explore the arena and preventing anxiety during testing. The next day, a baseline was determined as mice were given a five-minute

exposure to test for pre-existing quadrant preferences. Following this exposure, a familiarization phase was conducted, where mice were exposed to the arena in the presence of two identical objects. Each mouse was given five minutes to explore these objects. Short-term memory was assessed one hour later by placing the mouse back in the arena with one familiar and one new object. Finally, long-term memory was assessed 24 hours later when the mouse was placed in the arena with the previous "novel object" as the familiar object, and a second novel object (Fig. 3A). Objects consisted of candle holders (either red or silver) and hydrogen peroxide bottles. These objects were chosen for their similarities in size (approximately 15 cm high by 6 cm wide), weight (mouse is unable to knock it over – no risk for injury) and brightness yet variability in tactile surface. The location of the objects was kept constant between trials and mice. All objects were exchanged with replicas in subsequent trials. Mice began trials at the center of the arena with their backs facing the objects. Exploration time was defined as time spent with the head oriented towards and within 2 cm of the object. After each session, the arena and objects were cleaned thoroughly with 70% ethanol to mask odor cues.

III.4.9. RNA-Seq

Isolated mRNA from dentate gyri of mice exposed to either a locked disc, complex environment (locked disc, social interactions, and rotating toys) or exercise environment for a period of four weeks was used for the RNA-Seq. The exercise group was subdivided into low and high runners for a total of five groups. Sequenced mRNA fragments were trimmed for adapter sequences and then mapped to the reference mouse genome assembly version mm10 using Tophat (version 2.0.10) (Trapnell et al. 2009). Gene expressions were then estimated by using the HTSeq tool to compute read counts on RefSeq genes (Anders et al. 2015). For exploratory purposes, DESeq2 was used to normalize read counts, extract regularized log values and compute log fold changes (Love et al. 2014). Differentially expressed genes that are up or downregulated by a factor 0.3 or more (in log2, thus a fold change of 1.23 or more) were used to investigate enriched pathways and functions through the use of QIAGEN's Ingenuity Pathway Analysis (IPA®, QIAGEN Redwood City, www.qiagen.com/ingenuity). All samples were normalized at once, which allows us to produce comparisons between samples in the form of Principal Component Analysis (PCA) and hierarchical clustering. Genes were sorted according to their absolute fold

change, only genes with a log fold change >= 0.3 or <= -0.3 were analyzed further. The program Venny2.1 generated the list of genes specific to each group when compared with one another, and BioVenn generated the Venn diagrams. EnrichR was used to investigate signaling pathways, biological processes, cellular components, and molecular functions that were modulated.

III.4.10. Statistical analyses

For immunohistochemistry experiments, all experimental groups were analysed by One-way ANOVA and a multiple comparison test that compares the mean of each column (Social and running conditions) with the mean of a control column (Locked disc condition). For behavioural studies, the effect of the groups and trials were investigated by Two-way ANOVA and Tukey's multiple comparisons test. For the preference ratio for the NOR task, a one sample t-test was performed to detect performance above chance, set at 0.5. For all the experiments, significance level was set at α=0.05. Error bars represent standard error of the mean.

III.5. Results

III.5.1. Running mice and socially-enriched mice show learning and memory differences

We previously showed that running and socially-enriched complex environments have separable effects on the DG (Gregoire et al. 2014). Individually housed adult mice given access to a voluntary running wheel for 4 weeks (RUN) showed increased proliferation and neurogenesis, while group-housed mice (3/cage) exposed to tunnels that were rotated daily for the same period (socially enriched complex environment, CE) showed increased numbers of DG neurons expressing c-fos, a depolarization-induced immediate-early gene commonly used as a surrogate for neuronal activity. The effect of the CE on c-fos was likely attributable to the social enrichment component rather than the tunnels, as it also occurred in mice that had been exposed only to social enrichment (SOC). We therefore tested the hypothesis that RUN and SOC groups would have detectable differences in hippocampus-mediated behaviours. Mice were housed for 8 weeks in RUN, SOC, or locked disc (LD) conditions. After 4 weeks, they were sequentially tested in conditioned place preference (CPP), 8-arm radial maze (RAM), and novel object recognition (NOR) paradigms. RAM and NOR results are presented here, while CPP data are not reported due to experimental complications (**Fig. III.1A**). Notably, LD, SOC and RUN mice exhibited identical open-field locomotor activity, indicating that the distinct housing paradigms did not cause differences in baseline locomotion that might affect performance in these behavioural tests (**Fig. III.1B**).

RAM is an incentive-driven test of spatial learning and memory. Mice were exposed to 2 testing periods daily for 10 days (see Methods), using the 4-arm and 2-arm variations described by Clelland and colleagues (Clelland et al. 2009). Each testing period consisted of a sample phase (i.e., placed in the start arm with access to only one additional arm, which was baited) followed one minute later by a choice, or testing, phase (i.e., placed in the same start arm, but now with a choice between the previously baited arm and a new baited choice arm) (**Fig. III.1C**). "Success" in RAM is measured as greater Arm Accuracy (i.e., more time in the newly baited choice arm) and/or fewer Errors (i.e., fewer entries into the previously baited arm), and is indicative of better overall cognitive flexibility. On the first trial day following 41 days of EE, the SOC group showed strong trends to superior Arm Accuracy in the 2-arm paradigm ($p=0.0922$) compared to the LD

group, with no difference in Errors (**Fig. III.1D-E**). In contrast, although the RUN group showed a trend to increased Arm Accuracy in the 4-arm paraedigm (p=0.3515), they committed more Errors, reaching statistical significance in the 4-arm paradigm (p=0.0256) and in the 2-arm paradigm (day 6, p=0.0403). By the 10th trial, all groups showed similar Arm Accuracy and Errors (**Fig. III.1D-E**). These results suggest that the SOC group has greater overall cognitive flexibility (i.e., ability to abandon a previous association and establish a new one) while the RUN group has stronger recall/permanence of spatial memories. Furthermore, although a loss of neurogenesis led to a decrease in baseline spatial memory in this test (Clelland et al. 2009), we found that increased neurogenesis induced by running did not enhance the baseline spatial memory.

NOR is an incentive-independent test of object memory retention that capitalizes on an innate preference for novelty. Mice are initially exposed to two identical objects to assess any quadrant preferences. An hour later, short-term memory is analyzed as one familiar object is traded for a novel object and object preference is recorded. One day later, the previous novel object is used as a familiar object and a new novel object is introduced to assess long-term memory (**Fig. III.1F**). The LD control group showed a strong tendency for the novel object in the 1h paradigm (LD: p=0.0506) and no novel object preference in the 24h paradigm (LD: p=0.9528), indicating short-term but not long-term object memory in this test. Likewise, SOC mice exhibited object memory retention in the short-term (SOC: p=0.0125) but not long-term (SOC: p=0.2665) paradigms. The RUN group, in contrast, did not exhibit novel object preference in the short-term paradigm (p=0.7442), possibly indicative of the known rewarding effects of running, but showed a strong tendency for novel object preference in the long-term paradigm (p=0.0770) (**Fig. III.1G**) (Greenwood et al. 2011).

Post-behavioural testing assessment of neurogenesis demonstrated that the RUN but not SOC group showed increased numbers of neuroblasts after 8 weeks of EE, as reported previously after 4 weeks (**Fig. III.2A**)(Gregoire et al. 2014). NeuroD+ neuroblasts were unchanged between LD and SOC groups (p=0.9633) and remained significantly increased in the RUN group versus LD (p=0.0117). Ki67+ proliferating cells were not statistically different between LD (3116.0±431.3 cells), SOC (3003.0±364.5 cells), or RUN (3576.0±227.7 cells) groups, consistent

with previous work showing a time-dependent decay of running-induced proliferation during chronic running (**Fig. III.2B**)(Kronenberg et al. 2006).

Together, these data show that mice exposed to RUN and SOC environments exhibit detectable differences in hippocampus-regulated cognitive functions, with RUN mice displaying changes consistent with greater spatial and object memory and SOC mice displaying an overall improved cognitive flexibility.

III.5.2. Design of the transcriptomics paradigm

To test the hypothesis that the RUN and SOC groups have different genetic effect on the dentate gyrus (DG) niche, we performed a RNA-Seq analysis. CD1 mice were exposed to three different environments for four weeks (**Fig. III.3A**). The EE consisted in locked disc, running disc, and complex environment where mice were socially-housed, consistent with the behavioural group used previously (**Fig. III.3B**). At the end of the paradigm, the running group was divided in two sub-groups: low runners and high runners to investigate further the effect of exercise in a distance-dependent manner. Low runners ran in average 10.65±0.39 km in contrast to 17.00±0.45 km for high runners (**Fig. III.3C**). Mice were sacrificed and their dentate gyri microdissected to isolate RNA from the dissociated tissue and sent for sequencing (**Fig. III.3D**).

III.5.3. Validation of RNA-Seq results

The principal component analysis is a multivariate method that emphasizes variation from a data set and and it revealed significant group-wise differences in gene expression were indeed detectable (**Fig. III.4A**). The cluster dendrogram, a hierarchical gene clustering, is used to show similarities (fusion) and differences (split) between gene sets. LD baseline group was more similar to the CE group than to the running groups. Surprisingly, the Low Runners clustered as a distinct population from the Exercise and High Runners populations (**Fig.III.4B**). The heatmap showing log2 (FPKM) values transformed in Z-score of significantly different expressed genes demonstrates that each environment led to specific changes for each group (**Fig. III.4C**). The top 25 up-regulated and top 25 down-regulated genes are shown for each condition in **Tables III.1-8**. For the dissertation, the overall observations will be summarized and we explored bio-

informatically three main observations: differences between Low and High Runners, changes in CE, and changes unique to runners and CE, respectively.

III.5.4. Low and high runners have distinct genetic changes

To study the molecular changes following exposure to running, a Venn diagram was created with genes showing a log2 fold change of >0.3 or <-0.3 (Hulsen et al. 2008). This analysis showed that combining high and low runners' genes included most of the merged Exercise group genes (shown as the purple circle in Fig. **III.5A**). 41 and 262 genes were unique to the High and Low Runners, respectively, and they had 53 genes in common (**Fig. III.5A**). The large amount of genes specific to Low Runners is consistent with the cluster dendrogram result where it described this group as different as High Runners and Exercise. Given the substantial differences between Low and High Runners, we continued our analyses with these separate groups rather than with the Merged exercise group. We pursued our analyses with both Low and High Runners. An overview of the signaling pathways in which the modified genes are implicated shows differences between running groups and their combination. Notably, Low Runners were enriched in Calcium regulation (**Fig. III.5B**) whereas High Runners were enriched in serine/threonine mitogen-activated protein kinase (MAPK) signalling (**Fig. III.5C**), indicating that there are significant differences in intracellular signalling between Low and High runners (**Fig. III.5D**).

To gain deeper insights into the similarities and differences between Low and High runners, we used Enrichr to analyze these data sets with GO Biological Process (**Fig. III.6**), GO Cellular Component (**Fig. III.7**), and GO Molecular Function (**Fig. III.8**). Transcriptomic changes within the Low Runners revealed particular enrichments for synaptic transmission and regulation of neuron projection development *Biological Processes* (**Fig. III.6A**), which were associated with the synaptic and post-synaptic membrane *Cellular Components* (**Fig. III.7A**), and transmembrane transporter activity *Molecular Functions* (**Fig. III.8A**). In contrast, High Runners showed particular enrichments for organismal response to stress and behavioral defense response *Biological Processes* (**Fig. III.6B**), which were associated with receptor, ion channel, and transporter complex *Cellular Components* (**Fig. III.7B**), and transmembrane transporter activity and ion channel activity *Molecular Functions* (**Fig. III.8B**). Common changes occurring in both

Low and High Runners included glutamate receptor signaling pathway, but most interestingly neurogenesis *Biological Processes* (**Fig. III.6C**), which were associated with sarcoplasmic reticulum and kinesin complex *Cellular Components* (**Fig. III.7C**), and insulin-like growth factor binding and ionotropic glutamate receptor activity *Molecular Functions* (**Fig. III.8C**). These results suggest that synaptic transmission changes occur in Low Runners, while more varied changes are observed in High Runners. The fact that neurogenesis was found in the common pathways helps to validate our approach.

We then took a closer look to the transcriptomic changes that are unique to each running condition. Changes unique to High Runners included the MAPK signaling pathway (**Fig. III.9A**), enrichments in regulation of heart rate by cardiac conduction and behavioural defense and fear responses *Biological Processes* (**Fig. III.9B**), which were associated with lysosomal lumen and receptor complex *Cellular Components* (**Fig. III.9C**), and misfolded protein binding *Molecular Function* (**Fig. III.9D**). In contrast, changes unique to Low Runners included the calcium regulation (**Fig. III.10A**), enrichments in synaptic transmission *Biological Process* (**Fig. III.10B**), which were associated with synaptic and post-synaptic membrane *Cellular Components* (**Fig.III. 10C**), and transporter and microtubule *Molecular Functions* (**Fig. III.10D**). This more specific approach allowed to better define changes in cardiovascular functions for High Runners, whereas Low Runners showed consisted changes in calcium regulation and synaptic transmission.

These results suggest that Low and High Runners have distinct molecular changes and that Low Runners show the greatest difference from the LD, which will be explored in the future.

III.5.5. Genetic changes induced by the complex environment

To gain insights into the genetic changes induced by the exposure to a CE, the same analyses as before were performed. Transcriptomic changes within the CE group revealed serotonin receptor 2 and ELK-SRF/GATA4 signaling and inflammatory response pathways (**Fig. III.11A**). Moreover, enrichments in protein heterotrimerization and extracellular matrix organization *Biological Processes* (**Fig. III.11B**), were associated with collagen trimer and endoplasmic reticulum lumen *Cellular Components* (**Fig. III.11C**), and growth factor *Molecular*

Function (**Fig. III.11D**). These results suggest an influence of the CE in changes linked to serotonin transporters, which were shown to be found at the endoplasmic reticulum (Anderluh et al. 2014).

III.5.6. Running and CE induce distinct genetic changes in the dentate gyrus niche

The next logical step was to compare transcriptomic changes between running (Low and High runners combined) and the CE groups (including socially-housed mice). Another Venn diagram was created to determine both unique and common genes. Low Runners showed the highest number of genes specific to this condition with 340 genes, in contrast to 53 for High Runners. These results suggest that a certain level of exercise might be sufficient to modify the transcription and lead important changes (**Fig.III.12**). The CE condition showed a limited amount of specific genes with 31, suggesting that only a small difference between the LD and CE exists in terms of transcriptome changes, consistent with the cluster dendrogram (**Fig. III.12**). As done previously, we used Enrichr to analyze the similarities and differences between running and CE groups. Transcriptomic changes within the runners revealed particular enrichments in calcium regulation signaling pathways (**Fig. III.13A**). Synaptic transmission *Biological Process* (**Fig. III.13B**) was associated with synaptic and post-synaptic membrane *Cellular Components* (**Fig. III.13C**) and transmembrane transporter activity *Molecular Function* (**Fig. III.13D**). In contrast, changes specific to CE included serotonin receptor 2 signaling pathways once again (**Fig. III.14A**). Negative regulation of fibroblast growth factor receptor (FGF) signaling pathways *Biological Processes* (**Fig. III.14B**) were associated with extracellular matrix *Cellular Components* (**Fig.III. 14C**), and hormone and growth factor binding *Molecular Functions* (**Fig. III.14D**). Interestingly, BDNF came up in the running groups, which is not surprising as it is a well-known growth factor that is implicated in the exercise-induced changes (Choi et al. 2009). In contrast, the serotonin and FGF signaling pathways were modulated in the DG niche following CE exposure.

These results suggest that running and CE induce distinct molecular changes.

III.6. Discussion

EE has beneficial effects on neurogenesis, learning, and memory. Typical EE paradigms include several components such as physical exercise, environmental complexity, social interactions, and stress. Following investigation on the specific individual contribution of each EE variable in our previous study (Gregoire et al. 2014), we investigated further the behavioral outcome of exposure to a running and socially-housed environments during RAM and NOR tasks. Additionally, RNA-Seq was conducted to better understand the underlying mechanisms of changes (transcriptomics) orchestrated by these enriched environments on the DG niche. Our behavioral studies showed that running CD1 mice had stronger short-term recall, whereas socially-housed mice had enhanced cognitive flexibility on the RAM task. Mice showed no difference in locomotor activity between groups that could explain behavioural changes. Socially-housed mice performed better on the not reward-driven NOR short-term object recognition memory test, while runners showed an improvement for the long-term memory task. RNA-Seq analysis revealed that the distance ran influences transcriptomics and these changes differ from the one induced following CE exposure. These findings provide valuable insights in the importance of each component of the EE and reveal potential targets to promote beneficial cognitive activity.

In this study, we provided novel findings from the RAM task as Clelland and colleagues assessed the importance of hippocampal neurogenesis in the capacity to discriminate between closer arms, but did not look into the effect of enriched environment on task performance (Clelland et al. 2009). Runners showed improved short-term recall of the sample phase. These results suggest that the RUN group mice remember the previously-baited arm location, but learned again the new location as shown by the decreasing number of errors (learning curve) through time. Other studies have been conducted in the RAM, however, the paradigms differ and were performed with rats. Anderson and colleagues demonstrated that exercise improves RAM spatial learning in rats (Anderson et al. 2000). Another study suggested that exposure to both physical activity and cognitive stimulation (Hebb-Williams maze) led to an improved behavioral performance on the RAM baited configuration (Langdon and Corbett 2012). Socially-housed mice showed better cognitive flexibility. Some studies

have looked at the effect of isolation on learning and memory, but only a few looked at social interactions using the RAM (Silva et al. 2011).These results confirm our hypothesis that runners differ in their behavioral outcome from socially-housed mice.

The NOR paradigm was designed to assess both short-term and long-term object recognition memory. In the EE field, this test is not as common as reward-driven tasks, such as the MWM. However, we chose this test as it involves the mouse innate preference for novelty rather than reinforcement with a reward. Moreover, it was shown that NOR is hippocampus-dependent for a delayed NOR paradigm, defined as more than 15 minutes, which corresponds to both our memory paradigms (Hammond et al. 2004). In this study, we showed that socially-housed animals and runners performed better during the short-term and long-term task, respectively. These results were also consistent with our hypothesis for different behavioral outcome between these two enriched groups.

The most interesting results reside in our RNA-Seq transcriptomics findings. This method generated a lot of data, but for this dissertation, I limited myself to the three main following observations based on bioinformatics: 1) Difference between Low and High Runners, 2) Changes induced by CE, and 3) Changes unique to Runners (including Low and High Runners) and to CE. Unique molecular changes were observed for each enriched condition. Interestingly, Low and High runners were more different than we could have thought. The Low Runners showed enrichments in calcium-mediated synaptic processes. Many studies demonstrated the beneficial effects of exercise on cognitive functions such as long-term potentiation, the strengthening of synapses (Kramer et al. 1999; van Praag et al. 1999a). A proteomic analysis conducted in rats revealed that following voluntary running (with a resistance), 90% of the proteins were associated with energy metabolism and synaptic plasticity (Ding et al. 2006). This result is consistent with our observations, but this study did not take into consideration different level of running. In contrast, High Runners showed enrichments in serine/threonine MAPK-mediated processes. It was demonstrated that p38 MAPK activates PGC-1α, a transcriptional coactivator that induces mitochondrial biogenesis in skeletal muscles, that is increased following endurance exercise in human muscles (Little et

al. 2010). It would be interesting to investigate specific targets of this kinase in more detail to understand if it plays a role in the activation of NSCs. Lastly, CE induced changes in the extracellular matrix (ECM). It is well established that early embryonic NSCs first respond to FGF2, and then late embryonic and adult NSCs are responsive to both FGF2 and epidermal growth factor (EGF) (Reynolds and Weiss 1992). The EGF effect can be inhibited by BMP4. However, BMP4 can, in turn, be inhibited by Noggin, suggesting that different signals are present within the niche to balance between restriction and amplification (Lillien and Raphael 2000; Lim et al. 2000). A study showed that ECM molecules, such as tenascin C, can also control cell behavior such differentiation through growth factors (Garcion et al. 2004).

These experiments were conducted in the outbred CD1 mouse strain, whereas most EE studies use the inbred C57Bl/6 mice (Clark et al. 2011b). A concern might be that their genetic constitution is not stable. However, CD1 mice were chosen to maximize interanimal variation and hence the significance of any potential findings. The DG of eight mice were pooled in each condition to account for that variation in samples for RNA-Seq. Moreover, complete inbreeding can be considered as an unusual genetic state (Svenson et al. 2012). In regards to neurogenesis, a study showed that CD1 mice have the highest survival rate of newly-born neurons (Kempermann et al. 1997a). We have also shown that male CD1 mice exhibited a significant lower baseline proliferation rate compared to C57Bl/6 mice, permitting a running-induced increase to be detectable (Gregoire et al. 2014). Therefore, in order to be consistent with our previous studies, we decided to pursue these experiments in CD1 mice (Bednarczyk et al. 2009; Bednarczyk et al. 2011; Gregoire et al. 2014).

Overall, these results confirmed that runners and socially-housed have different behavioral outcomes and the underlying molecular changes are unique to each condition. Moreover, within the exercise group, Low and High runners showed specific modifications implying that different mechanisms are underlying changes present at various levels of exercise.

III.7. Future directions

The next logical experiment is to perform qPCR in order to validate transcriptome sequencing results (RNA-Seq). The targeted genes will be chosen based on the findings from the Wikipathways and GO analyses (Biological Process, Cellular Component, and Molecular Function) from EnrichR. The program Panther will also be useful to determine the genes that are included in the different classes of processes or pathways. Then, in order to define the importance of the targeted genes and proteins in adult neurogenesis, we will perform tissue culture using transfections by shRNA or dominant-negative plasmid to study loss-of-function and overexpression plasmids to study gain-of-function and assess for proliferation, differentiation, and survival *in vitro*. Then, we would study the importance of these genes / pathways *in vivo* by performing electroporation, a technique that has been developed in the laboratory with the help of our collaborator Dr. Fanie Barnabé-Heider (Barnabe-Heider et al. 2008).

III.8. Figures and legends

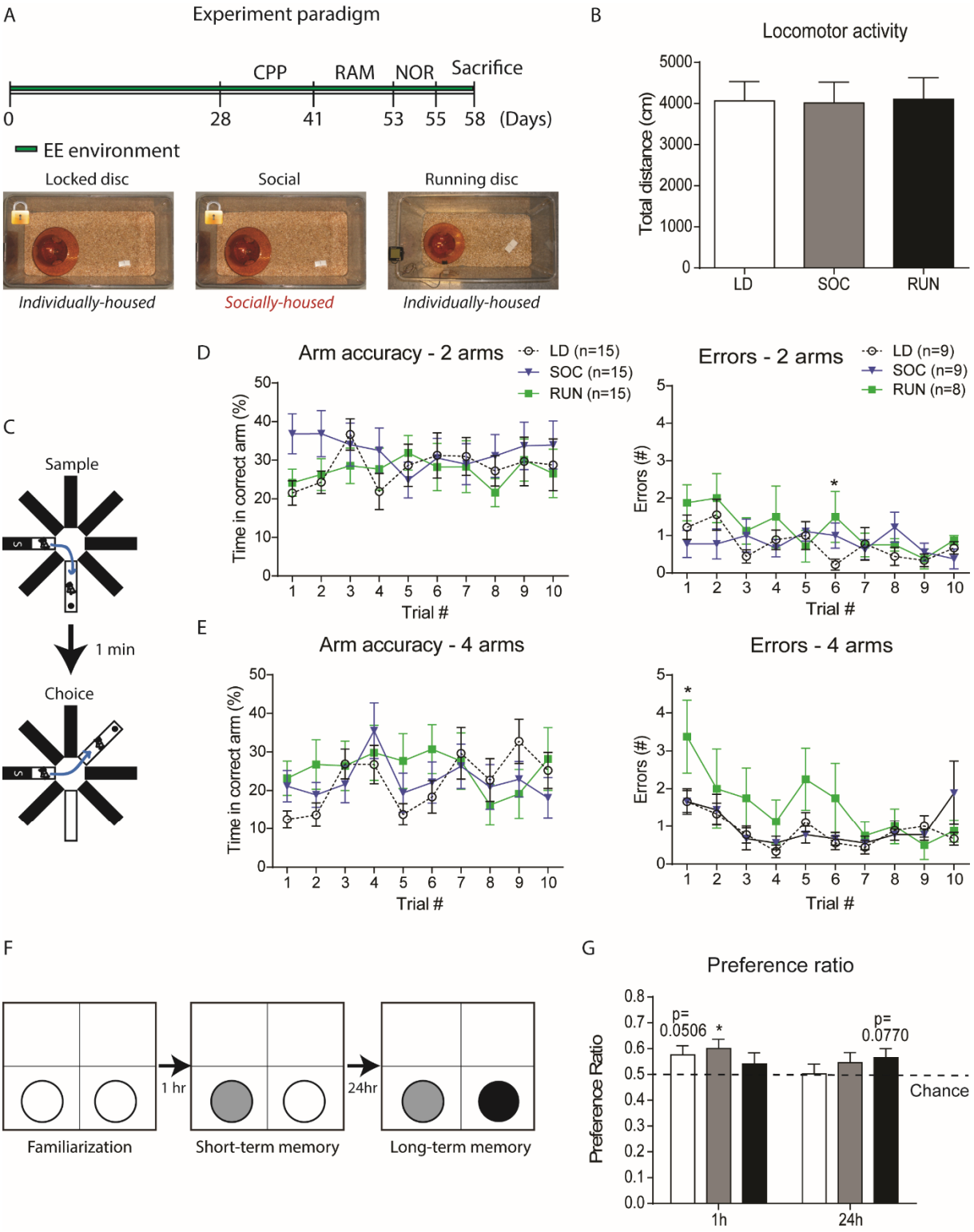


Figure III.1. Behavioural tests

Experimental paradigm for behavioural testing. Mice were exposed to enriched environments for a period of four weeks, followed by exposure to four behavioural tasks over a period of another four weeks: conditioned place preference (CPP) test, eight-arm radial maze (RAM), open-field (OF), and novel object recognition (NOR). The enriched environments were maintained as home cages throughout behavioural testing (**A**). Basic environments used in this paradigm: *Locked disc* (containing a running disc that has been locked to prevent running exercise), *Social* (a *Locked disc* cage where 3 mice are socially housed), and *Running disc* (containing a normal running disc) (**A**). The open field task was performed to measure the total distance travelled and assess the baseline locomotor activity (**B**). The RAM trial paradigm consisted of two phases: a sample phase with one baited arm followed by a choice arm where mice had the choice between the previously baited arm and the new baited arm (**C**). The two-arm separation task was performed by all three groups: locked disc (LD), socially-housed (SOC), and runners (RUN). Arm accuracy (time spent in correct arm), and errors defined as entries into the previously baited arm (**D**). Similarly, the four-arm separation task was also performed and both arm accuracy and errors were analyzed (**E**). The NOR paradigm consisted of three phases: familiarization (similar objects) followed an hour later by a task assessing short-term object recognition memory (similar and novel objects), then one day later mice were tested on their long-term memory (old novel and novel objects) (**F**). Preference ratio where chance was set at 0.5 (**G**).

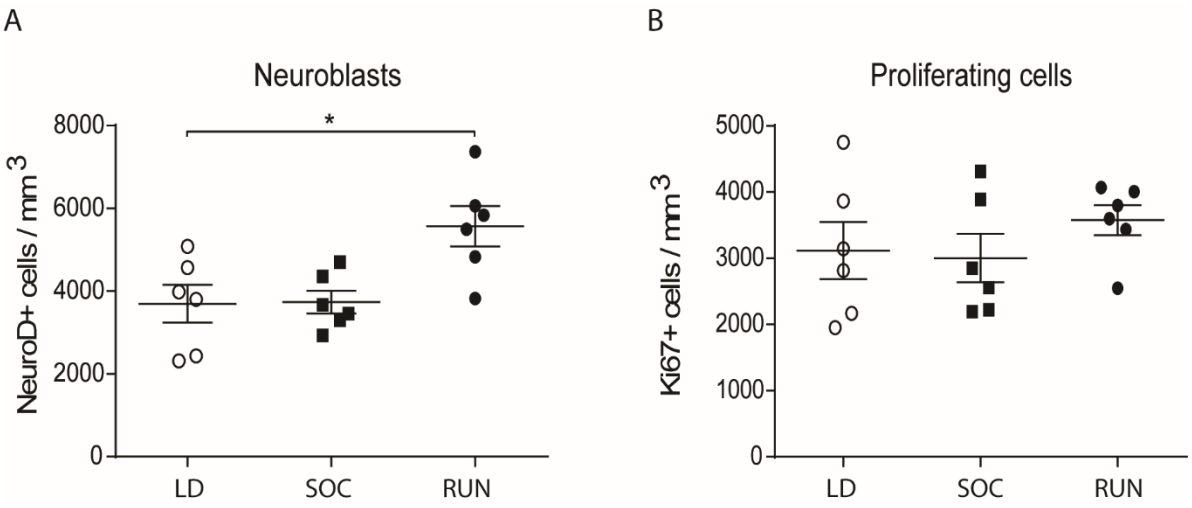


Figure III.2. Proliferating cells and neuroblasts numbers after eight weeks

Quantification of NeuroD+ neuroblasts (**A**) and Ki67+ proliferating cells (**B**) exposure to an eight-week paradigm.

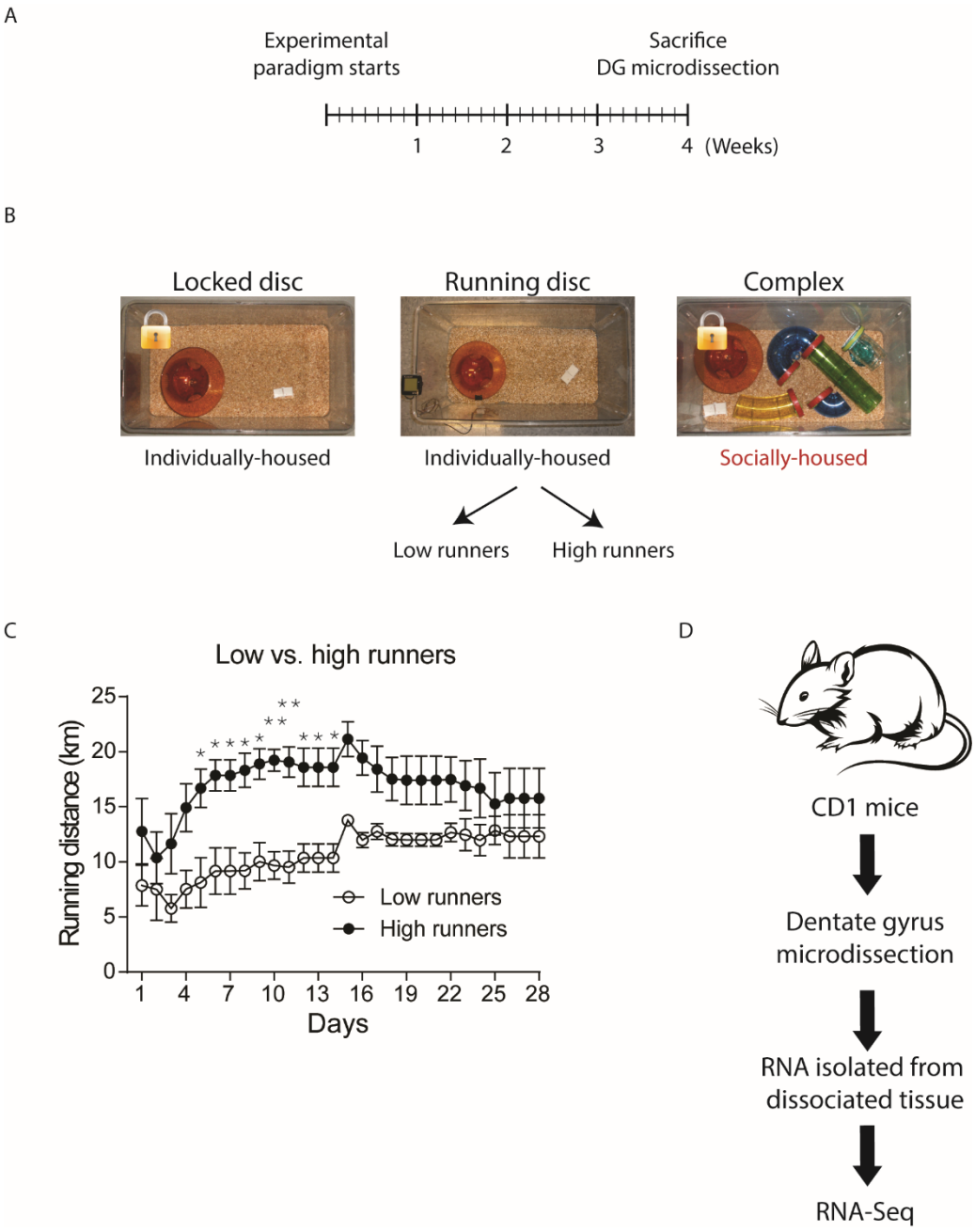


Figure III.3. RNA-Seq experimental design

Experimental paradigm of four-weeks (**A**) when mice were exposed to three different environments: Locked disc (individually-housed), Running disc (individually-housed), and Complex (socially-housed) (**B**). The runners were subdivided into two groups of low and high runners based on their average distance ran in km (**C**). Mice were sacrificed at the end of the paradigm, their dentate gyri were microdissected and the tissue was dissociated to extract RNA and submitted for sequencing at the IRIC genomics platform (RNA-Seq) (**D**).

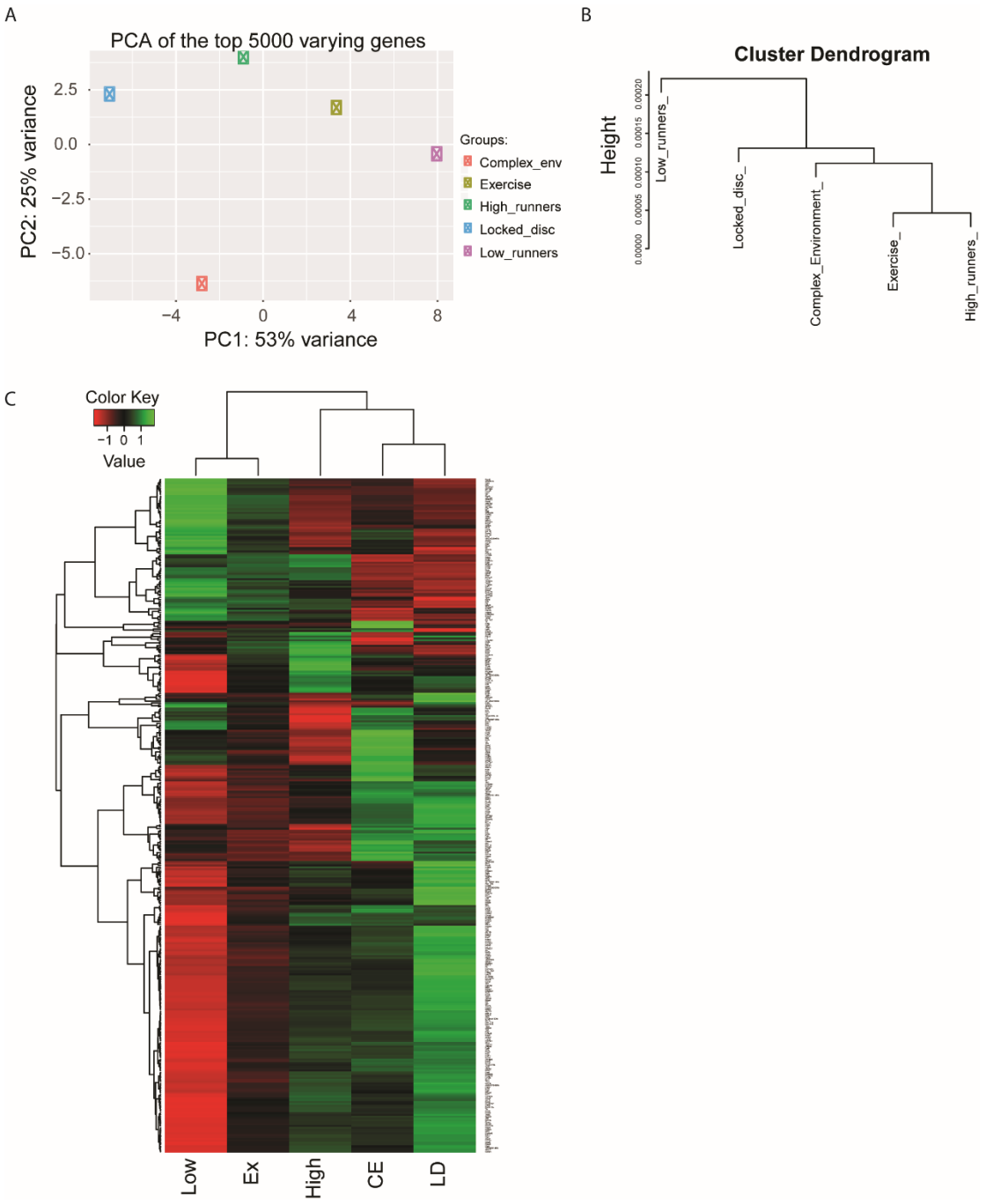


Figure III.4. RNA-Seq overview

Principal component analysis, showing 5 distinct gene populations (**A**). Cluster dendrogram, showing that the locked disc is similar to CE and that Low Runners are distinct from other exercise groups (**B**). Heatmap showing log2 (FPKM) values transformed in Z-score of significantly different expressed genes (**C**).

Table III.1. Top 25 of up-regulated genes in Low Runners

Ensembl gene ID	Gene name	Description	Log2 fold	Fold
ENSMUSG00000015090	Ptgds	prostaglandin D2 synthase (brain)	1.969967	3.918
ENSMUSG00000048583	Igf2	insulin-like growth factor 2	1.095856	2.137
ENSMUSG00000041559	Fmod	fibromodulin	1.013043	2.018
ENSMUSG00000045573	Penk	preproenkephalin	0.905162	1.873
ENSMUSG00000029843	Slc13a4	solute carrier family 13 (sodium/sulfate symporters), member 4	0.827054	1.774
ENSMUSG00000022548	Apod	apolipoprotein D	0.780339	1.718
ENSMUSG00000013584	Aldh1a2	aldehyde dehydrogenase family 1, subfamily A2	0.759932	1.693
ENSMUSG00000020473	Aebp1	AE binding protein 1	0.713043	1.639
ENSMUSG00000030218	Mgp	matrix Gla protein	0.705284	1.630
ENSMUSG00000030108	Slc6a13	solute carrier family 6 (neurotransmitter transporter, GABA), member 13	0.693914	1.618
ENSMUSG00000046352	Gjb2	gap junction protein, beta 2	0.675901	1.598
ENSMUSG00000029661	Col1a2	collagen, type I, alpha 2	0.674183	1.596
ENSMUSG00000024650	Slc22a6	solute carrier family 22 (organic anion transporter), member 6	0.627399	1.545
ENSMUSG00000026043	Col3a1	collagen, type III, alpha 1	0.592637	1.508
ENSMUSG00000047507	Baiap3	BAI1-associated protein 3	0.573576	1.488
ENSMUSG00000024793	Tnfrsf25	tumor necrosis factor receptor superfamily, member 25	0.561098	1.475
ENSMUSG00000023046	Igfbp6	insulin-like growth factor binding protein 6	0.557626	1.472
ENSMUSG00000075334	Rprm	reprimo, TP53 dependent G2 arrest mediator candidate	0.537535	1.451
ENSMUSG00000037406	Htra4	HtrA serine peptidase 4	0.526601	1.441
ENSMUSG00000032128	Robo3	roundabout guidance receptor 3	0.524813	1.439
ENSMUSG00000048126	Col6a3	collagen, type VI, alpha 3	0.506953	1.421
ENSMUSG00000035202	Lars2	leucyl-tRNA synthetase, mitochondrial	0.483818	1.398
ENSMUSG00000067786	Nnat	neuronatin	0.482454	1.397
ENSMUSG00000001119	Col6a1	collagen, type VI, alpha 1	0.460593	1.376
ENSMUSG00000052407	Ccdc171	coiled-coil domain containing 171	0.449059	1.365

Using a threshold of -0.3 > log2 fold > 0.3, we identified 424 genes that were significantly changes in the Low Runners when compared to the Locked Disc condition. Within the 108 up-regulated genes, the table shows the top 25 genes.

Table III.2. Top 25 of down-regulated genes in Low Runners

Ensembl gene ID	Gene name	Description	Log2 fold	Fold
ENSMUSG00000027014	Cwc22	CWC22 spliceosome-associated protein	-1.14831	0.451
ENSMUSG00000034731	Dgkh	diacylglycerol kinase, eta	-0.88547	0.541
ENSMUSG00000059003	Grin2a	glutamate receptor, ionotropic, NMDA2A (epsilon 1)	-0.87996	0.543
ENSMUSG00000049313	Sorl1	sortilin-related receptor, LDLR class A repeats-containing	-0.76557	0.588
ENSMUSG00000051375	Pcdh1	protocadherin 1	-0.76374	0.589
ENSMUSG00000039270	Megf9	multiple EGF-like-domains 9	-0.75501	0.593
ENSMUSG00000029361	Nos1	nitric oxide synthase 1, neuronal	-0.73796	0.600
ENSMUSG00000031292	Cdkl5	cyclin-dependent kinase-like 5	-0.73606	0.600
ENSMUSG00000034342	Cbl	Casitas B-lineage lymphoma	-0.73352	0.601
ENSMUSG00000003316	Glg1	golgi apparatus protein 1	-0.72959	0.603
ENSMUSG00000019852	D10Bwg1379e	ARFGEF family member 3	-0.72718	0.604
ENSMUSG00000023845	Lnpep	leucyl/cystinyl aminopeptidase	-0.72153	0.606
ENSMUSG00000030209	Grin2b	glutamate receptor, ionotropic, NMDA2B (epsilon 2)	-0.71522	0.609
ENSMUSG00000056258	Kcnq3	potassium voltage-gated channel, subfamily Q, member 3	-0.68842	0.621
ENSMUSG00000085438	1700020I14Rik	RIKEN cDNA 1700020I14 gene	-0.68412	0.622
ENSMUSG00000027784	Ppm1l	protein phosphatase 1 (formerly 2C)-like	-0.67791	0.625
ENSMUSG00000045589	Frrs1l	ferric-chelate reductase 1 like	-0.66118	0.632
ENSMUSG00000067336	Bmpr2	bone morphogenetic protein receptor, type II (serine/threonine kinase)	-0.65997	0.633
ENSMUSG00000024294	Mib1	mindbomb E3 ubiquitin protein ligase 1	-0.65623	0.635
ENSMUSG00000015829	Tnr	tenascin R	-0.65589	0.635
ENSMUSG00000052387	Trpm3	transient receptor potential cation channel, subfamily M, member 3	-0.64795	0.638
ENSMUSG00000030806	Stx1b	syntaxin 1B	-0.63193	0.645
ENSMUSG00000017639	Rab11fip4	RAB11 family interacting protein 4 (class II)	-0.62704	0.648
ENSMUSG00000036698	Eif2c2	argonaute RISC catalytic subunit 2	-0.6203	0.651
ENSMUSG00000073557	Ppp1r12b	protein phosphatase 1, regulatory (inhibitor) subunit 12B	-0.61826	0.651

Using a threshold of -0.3 > log2 fold > 0.3, we identified 424 genes that were significantly changes in the Low Runners when compared to the Locked Disc condition. Within the 316 down-regulated genes, the table shows the top 25 genes.

Table III.3. Top 25 of up-regulated genes in Exercise

Ensembl gene ID	Gene name	Description	Log2 fold	Fold
ENSMUSG00000015090	Ptgds	prostaglandin D2 synthase (brain)	1.337462	2.527
ENSMUSG00000045573	Penk	preproenkephalin	0.85469	1.808
ENSMUSG00000041559	Fmod	fibromodulin	0.598438	1.514
ENSMUSG00000048583	Igf2	insulin-like growth factor 2	0.574986	1.490
ENSMUSG00000037406	Htra4	HtrA serine peptidase 4	0.544093	1.458
ENSMUSG00000075334	Rprm	reprimo, TP53 dependent G2 arrest mediator candidate	0.535577	1.450
ENSMUSG00000022548	Apod	apolipoprotein D	0.473458	1.388
ENSMUSG00000032128	Robo3	roundabout guidance receptor 3	0.459296	1.375
ENSMUSG00000023046	Igfbp6	insulin-like growth factor binding protein 6	0.454045	1.370
ENSMUSG00000013584	Aldh1a2	aldehyde dehydrogenase family 1, subfamily A2	0.441239	1.358
ENSMUSG00000029843	Slc13a4	solute carrier family 13 (sodium/sulfate symporters), member 4	0.432079	1.349
ENSMUSG00000039457	Ppl	periplakin	0.429833	1.347
ENSMUSG00000029661	Col1a2	collagen, type I, alpha 2	0.422054	1.340
ENSMUSG00000030218	Mgp	matrix Gla protein	0.415232	1.334
ENSMUSG00000030108	Slc6a13	solute carrier family 6 (neurotransmitter transporter, GABA), member 13	0.412797	1.331
ENSMUSG00000031557	Plekha2	pleckstrin homology domain-containing, family A member 2	0.410648	1.329
ENSMUSG00000037872	Darc	atypical chemokine receptor 1 (Duffy blood group)	0.401604	1.321
ENSMUSG00000042793	Lgr6	leucine-rich repeat-containing G protein-coupled receptor 6	0.400424	1.320
ENSMUSG00000028843	Sh3bgrl3	SH3 domain binding glutamic acid-rich protein-like 3	0.399768	1.319
ENSMUSG00000023232	Serinc2	serine incorporator 2	0.389887	1.310
ENSMUSG00000020473	Aebp1	AE binding protein 1	0.387751	1.308
ENSMUSG00000074578	1500012F01Rik	zinc finger, NFX1-type containing 1, antisense RNA 1	0.387608	1.308
ENSMUSG00000051617	Krt9	keratin 9	0.386592	1.307
ENSMUSG00000024793	Tnfrsf25	tumor necrosis factor receptor superfamily, member 25	0.377948	1.299
ENSMUSG00000038530	Rgs4	regulator of G-protein signaling 4	0.370484	1.293

Using a threshold of -0.3 > log2 fold > 0.3, we identified 178 genes that were significantly changes in the Low Runners when compared to the Locked Disc condition. Within the 49 up-regulated genes, the table shows the top 25 genes.

Table III.4. Top 25 of down-regulated genes in Exercise

Ensembl gene ID	Gene name	Description	Log2 fold	Fold
ENSMUSG00000029361	Nos1	nitric oxide synthase 1, neuronal	-0.57847	0.670
ENSMUSG00000059003	Grin2a	glutamate receptor, ionotropic, NMDA2A (epsilon 1)	-0.5747	0.671
ENSMUSG00000056258	Kcnq3	potassium voltage-gated channel, subfamily Q, member 3	-0.54011	0.688
ENSMUSG00000034981	Parm1	prostate androgen-regulated mucin-like protein 1	-0.51962	0.698
ENSMUSG00000039270	Megf9	multiple EGF-like-domains 9	-0.50657	0.704
ENSMUSG00000034342	Cbl	Casitas B-lineage lymphoma	-0.50575	0.704
ENSMUSG00000034731	Dgkh	diacylglycerol kinase, eta	-0.50508	0.705
ENSMUSG00000026185	Igfbp5	insulin-like growth factor binding protein 5	-0.50163	0.706
ENSMUSG00000045589	Frrs1l	ferric-chelate reductase 1 like	-0.49603	0.709
ENSMUSG00000028842	Eif2c3	argonaute RISC catalytic subunit 3	-0.48981	0.712
ENSMUSG00000022324	Matn2	matrilin 2	-0.48023	0.717
ENSMUSG00000030102	Itpr1	inositol 1,4,5-trisphosphate receptor 1	-0.48018	0.717
ENSMUSG00000023845	Lnpep	leucyl/cystinyl aminopeptidase	-0.46423	0.725
ENSMUSG00000036698	Eif2c2	argonaute RISC catalytic subunit 2	-0.46115	0.726
ENSMUSG00000026841	Fibcd1	fibrinogen C domain containing 1	-0.46022	0.727
ENSMUSG00000052387	Trpm3	transient receptor potential cation channel, subfamily M, member 3	-0.46008	0.727
ENSMUSG00000049313	Sorl1	sortilin-related receptor, LDLR class A repeats-containing	-0.4575	0.728
ENSMUSG00000016150	Tenm1	teneurin transmembrane protein 1	-0.45616	0.729
ENSMUSG00000046318	Ccbe1	collagen and calcium binding EGF domains 1	-0.45559	0.729
ENSMUSG00000001985	Grik3	glutamate receptor, ionotropic, kainate 3	-0.45528	0.729
ENSMUSG00000046607	Hrk	harakiri, BCL2 interacting protein (contains only BH3 domain)	-0.44563	0.734
ENSMUSG00000019852	D10Bwg1379e	ARFGEF family member 3	-0.44303	0.736
	E130008D07Rik	RIKEN cDNA E130008D07 gene	-0.44296	0.736
ENSMUSG00000030209	Grin2b	glutamate receptor, ionotropic, NMDA2B (epsilon 2)	-0.44121	0.737
ENSMUSG00000102697	Pcdhac2	protocadherin alpha subfamily C, 2	-0.43957	0.737

Using a threshold of -0.3 > log2 fold > 0.3, we identified 178 genes that were significantly changes in the Low Runners when compared to the Locked Disc condition. Within the 129 down-regulated genes, the table shows the top 25 genes.

Table III.5. Top 25 of up-regulated genes in High Runners

Ensembl gene ID	Gene name	Description	Log2 fold	Fold
ENSMUSG00000045573	Penk	preproenkephalin	0.834299	1.783
ENSMUSG00000037406	Htra4	HtrA serine peptidase 4	0.571414	1.486
ENSMUSG00000019970	Sgk1	serum/glucocorticoid regulated kinase 1	0.546006	1.460
ENSMUSG00000075334	Rprm	reprimo, TP53 dependent G2 arrest mediator candidate	0.511043	1.425
ENSMUSG00000023046	Igfbp6	insulin-like growth factor binding protein 6	0.458461	1.374
ENSMUSG00000028370	Pappa	pregnancy-associated plasma protein A	0.456722	1.372
ENSMUSG00000031557	Plekha2	pleckstrin homology domain-containing, family A member 2	0.456199	1.372
ENSMUSG00000042793	Lgr6	leucine-rich repeat-containing G protein-coupled receptor 6	0.450569	1.367
ENSMUSG00000052557	Gan	giant axonal neuropathy	0.449415	1.365
ENSMUSG00000021478	Drd1a	dopamine receptor D1	0.432725	1.350
ENSMUSG00000032128	Robo3	roundabout guidance receptor 3	0.426911	1.344
ENSMUSG00000035202	Lars2	leucyl-tRNA synthetase, mitochondrial	0.420399	1.338
ENSMUSG00000059991	Nptx2	neuronal pentraxin 2	0.419791	1.338
ENSMUSG00000038530	Rgs4	regulator of G-protein signaling 4	0.416446	1.335
ENSMUSG00000026278	Bok	BCL2-related ovarian killer	0.410005	1.329
ENSMUSG00000090291	Lrrc10b	leucine rich repeat containing 10B	0.405425	1.324
ENSMUSG00000036907	C1ql2	complement component 1, q subcomponent-like 2	0.404165	1.323
ENSMUSG00000056596	Trnp1	TMF1-regulated nuclear protein 1	0.400567	1.320
ENSMUSG00000023232	Serinc2	serine incorporator 2	0.399382	1.319
ENSMUSG00000032487	Ptgs2	prostaglandin-endoperoxide synthase 2	0.39874	1.318
ENSMUSG00000013367	Iglon5	IgLON family member 5	0.392747	1.313
ENSMUSG00000041695	Kcnj2	potassium inwardly-rectifying channel, subfamily J, member 2	0.387747	1.308
ENSMUSG00000039457	Ppl	periplakin	0.385232	1.306
ENSMUSG00000028843	Sh3bgrl3	SH3 domain binding glutamic acid-rich protein-like 3	0.383821	1.305
ENSMUSG00000059149	Mfsd4	major facilitator superfamily domain containing 4A	0.363023	1.286

Using a threshold of -0.3 > log2 fold > 0.3, we identified 112 genes that were significantly changes in the Low Runners when compared to the Locked Disc condition. Within the 48 up-regulated genes, the table shows the top 25 genes.

Table III.6. Top 25 of down-regulated genes in High Runners

Ensembl gene ID	Gene name	Description	Log2 fold	Fold
ENSMUSG00000021390	Ogn	osteoglycin	-0.6777	0.625
ENSMUSG00000026185	Igfbp5	insulin-like growth factor binding protein 5	-0.5506	0.683
ENSMUSG00000001985	Grik3	glutamate receptor, ionotropic, kainate 3	-0.53562	0.690
ENSMUSG00000019929	Dcn	decorin	-0.52237	0.696
ENSMUSG00000026841	Fibcd1	fibrinogen C domain containing 1	-0.51904	0.698
ENSMUSG00000093880	Tmem181c-ps	transmembrane protein 181C, pseudogene	-0.49922	0.707
ENSMUSG00000029361	Nos1	nitric oxide synthase 1, neuronal	-0.4538	0.730
ENSMUSG00000020251	Glt8d2	glycosyltransferase 8 domain containing 2	-0.43648	0.739
ENSMUSG00000046607	Hrk	harakiri, BCL2 interacting protein (contains only BH3 domain)	-0.43517	0.740
ENSMUSG00000034981	Parm1	prostate androgen-regulated mucin-like protein 1	-0.43032	0.742
ENSMUSG00000007613	Tgfbr1	transforming growth factor, beta receptor I	-0.42432	0.745
ENSMUSG00000021803	Cdhr1	cadherin-related family member 1	-0.42117	0.747
ENSMUSG00000046743	Fat4	FAT atypical cadherin 4	-0.41929	0.748
ENSMUSG00000051726	Kcnf1	potassium voltage-gated channel, subfamily F, member 1	-0.41309	0.751
ENSMUSG00000019966	Kitl	kit ligand	-0.40791	0.754
ENSMUSG00000051497	Kcnj16	potassium inwardly-rectifying channel, subfamily J, member 16	-0.4031	0.756
ENSMUSG00000031343	Gabra3	gamma-aminobutyric acid (GABA) A receptor, subunit alpha 3	-0.40263	0.756
ENSMUSG00000024610	Cd74	CD74 antigen	-0.40232	0.757
ENSMUSG00000022324	Matn2	matrilin 2	-0.40078	0.757
ENSMUSG00000030102	Itpr1	inositol 1,4,5-trisphosphate receptor 1	-0.4002	0.758
ENSMUSG00000025384	2310003H01Rik	Fanconi anemia core complex associated protein 100	-0.39494	0.761
ENSMUSG00000056258	Kcnq3	potassium voltage-gated channel, subfamily Q, member 3	-0.3921	0.762
ENSMUSG00000020032	Nuak1	NUAK family, SNF1-like kinase, 1	-0.39095	0.763
ENSMUSG00000030889	Vwa3a	von Willebrand factor A domain containing 3A	-0.39011	0.763
ENSMUSG00000003949	Hlf	hepatic leukemia factor	-0.38084	0.768

Using a threshold of -0.3 > log2 fold > 0.3, we identified 112 genes that were significantly changes in the Low Runners when compared to the Locked Disc condition. Within the 64 down-regulated genes, the table shows the top 25 genes.

Table III.7. Top 25 of up-regulated genes in Complex Environment

Ensembl gene ID	Gene name	Description	Log2 fold	Fold
ENSMUSG00000040152	Thbs1	thrombospondin 1	0.686071	1.609
ENSMUSG00000061808	Ttr	transthyretin	0.621779	1.539
ENSMUSG00000024121	Atp6v0c	ATPase, H+ transporting, lysosomal V0 subunit C	0.599845	1.516
ENSMUSG00000036357	Gpr101	G protein-coupled receptor 101	0.526296	1.440
ENSMUSG00000001119	Col6a1	collagen, type VI, alpha 1	0.518645	1.433
ENSMUSG00000069939	Gm12070	predicted gene 12070	0.517298	1.431
ENSMUSG00000042793	Lgr6	leucine-rich repeat-containing G protein-coupled receptor 6	0.505229	1.419
ENSMUSG00000034796	Cpne7	copine VII	0.483809	1.398
ENSMUSG00000096768	Erdr1	erythroid differentiation regulator 1	0.48279	1.397
ENSMUSG00000041380	Htr2c	5-hydroxytryptamine (serotonin) receptor 2C	0.470805	1.386
ENSMUSG00000034361	Cpne2	copine II	0.460211	1.376
ENSMUSG00000029661	Col1a2	collagen, type I, alpha 2	0.451562	1.368
ENSMUSG00000019817	Plagl1	pleiomorphic adenoma gene-like 1	0.440868	1.357
ENSMUSG00000047507	Baiap3	BAI1-associated protein 3	0.437943	1.355
ENSMUSG00000026837	Col5a1	collagen, type V, alpha 1	0.437622	1.354
ENSMUSG00000020241	Col6a2	collagen, type VI, alpha 2	0.427941	1.345
ENSMUSG00000031765	Mt1	metallothionein 1	0.392772	1.313
ENSMUSG00000039059	Hrh3	histamine receptor H3	0.388785	1.309
ENSMUSG00000001506	Col1a1	collagen, type I, alpha 1	0.387417	1.308
ENSMUSG00000022577	Ly6h	lymphocyte antigen 6 complex, locus H	0.384061	1.305
ENSMUSG00000040860	Crocc	ciliary rootlet coiled-coil, rootletin	0.380064	1.301
ENSMUSG00000052557	Gan	giant axonal neuropathy	0.375011	1.297
ENSMUSG00000017466	Timp2	tissue inhibitor of metalloproteinase 2	0.368923	1.291
ENSMUSG00000026247	Ece1	endothelin converting enzyme-like 1	0.368912	1.291
ENSMUSG00000019929	Dcn	decorin	0.368482	1.291

Using a threshold of -0.3 > log2 fold > 0.3, we identified 81 genes that were significantly changes in the Low Runners when compared to the Locked Disc condition. Within the 45 up-regulated genes, the table shows the top 25 genes.

Table III.8. Top 25 of down-regulated genes in Complex Environment

Ensembl gene ID	Gene name	Description	Log2 fold	Fold
ENSMUSG00000094786	Gm14403	predicted gene 14403	-0.53446	0.690
ENSMUSG00000063415	Cyp26b1	cytochrome P450, family 26, subfamily b, polypeptide 1	-0.4789	0.718
ENSMUSG00000027014	Cwc22	CWC22 spliceosome-associated protein	-0.4436	0.735
ENSMUSG00000034731	Dgkh	diacylglycerol kinase, eta	-0.43212	0.741
ENSMUSG00000024610	Cd74	CD74 antigen (invariant polypeptide of major histocompatibility complex)	-0.43192	0.741
ENSMUSG00000024026	Glo1	glyoxalase 1	-0.42736	0.744
ENSMUSG00000059003	Grin2a	glutamate receptor, ionotropic, NMDA2A (epsilon 1)	-0.41624	0.749
ENSMUSG00000014956	Ppp1cb	protein phosphatase 1, catalytic subunit, beta isoform	-0.40477	0.755
ENSMUSG00000021948	Prkcd	protein kinase C, delta	-0.40245	0.757
ENSMUSG00000078877	Gm14295	predicted gene 14295	-0.39347	0.761
ENSMUSG00000028842	Eif2c3	argonaute RISC catalytic subunit 3	-0.39314	0.761
ENSMUSG00000085438	1700020I14Rik	RIKEN cDNA 1700020I14 gene	-0.38812	0.764
ENSMUSG00000033623	Pcgf3	polycomb group ring finger 3	-0.38809	0.764
	E130008D07Rik	RIKEN cDNA E130008D07 gene	-0.37811	0.769
ENSMUSG00000030350	Prmt8	protein arginine N-methyltransferase 8	-0.3746	0.771
ENSMUSG00000035236	Scai	suppressor of cancer cell invasion	-0.37417	0.772
ENSMUSG00000027347	Rasgrp1	RAS guanyl releasing protein 1	-0.36928	0.774
ENSMUSG00000030102	Itpr1	inositol 1,4,5-trisphosphate receptor 1	-0.36767	0.775
ENSMUSG00000035284	Vps13c	vacuolar protein sorting 13C	-0.36363	0.777
ENSMUSG00000023845	Lnpep	leucyl/cystinyl aminopeptidase	-0.3479	0.786
ENSMUSG00000027499	Pkia	protein kinase inhibitor, alpha	-0.33797	0.791
ENSMUSG00000048732	Klhl11	kelch-like 11	-0.33499	0.793
ENSMUSG00000033454	Zbtb1	zinc finger and BTB domain containing 1	-0.32723	0.797
ENSMUSG00000075470	Alg10b	asparagine-linked glycosylation 10B (alpha-1,2-glucosyltransferase)	-0.32077	0.801
ENSMUSG00000017418	Arl5b	ADP-ribosylation factor-like 5B	-0.31903	0.802

Using a threshold of -0.3 > log2 fold > 0.3, we identified 81 genes that were significantly changes in the Low Runners when compared to the Locked Disc condition. Within the 36 down-regulated genes, the table shows the top 25 genes.

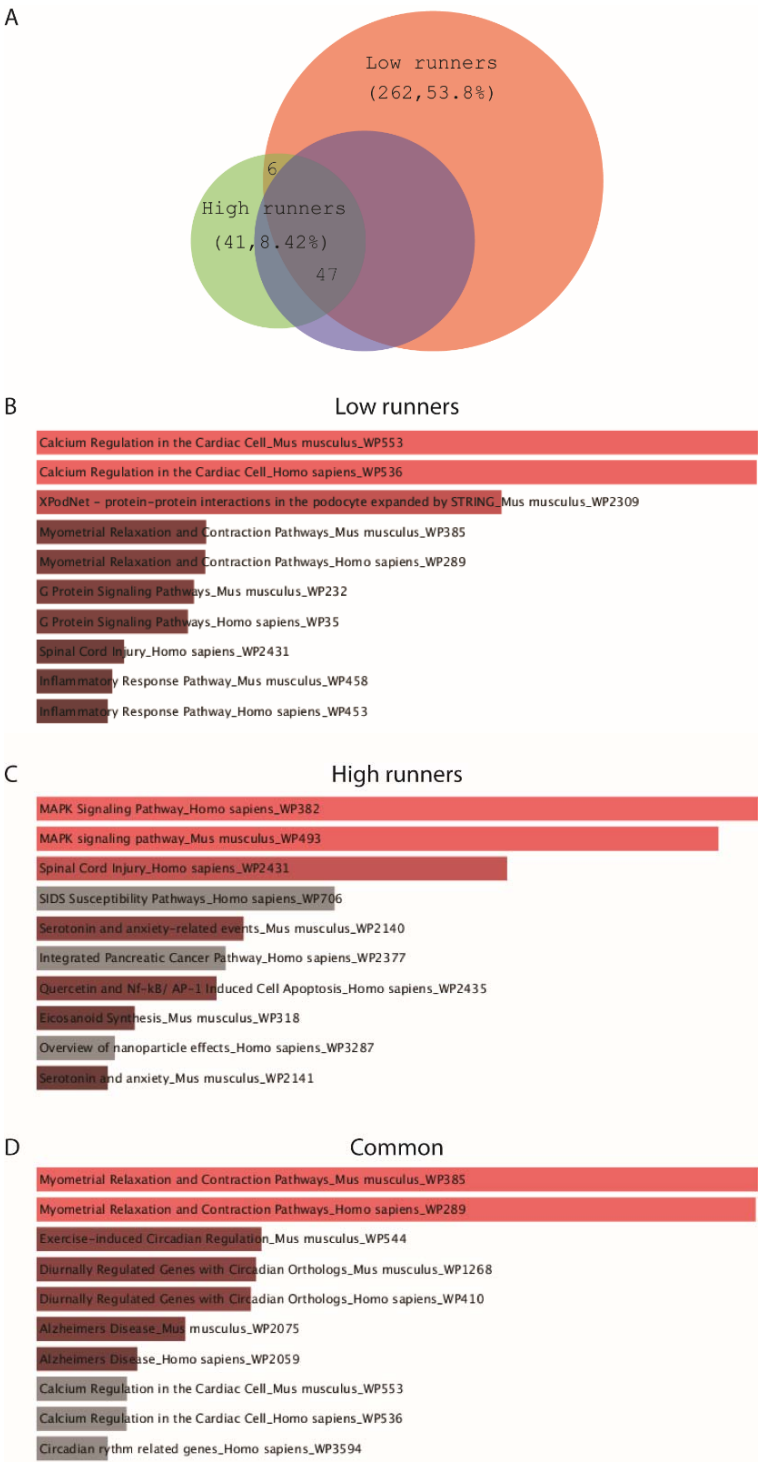


Figure III.5. Signaling pathways of Low and High runners

Venn diagram (BioVenn) showing the Low and High Runners as two distinct sets of genes when compared to the merged exercise group (purple) (**A**). Wikipathways (Enrichr) for the full list of significant genes for Low Runners (**B**), High Runners (**C**), and Low and High runners combined (**D**).

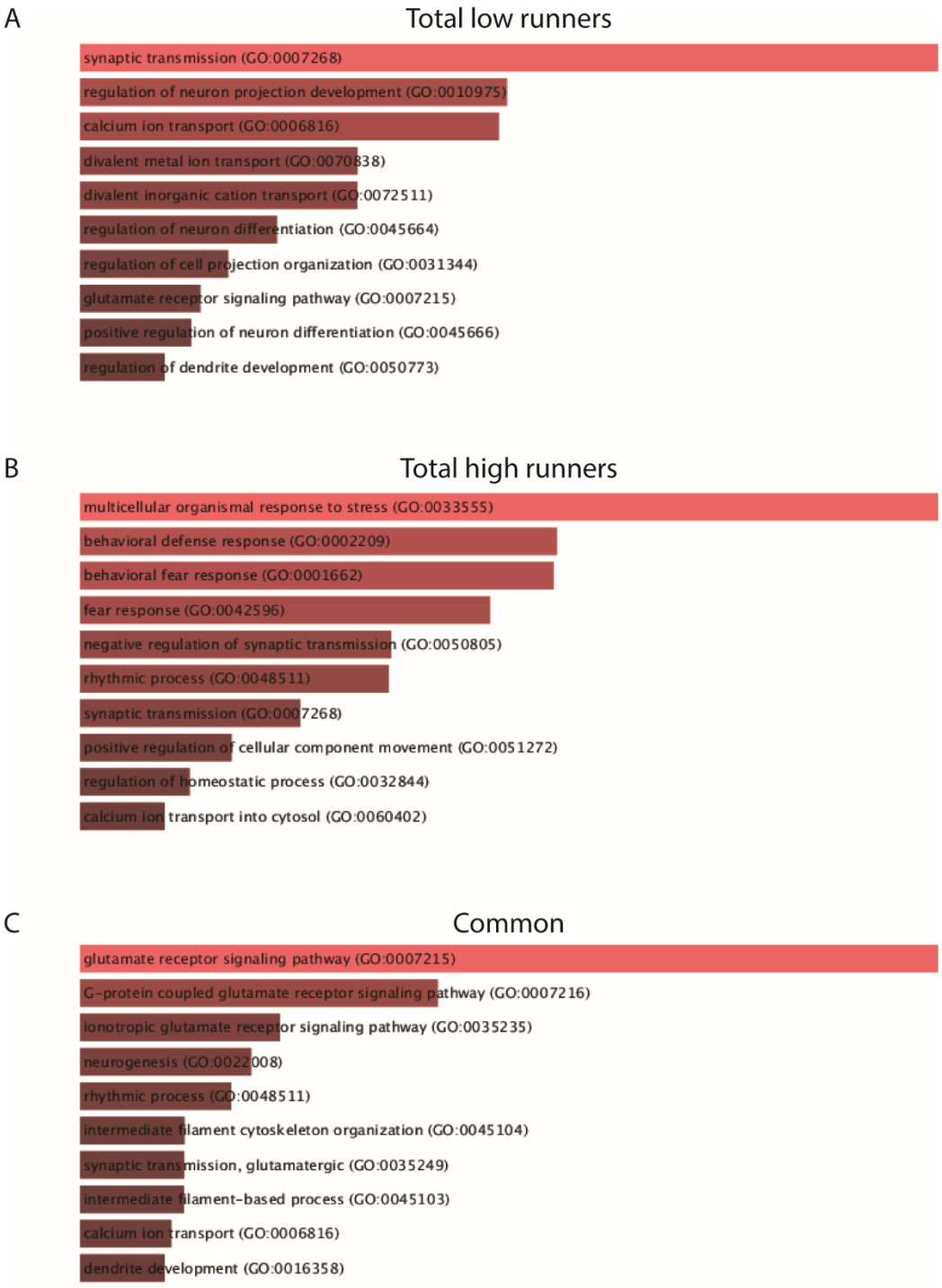


Figure III.6. Biological Processes of Low and High Runners

The total list of significant genes for Low Runners (**A**), High Runners (**B**), and their combination (**C**) was analyzed through GO Biological Process (Enrichr).

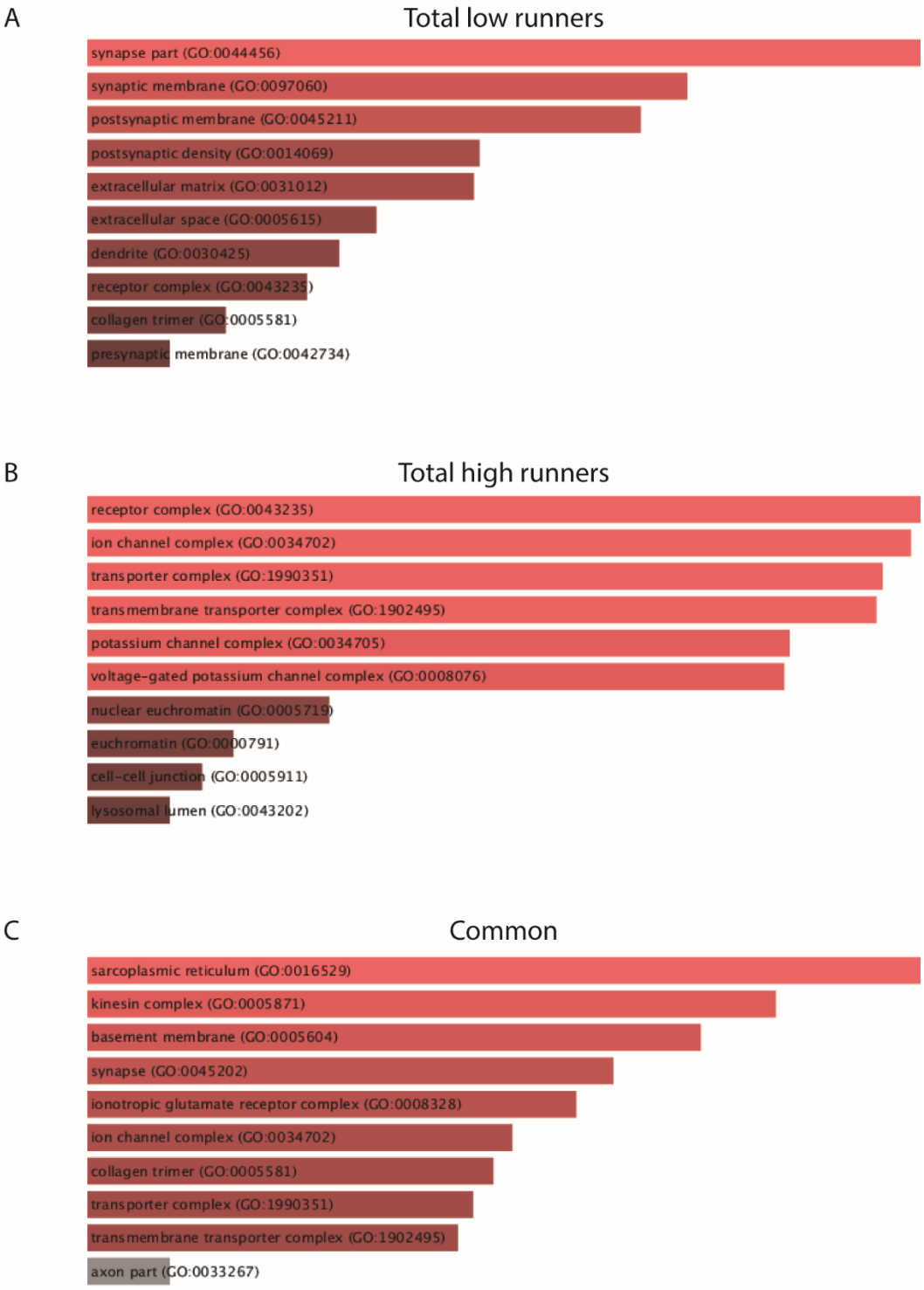


Figure III.7. Cellular Components for Low and High Runners

The total list of significant genes for Low Runners (**A**), High Runners (**B**), and their combination (**C**) was analyzed through GO Cellular Component (Enrichr).

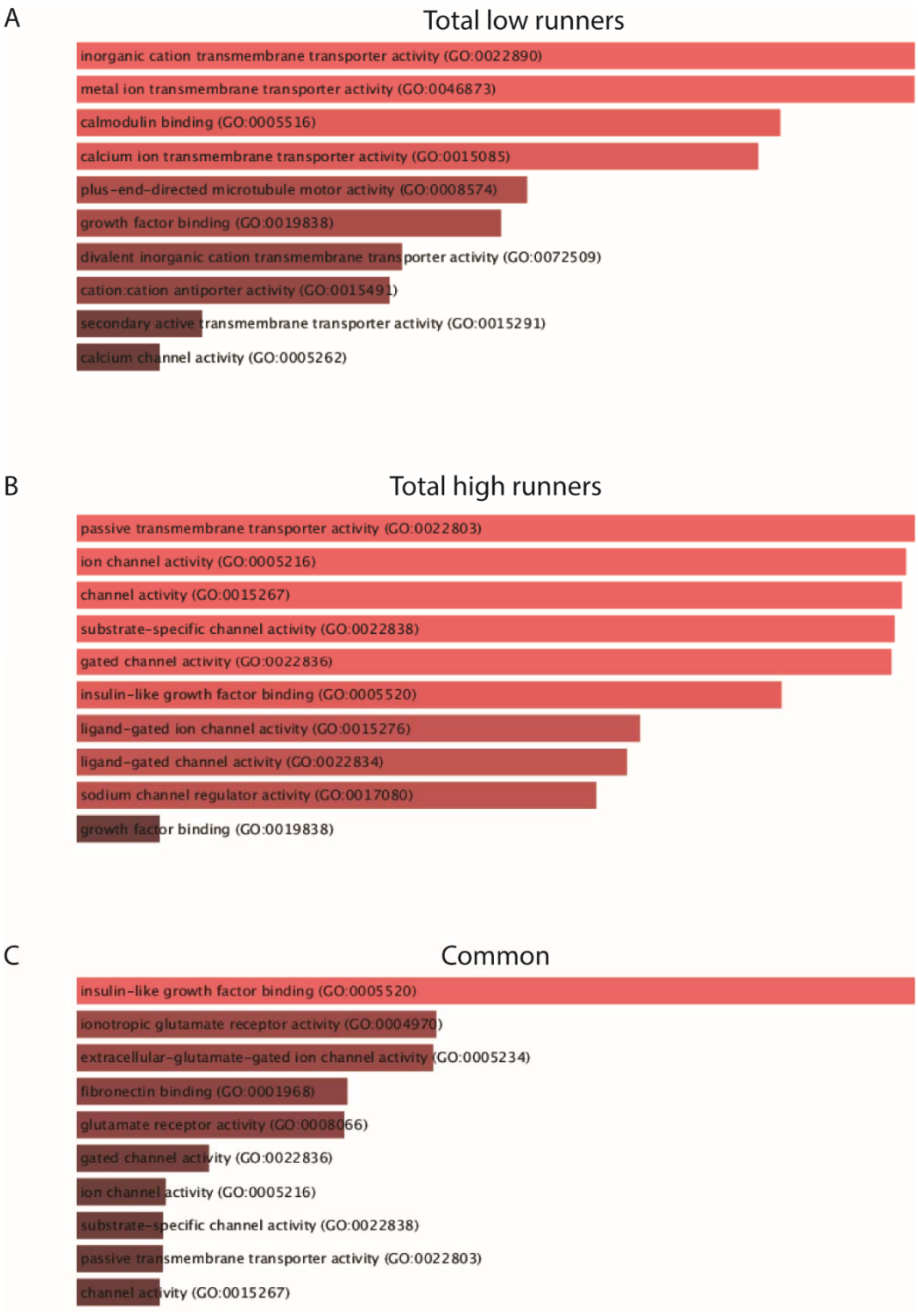


Figure III.8. Molecular Functions for Low and High Runners

The total list of significant genes for Low Runners (**A**), High Runners (**B**), and their combination (**C**) was analyzed through GO Molecular Function (Enrichr).

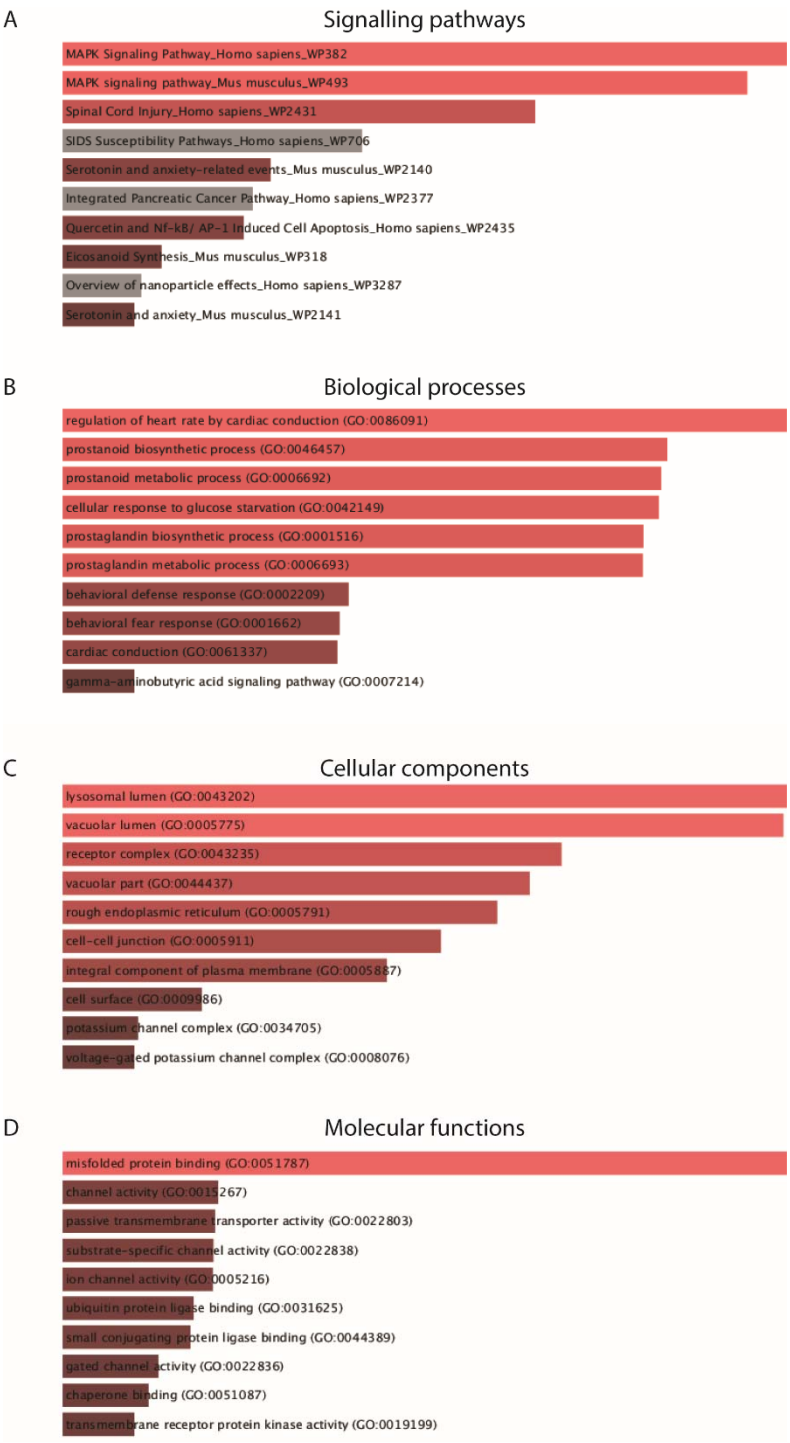


Figure III.9. Enrichr analysis for genes unique to High Runners

Genes unique to High Runners (see Venn diagram Fig. 7) were analyzed for signalling pathways (**A**), Biological Processes (**B**), Cellular Components (**C**), and Molecular Functions (**D**) using Enrichr.

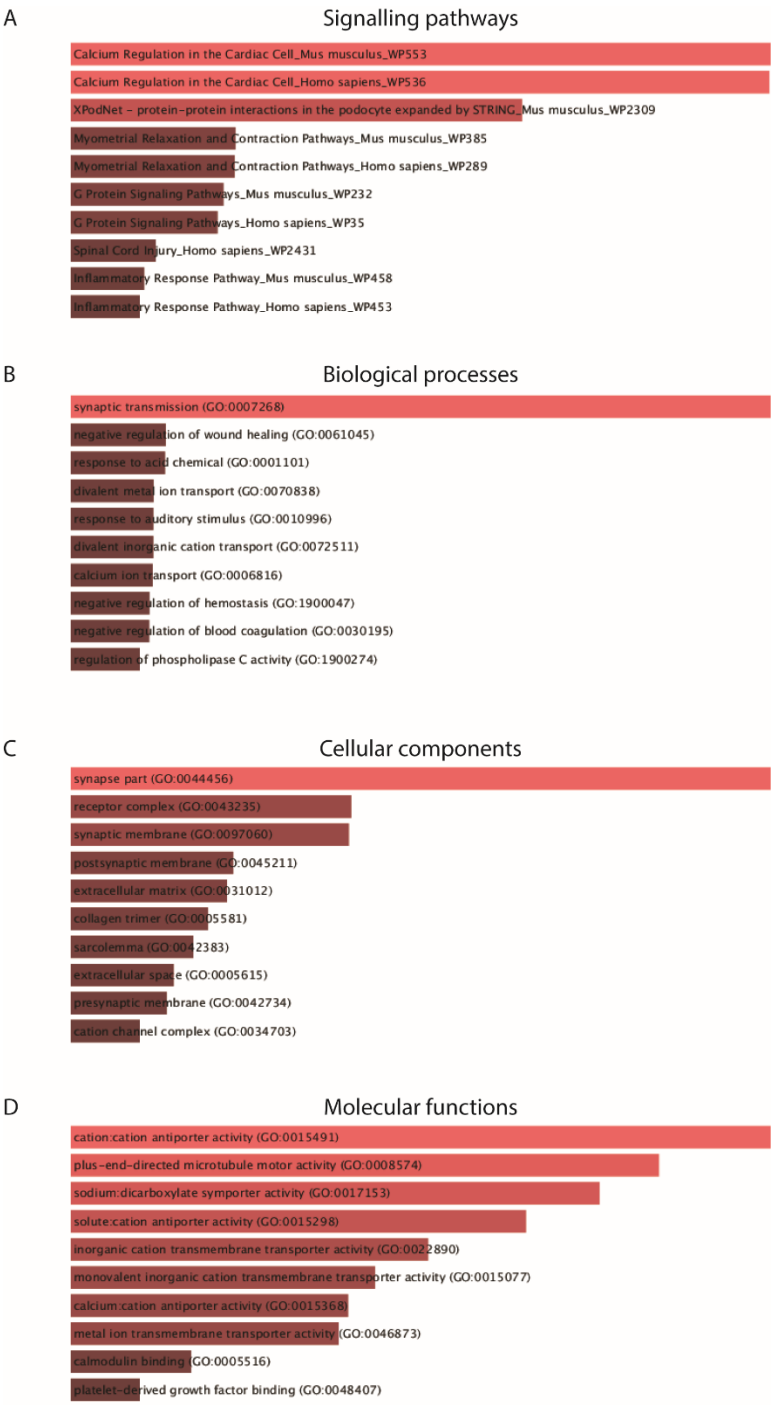


Figure III.10. Enrichr analysis for genes unique to Low Runners

Genes unique to Low Runners (see Venn diagram Fig. 7) were analyzed for signalling pathways (**A**), Biological Processes (**B**), Cellular Components (**C**), and Molecular Functions (**D**) using Enrichr.

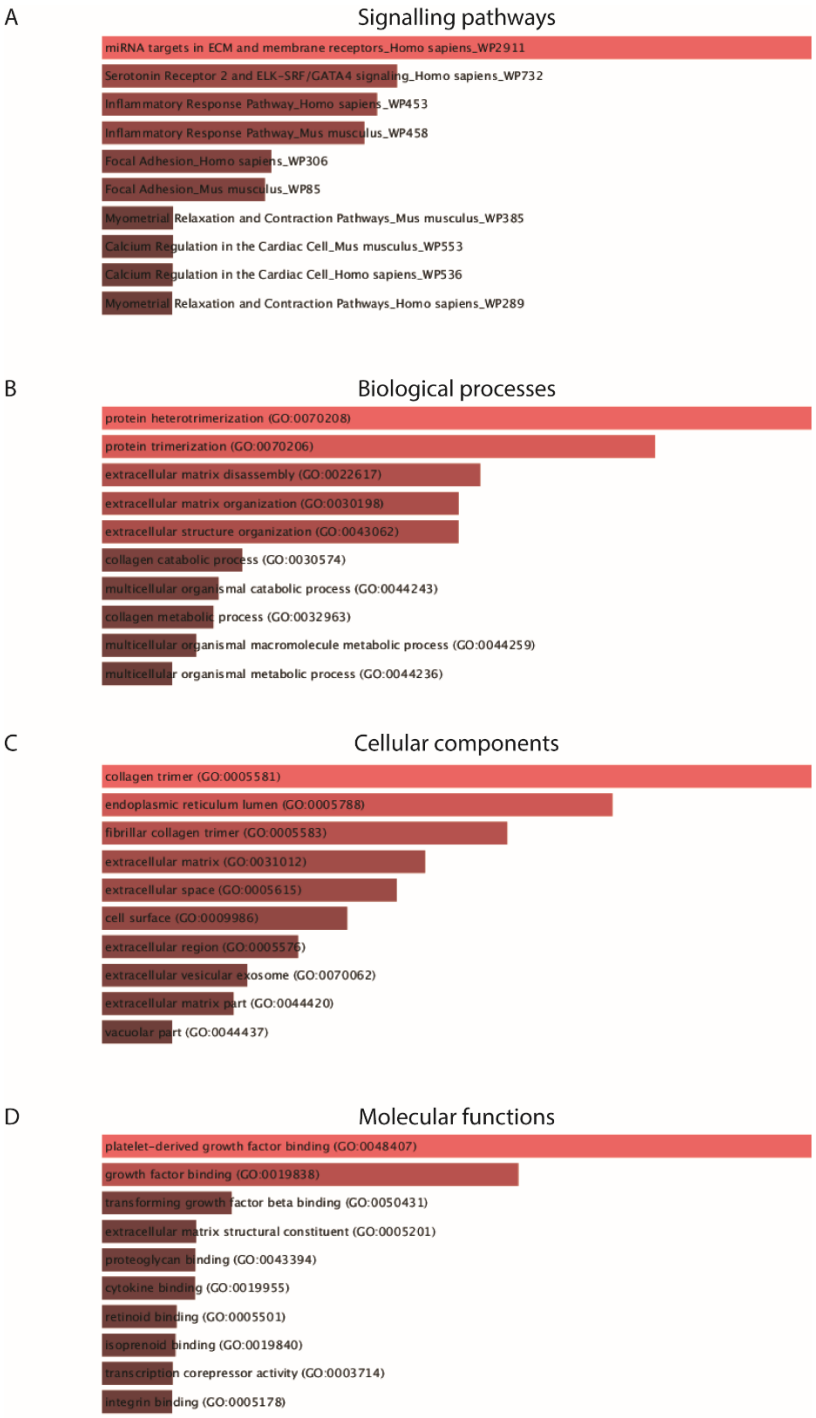


Figure III.11. Genes modified in CE

Genes significantly changed in the DG niche after CE exposure were analyzed for signalling pathways (**A**), Biological Processes (**B**), Cellular Components (**C**), and Molecular Functions (**D**) using Enrichr.

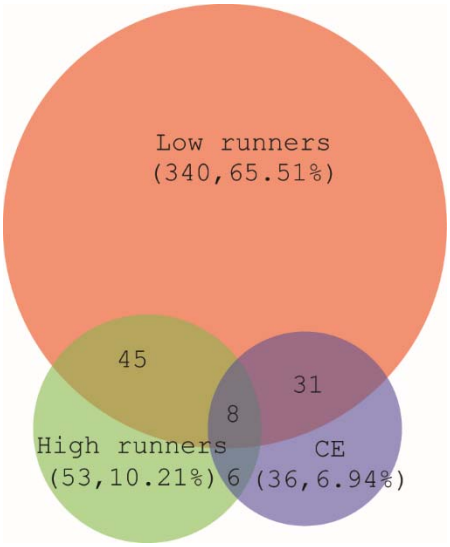


Figure III.12. Modified genes comparison between Low and High Runners and CE

Venn diagram (BioVenn) showing significant modified genes that are unique to Runners (Low and High runners combined (438 genes), and CE (36 genes).

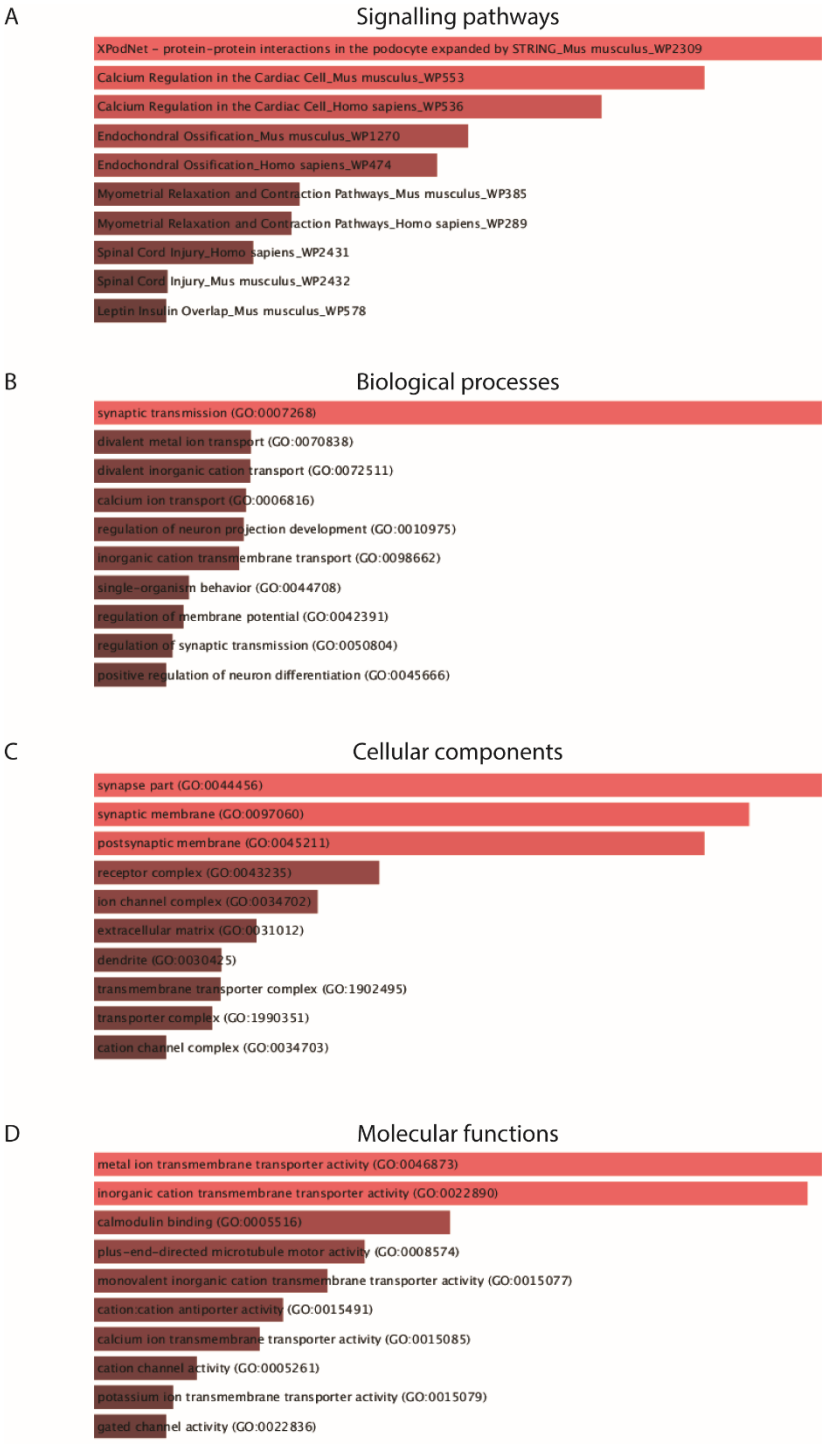


Figure III.13. Enrichr analysis for genes unique to runners

Genes unique to runners (Unique to Low and High Runners and combined, see Venn diagram Fig. 7) were analyzed for signalling pathways (**A**), Biological Processes (**B**), Cellular Components (**C**), and Molecular Functions (**D**) using Enrichr.

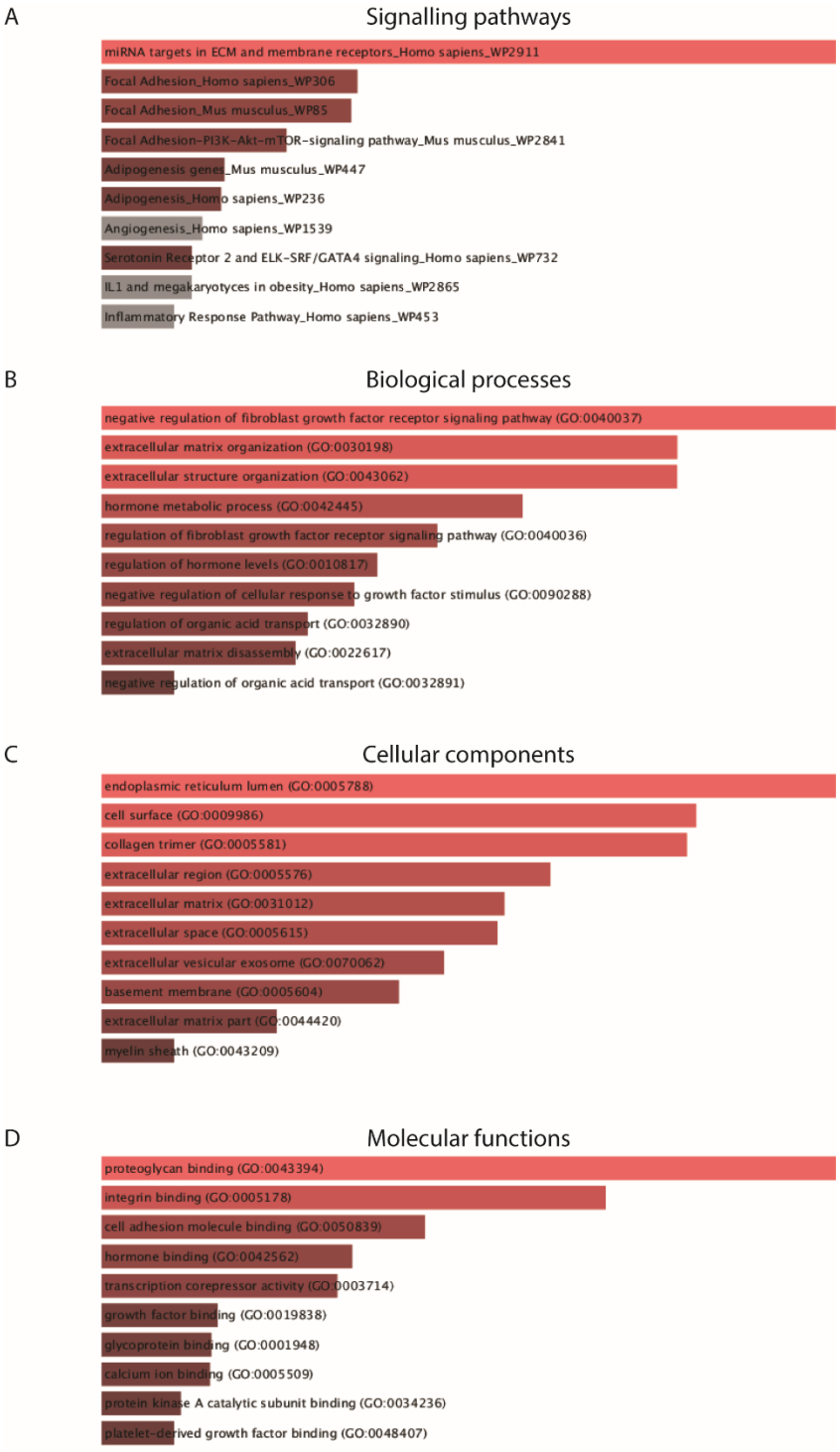


Figure III.14. Enrichr analysis for genes unique to CE

Genes unique to CE (see Venn diagram Fig. 7) were analyzed for signalling pathways (**A**), Biological Processes (**B**), Cellular Components (**C**), and Molecular Functions (**D**) using Enrichr.

Chapter IV. Impact of transforming growth factor-β1 on ependymal cells

IV.1. Chapter context

For this last project, we collaborated with Dr. Steve Lacroix's laboratory to isolate ependymal cells from FoxJ1-eGFP mice using fluorescence-activated cell sorting following Sham treatment or SCI. A RNA-Seq of these purified cells before and after injury was performed to obtain the transcriptome of activated ependymal cells. One of the main players that came out of this analysis was TGF-β1. We, therefore, investigated the effects of this cytokine on the ependymal cell population.

IV.2. Author's contributions

In this study, I designed and performed most of the experiments with the helpful advices of my research director, Dr. Karl Fernandes. Dr. Louis-Charles Levros, a post-doctoral fellow helped me design and analyze experiments from Figures IV.5C-G and IV.7, as well as perform the self-renewal experiment and took pictures of neurospheres for me to measure from Figure IV.8. Dr. Brianna Goldenstein, a former post-doctoral fellow, performed the *in vivo* surgeries to install osmotic pumps from Figure IV.4B-C, and took pictures of neurospheres for me to measure from Figures IV.5 and IV.7 (data not shown). Sandra Joppé, a Ph.D student, performed flow cytometry analyses on samples prepared by myself from Figure IV.9E-I. Anne Aumont, our laboratory technician, performed some culture experiments from Figure IV.5A-B and IV.6. Nadia Fortin, an animal health technician from Dr. Steve Lacroix's laboratory, a collaborator in Quebec City, performed the spinal cord injury from Figure IV.1B and IV.3. Jorge Barreto, a Ph. D student in Dr. Steve Lacroix's laboratory, isolated ependymal cells by fluorescence-activated cell sorting (FACS) and performed RNA isolation from purified cells from Figure IV.1B. Dr. Steve Lacroix also provided the *in situ* hybridization pictures from Figure IV.3. Patrick Gendron, a biostatistician from the IRIC genomics platform, performed the RNA-Seq alignments and basic bio-informatics from Figure IV.1C-D, and guided me in performing the deeper bio-informatics analyses from Figures IV.1E, IV.2, and Tables IV.1-4. Alexandre Vaugeois, a former M.Sc student in the laboratory, designed and performed the preliminary data for IL-6, IL-1β, and TNF-α from Figure IV.11. The manuscript was written by myself in collaboration with Dr. Karl Fernandes.

TGF-β1 exerts opposing effects on recruitment of neurosphere-initiating cells and proliferation within the ependymal niche of the adult mouse spinal cord

Catherine-Alexandra Grégoire[1,2,3], Louis-Charles Levros[1,2,4], Brianna L. Goldenstein[1,2,4], Sandra E. Joppé [1,2,3], Anne Aumont [1], Steve Lacroix[5], Karl J. L. Fernandes[1,2,4]*

[1]Research Center of the University of Montreal Hospital (CRCHUM),
[2]CNS Research Group (GRSNC), University of Montreal
[3]Department of Pathology and Cell Biology, Faculty of Medicine, Université de Montréal, QC, Canada, H3T1J4
[4]Department of Neurosciences, Faculty of Medicine, Université de Montréal, QC, Canada, H2X0A9
[5]Centre de recherche du Centre hospitalier universitaire (CHU) de Québec – CHUL et Département de médecine moléculaire, Faculté de médecine, Université Laval, Québec, Canada

IV.3. Introduction

The EZ of the adult spinal cord is a unique niche of latent neural stem cell activity. The EZ niche is comprised of ciliated ependymal cells that line the central canal, and a mixture of adjacent sub-ependymal astrocytes, post-mitotic neurons, and oligodendrocyte lineage cells (Hamilton et al. 2009; Meletis et al. 2008). Unlike other central nervous system niches, the EZ niche does not normally display NSC activity. However, when the adult spinal cord is dissociated and cultured, a subpopulation of EZ cells gives rise to epidermal growth factor/fibroblast growth factor-2 (EGF/FGF2)-dependent neurosphere colonies that contain self-renewing, tripotential NSCs (Martens et al. 2002; Weiss et al. 1996). Transgenic fate-mapping studies have traced the origin of these NSCs to the FoxJ1-expressing ependymal cell population (and not to Connexin+ astrocytes or Olig2+ oligodendrocyte precursors) (Barnabe-Heider et al. 2010; Meletis et al. 2008). Interestingly, ependymal cells *in vivo* divide infrequently and are normally unipotential, undergoing symmetrical divisions that result in new ependymal cells (Alfaro-Cervello et al. 2012; Barnabe-Heider et al. 2010; Hamilton et al. 2009). However, in response to SCI, ependymal cells undergo dramatic changes in their cellular properties that include increased proliferation, migration of ependymal progeny out of the EZ and into areas of degeneration, and multi-lineage glial differentiation into astrocytes and oligodendrocytes (Barnabe-Heider et al. 2010; Johansson et al. 1999). Understanding how ependymal cells are recruited into a proliferative and multipotential NSC state may help to develop endogenous spinal cord repair strategies since neurosphere-initiating cells (NICs) are also present in the human spinal cord (Dromard et al. 2008; Mothe et al. 2011).

Following SCI, central canal ependymal cell proliferation is increased at long distances from the site of injury, is maintained for several weeks, and is associated with significantly enhanced formation of ependymal cell-derived tripotential neurospheres (Barnabe-Heider et al. 2010; Lacroix et al. 2014). These ependymal cell reactions are context-specific, as they occur following traumatic lesions but not in autoimmune- or chemical-mediated models of demyelination (Lacroix et al. 2014). Little is known about the nature of the culture- or injury-induced signals mediating initial acquisition of NSC characteristics, proliferative expansion, or glial differentiation by quiescent ependymal cells (reviewed in (Gregoire et al. 2015)). In this

regard, TGF-β1 is a prominently expressed injury-induced factor that plays a central role in wound healing across many tissues. TGF-β1 has a role in cell growth, differentiation, organ development, migration, pro-inflammatory and anti-inflammatory processes, and wound repair (Kleiter et al. 2007; McCartney-Francis and Wahl 1994; Taylor 2009). Following SCI, it is highly expressed by inflammatory cells, with levels that peak at 48 hours post-SCI and that are maintained for several days (Buss et al. 2008; McTigue et al. 2000). Previous studies have shown that this cytokine plays important and multi-faceted roles after SCI, as TGF-β1-treated animals showed a lesion volume reduced by 50% 48 hours post-SCI (Tyor et al. 2002), while TGF-β1-blocking antibodies inhibit glial scar formation and improve locomotor activity (Kohta et al. 2009). Here, we asked whether TGF-β1 affects the recruitment, expansion, and differentiation of spinal cord-derived NSCs.

IV.4. Materials and methods

IV.4.1. Mice

Experiments were conducted in accordance with the guidelines of the Canadian Council of Animal Care and were approved by the institutional animal care committee of the University of Montreal and the CRCHUM. Experiments were performed using two- to three-month-old male C57BL/6 mice from Charles River Laboratories (St-Constant, QC, Canada) and four-month-old female B6;C3-Tg (FOXJ1-EGFP)85Leo/J mice (The Jackson Laboratory). The animals had free access to food and water.

IV.4.2. Spinal cord injury

C57BL/6 adult mice were anesthetized with isoflurane and underwent a laminectomy at vertebral level T9-10, which corresponds to spinal segment T10-11. Briefly, the vertebral column was stabilized and a contusion of 50 kdyn was performed using the Infinite Horizon SCI device (Precision Systems & Instrumentation). For the sham-operated mice (laminectomy), the exposed spinal cord was left untouched. Overlying muscular layers were then sutured and cutaneous layers stapled. Post-operatively, animals received manual bladder evacuation twice daily to prevent

urinary tract infections. Mice were killed by transcardiac perfusion at seven (Figure 1) or four days post-surgery (Figure 2).

IV.4.3. Tissue fixation and processing

Mice received a lethal dose of ketamine (Bimeda-MTC), xylazine (Bayer Healthcare), and acepromazine (Boehringer Ingelheim Canada Ltd). Tissues were prepared by perfusing transcardially with phosphate-buffered saline (PBS) containing heparin at 40mg/mL (Fisher), followed by 4% formaldehyde, pH 7.4, freshly prepared from paraformaldehyde (Fisher). Spinal cords were removed and post-fixed in 4% formaldehyde overnight. The spinal cord of each animal was cut into 40 µm coronal sections using a vibrating microtome (Leica VT1000S) and the sections were stored at -20°C in an antifreeze solution (glycerol:ethylene glycol:PBS, 3:3:4) until use.

IV.4.4. Intracerebroventricular (ICV) osmotic pumps

For ICV infusions, mice were locally injected with bupivacaine (Hospira) and operated under isoflurane anesthesia (Baxter). Cannulae attached to seven day Alzet osmotic pumps (0.5 µl/h infusion rate, model 1007D; Durect), were stereotaxically implanted at 0.1mm anteroposterior and 0.9mm lateral to Bregma according to manufacturer`s instructions. TGF-β1 was diluted in a vehicle solution (PBS) and infused at 400ng/day. A pilot study was performed previously and showed that ICV infusion of EGF increased proliferation of spinal cord ependymal cells, confirming that ICV delivery of growth factors reaches the central canal (data not shown). Animals were sacrificed after five days.

IV.4.5. Culture experiments

Preparation and treatment with TGF-β1

TGF-β1 (10ug, cell signalling) was reconstituted in 20mM citrate buffer pH 3.0 and used for subsequent working dilutions. The citrate buffer was used as the Vehicle in all culture experiments.

Alk5 inhibitor treatment

In experiments where TGF-β1 signaling needed to be blocked, a type 1 receptor kinase Alk5 inhibitor II, 2-(3-(6-methylpyridin-2-yl)-1*H*-pyrazol-4-yl)-1,5-naphthyridine, was diluted in dimethyl sulfoxide (DMSO) (Sigma) to reach a final concentration of 5μM (Enzo life sciences).

Neurosphere assays

Neurospheres were generated according to a procedure modified from Weiss and colleagues, 1996 (Weiss et al. 1996). Two- to three-month-old male C57BL/6 mice were euthanized by isoflurane followed by decapitation. Spinal cords were rapidly extracted by placing a needle and 10mL syringe filled with sterile HBSS into the severed spinal column at the lumbar level, and applying hydraulic pressure until the cord emerged at the cervical level. The isolated cord was placed in ice-cold HBSS (Wisent), cut to a 20mm length, minced with a scalpel, and both enzymatically and mechanically dissociated into single cells using Papain (Worthington), and rapid pipetting. The dissociated cells were diluted to 30 mL in DMEM/F-12 (3:1; both from Life technologies) supplemented with 2% B27 (Life technologies), 1μg/mL fungizone (Life technologies), 1% penicillin and streptomycin (Wisent), 20 ng/mL EGF (Feldan), 25 ng/mL FGF2 (Feldan) and seeded in 75cm^2 flasks for 13-14 days to generate primary neurospheres. Primary neurospheres were mechanically dissociated and re-seeded at 1.5cells/μL in 48-well plates for nine-to-ten days (in the same media as above) to generate secondary neurospheres. Tertiary neurospheres, were generated by a similar procedure as previously described. The cultures were re-fed with growth factors and B27 every three-to-four days. Neurosphere numbers were quantified by plating cells in 48-well plates at a clonal density (1.5 cells/μL) (12 wells/treatment/n, but 48 wells/n for the osmotic pump experiment, Fig.1). Neurosphere sizes were quantified by measuring the diameter of at least 100 neurospheres per condition using ImageJ software (version 1.47v, NIH, USA), and the data expressed using frequency histograms (GraphPad Prism, Version 5.02, GraphPad Software, Inc). For experiments assessing neural differentiation, neurospheres were mechanically dissociated and plated at a density of 25,000 cells/cm^2 in a basal differentiation medium (i.e., neurosphere growth medium in which EGF and FGF2 were replaced with 2% Fetal Bovine Serum (Wisent) supplemented with either Vehicle or 10ng/ml of TGF-β1).

TUNEL and proliferation assay

The TUNEL and proliferation assay was performed as described previously (Hamilton et al. 2015; Joppé et al. 2015), in medium supplemented with Vehicle or 10ng/mL TGF-β1. Quantification of Ki67-TUNEL double-labeling was performed by counting the number of Hoechst-positive nuclei also double-labelled with either Ki67 or TUNEL from 200X pictures (9 fields of view by condition).

Carboxyfluorescein Diacetate, Succinimidyl Ester (CFSE) cell division assay

The CFSE cell division assay was performed as described previously (Hamilton et al. 2015; Joppé et al. 2015), except that the proliferation medium was supplemented with either Vehicle or TGF-β1 at 10ng/mL.

Propidium Iodide (PI) cell cycle assay

Dissociated neurosphere cells were plated at 25,000 cells/cm^2 in medium containing the indicated treatments. Cells were harvested by trypsinization after 24h of treatment, rinsed and frozen in 70% ethanol in PBS until analysis. Before flow cytometry analysis, samples were treated with 0.5 mg/mL of DNAse-free RNAse (Sigma) (30 min at room temperature) and incubated with 50 mg/mL of PI (Sigma). The cells were then harvested, washed, and run through an LSRII cytometer (BD Biosciences). Data was analyzed using FlowJo v7.6.5 (Tree Star).

Self-renewal assay

Dissociated primary neurospheres were grown into secondary neurospheres in the presence of Vehicle (Citrate 20mM pH 3.0) or 10 ng/mL TGF-β1 for nine days (as above) (n=4 experiments). 60 secondary neurospheres were chosen from each condition using a micropipette and inverted microscope (40X total magnification), transferred to a 1.5 mL tube, and mechanically dissociated using a p200 pipette set to 100µL for five min in 250µL of proliferating medium. The cell density was determined by using a hemocytometer, and the cells were re-plated at 1.5 cells/µL in proliferation medium (as above). Tertiary neurospheres were counted and measured after nine days, allowing quantification and retrospective calculation of the mean number of neurosphere-initiating cells that were present per secondary neurosphere.

IV.4.6. In situ hybridization

Animals were perfused with ice-cold saline followed by 4% PFA, pH 9.5, in Borax buffer. Spinal cords were removed, post-fixed for 2 days in 4% PFA and subsequently transferred in a solution of 4% PFA with 10% sucrose for 24 h. Spinal cords were divided into three 4 mm-segments corresponding to the thoracic and lumbar spine, sliced into 30-µm-thick coronal sections, and collected directly onto Surgipath X-tra® microslides (Leica Biosystems). All sections were pre-hybridized, hybridized and post-hybridized as before (Vallieres et al. 2006). The antisense riboprobe was transcribed *in vitro* from a linearized cDNA coding for mouse TGF-β1 with T3 RNA polymerase in the presence of [S35]-UTP (Perkin Elmer). For the latter cDNA, a 1173-bp sequence corresponding to nucleotides 315-1487 from GenBank: BC013738 was amplified using the following primers: TGF-β1 (forward, 5'- catgccgccctcggggctgcggctac -3'; reverse, 5'-tcagctgcacttgcaggagcgcac -3'). The sequence chosen for riboprobe synthesis was selected to match only the intended gene, as verified by BLAST analysis in Genbank. All ISH images were acquired at 20X magnification using the Bioquant Nova Prime software on video images of tissue sections transmitted by a high-resolution Retiga QICAM fast color 1394 camera (1392 x 1040 pixels; QImaging) installed on a Nikon (Tokyo, Japan) Eclipse 80i microscope. All files were exported as TIFF.

IV.4.7. Immunohistochemistry

Immunohistochemical procedures were performed as detailed previously (Gregoire et al. 2014). Mouse anti-human Ki67 was used at 1:200 (BD Biosciences), rabbit anti-glial fibrillary acidic protein at 1:2000 (Dako), and chicken anti-GFP at 1:2000 (Aves Labs). Microscopy was performed using a motorized Olympus IX81 microscope (Fig.2, 40X objective) or with a Quorum Technologies spinning disk confocal microscope with a CSU10B (Yokogawa) spinning head mounted on an Olympus BX61W1 fluorescence microscope and connected to a Hamamatsu ORCA-ER camera (Fig.1, 60X objective). All quantifications were performed on coded slides by a blinded experimenter.

IV.4.8. Western blotting

Western blotting was performed as previously described (Hamilton et al. 2010). The following antibodies were used: mouse anti-Class III β-tubulin (1:1000, Covance), mouse anti-beta actin (1:20,000, Abcam), mouse anti-CNPase (1:250, Millipore), rabbit anti-glial fibrillary acidic protein (1:1000, Dako), rabbit anti-Olig-2 (1:1000, Millipore), and mouse anti-human PCNA (1:500, BD Biosciences). HRP-conjugated secondary antibodies were used at the following dilutions: anti-mouse IgG (1:5000, Bio-Rad) or anti-rabbit IgG (1:5000, Millipore). Secondary antibodies were detected using Clarity (Bio-Rad) and ChemiDoc (Bio-Rad). Quantitative densitometry of bands was performed using Image Lab version 4.1 (Bio-Rad).

IV.4.9. RNA-Seq

Female B6;C3-Tg (FOXJ1-EGFP)85Leo/J mice (The Jackson Laboratory) (8 mice per group x 2 groups (Sham, SCI)) were contused with the Infinite Horizon impactor (50 kdyn) at T9-T10. Three days after SCI, mice were killed and 8mm of their spinal cord extracted. Then, tissue is mechanically and enzymatically dissociated and cells enriched by continuous percoll gradient. Ependymal GFP+ cells were isolated using a cell sorter and markers such as CD24+, CD133+, FoxJ1 (eGFP)+, and CD45-. RNA was isolated from purified cells and sequenced mRNA fragments were trimmed for adapter sequences and then mapped to the reference mouse genome assembly version mm10 using Tophat (version 2.0.10) (Trapnell et al. 2009). Gene expressions were then estimated by using the HTSeq tool to compute read counts on RefSeq genes (Anders et al. 2015). For exploratory purposes, DESeq2 was used to normalize read counts, extract regularized log values and compute log fold changes (Love et al. 2014). Differentially expressed genes that are up or downregulated by a factor 0.3 or more (in log2, thus a fold change of 1.23 or more) were used to investigate enriched pathways and functions through the use of QIAGEN's Ingenuity Pathway Analysis (IPA®, QIAGEN Redwood City, www.qiagen.com/ingenuity). All samples were normalized at once, which allows us to produce comparisons between samples in the form of Principal Component Analysis (PCA) and hierarchical clustering. Genes were sorted according to their absolute fold change, only genes with a log fold change ≥ 1 or ≤ -1 were analyzed further. Further biostatistical analyses were performed by EnrichR.

IV.4.10. Statistical Analyses

All statistical analyses were performed using GraphPad Prism, Version 6.01 (GraphPad Software, Inc). Statistical analysis used two-tailed unpaired Student's *t*-test, One-way ANOVA with Dunnett's multiple comparison test, or one sample t-test as indicated in figure legends. Error bars represent mean ± standard error of the mean. The significance level was set at $p \leq 0.05$.

IV.5. Results

IV.5.1. RNA-Seq transcriptomic profiling of ependymal cells following SCI

SCI induces the proliferation, migration and multi-lineage differentiation of ependymal cells (Barnabe-Heider et al. 2010; Gregoire et al. 2015) (**Fig. IV.1A**). To attempt to gain insights into the genetic program associated with this injury-induced acquisition of NSC properties, we used an RNA-Seq-based strategy to perform a transcriptomic analysis of the reactive ependymal cells (**Fig. IV.1B**). Laminectomy at the vertebral level T9-10 was performed on FoxJ1-eGFP sham mice (n=8) and the vertebral column stabilized to perform a contusion of 50 kdyn on FoxJ1-eGFP SCI mice (n=8). Three days following SCI, mice were sacrificed and 8mm of the spinal cord was extracted. Tissue was dissociated and GFP+CD24+CD133+CD45- ependymal cells were isolated by FACS. RNA was then isolated from the purified cells and sequenced by RNA-Seq. Using a threshold of $-1 >$ log2 fold > 1 and $p(adj) < 0.05$, we identified 1,241 SCI-induced significantly changed genes, including 824 up-regulated and 417 down-regulated (**Fig. IV.1C**). Hierarchical clustering and heatmap analysis in log readcount values (IRIC genomics platform) showed consistent clustering of the control groups and SCI groups (**Fig. IV.1D**). Using WikiPathways (EnrichR), the principal biological pathway identified by the differentially expressed genes was Spinal Cord Injury (**Fig. IV.1E**). The top 25 up-regulated and top 25 down-regulated genes are shown in **Tables IV.1 and IV.2** respectively. Notably within the list of up-regulated genes the *glial fibrillary acidic protein (gfap)* came up, known to be expressed by astrocytes (Frisen et al. 1995). This result shows that cells are rapidly directed towards the astrocytic fate after injury. The gene *thrombospondin 2 (Thbs2)*, which is astrocyte-derived, is also up-regulated and is known to be implicated in functional recovery after stroke and is involved in repair of the blood-brain barrier (Liauw et al. 2008; Tian et al. 2011). Moreover, a closely related family member of Thbs2 (Thbs4) has recently been implicated as a regulator of Notch signalling, and in directing forebrain stem cells to an astrocytic fate following stroke (Benner et al. 2013).

IV.5.2. Bio-informatics analyses of injured ependymal cells

To study the global patterns of effects of SCI on ependymal cell gene expression, we submitted the differentially expressed genes to GO enrichment analysis (EnrichR) for Biological Processes, Cellular Components, and Molecular Functions. The main Biological Processes (**Fig. IV.2A**) identified involved the extracellular matrix (GO:0030198, 0043062) and cell movement and migration (GO:0051272, 0030335, 2000147). The principal Cellular Components (**Fig. IV.2B**) where the differentially expressed genes are active are related to cell-substrate interactions (GO: 0030055, 0005924) and cellular junctions (GO:0070161, 0005912). The main Molecular Functions (**Fig. IV.2C**) of the differentially expressed genes are in binding to the extracellular matrix (GO: 0050840), to cells (GO: 0050839), or to cytokines (GO: 0019955). Thus, these patterns of gene expression are consistent with the observed SCI-induced migratory behaviour of ependymal-derived cells, and reveal a strong interaction with surrounding extracellular components during this process (Gregoire et al. 2015).

IV.5.3. Identification of TGF-β as a potential upstream regulator of ependymal responses to SCI

To gain insight into SCI-induced factors that may be responsible for the observed transcriptomic changes, we used Ingenuity Pathway Analysis to perform an Upstream Regulator Analysis of the differentially expressed genes. The 5 top predicted upstream regulators were lipopolysaccharide (p=3.24E-35), TGF-β1 (p=8.60E-35), TNF (p=3.03E-29), IFNg (p=9.64E-29), and tretinoin (6.03E-25). **Tables IV.3 and IV.4** list the top predicted 10 growth factors and top 10 cytokines.

We focused on TGF-β1 as it was the most predicted endogenous protein. *In situ* hybridization of the spinal cord at 4 days post-SCI confirmed a strong up-regulation of TGF-β1 in the region surrounding the central canal (**Fig. IV.3A-D**), consistent with its potential role as an endogenous regulator of ependymal cell injury responses.

IV.5.4. Exogenous TGF-β1 infusion reduces NIC numbers but increases ependymal proliferation within the intact spinal cord

Previous lineage-tracing studies have determined that spinal cord neurospheres derive from a sub-population of FoxJ1-expressing ependymal cells (Meletis et al. 2008), that the number of ependymal-derived neurosphere-initiating cells (NICs) is increased upon SCI (Barnabe-Heider et al. 2010), and that ependymal-derived proliferating progenitors subsequently exhibit multi-lineage glial differentiation (primarily astrocytes). We defined the conversion of an ependymal cell to a NIC as NIC recruitment and the ability to create neurospheres as NIC expansion and progenitor proliferation (**Fig. IV.4A**). We next asked what the impact of elevated levels of TGF-β1 might be on the number of ependymal-derived NICs.

To assess the impact of increased TGF-β1 on NIC numbers, osmotic pumps containing either Vehicle or TGF-β1 were implanted into the lateral ventricles of adult mice, and after five days of infusions, the spinal cords were dissociated and processed for clonal neurosphere assays using EGF/FGF2 (**Fig. IV.4B**). The mean number of neurospheres grown from the thoracic spinal cord decreased by 75%, from 17.19±4.02 neurospheres/mm of spinal cord in Vehicle-infused mice to 4.37±0.42 neurospheres/mm in TGF-β1-infused mice (**Fig. IV.4C**). There was no significant difference in neurosphere size between groups (data not shown), indicating that an increase in TGF-β1 within the intact spinal cord results in a reduction in the number of NICs.

To assess whether the TGF-β1-induced reduction in NICs correlates with altered numbers of proliferating ependymal-derived progenitors, Vehicle or TGF-β1-containing pumps were implanted (as above) into the lateral ventricles of adult FoxJ1-EGFP mice, which express GFP in ependymal cells (**Fig. IV.4D**), and the animals sacrificed after five days for immunohistochemical analysis of the thoracic spinal cord. TGF-β1 administration resulted in a pronounced increase in the number of $Ki67^{+}$ proliferating cells within the EZ (defined by FoxJ1-GFP expression, **Fig. IV.4E**) and the immediately adjacent subependymal zone (SEZ) compared to Vehicle (**Fig. IV.4F**). Mean number of $Ki67^{+}$ cells increased 2.8-fold in the EZ (from 1.29±0.25 to 3.60±0.81 cells per section, p=0.0258) and 23.2-fold in the SEZ (from 0.05±0.05 to 1.16±0.15 cells per section, p=0.0004) (**Fig. IV.4F**). Plotting the data as frequency histograms demonstrated a marked TGF-β1-induced shift to higher numbers of $Ki67^{+}$ cells (**Fig. IV.4G**). Specifically, while 40.0% of sections in Vehicle-infused mice were devoid of $Ki67^{+}$ cells (similar to previous results in naive mice (Hamilton et al. 2009), all sections from TGF-β1-treated mice contained $Ki67^{+}$ cells

(**Fig. IV.4G**). Moreover, the frequency of sections containing three or more Ki67+ cells increased from 17.5% of sections in Vehicle-infused mice to 66.7% in TGF-β1-infused mice). The largest proportional increase in Ki67+ cells in the EZ occurred at the dorsal pole of the central canal (**Fig. IV.4H**).

Together, these data unexpectedly reveal opposing effects of TGF-β1 on *in vivo* numbers of NICs and proliferating ependymal-derived progenitors.

IV.5.5. NIC expansion: TGF-β1 treatment in vitro suppresses growth factor-mediated formation of primary neurospheres

We next used *in vitro* assays to investigate the TGF-β1-mediated suppression of NICs. To determine whether TGF-β1 influences the ability of NICs to expand in number in response to mitogens, dissociated spinal cord cells were grown in the presence of EGF+FGF2 to generate neurospheres, in media supplemented with either Vehicle or TGF-β1 (**Fig. IV.5A**). 30 ng/mL TGF-β1 abolished the formation of EGF/FGF2-dependent primary neurospheres from the dissociated spinal cord (**Fig. IV.5B**). We further reduced TGF-β1 concentration to 5 ng/mL and still observed identical effects. Since neurospheres consist of approximately 5% NICs and 95% NIC-derived progenitors, the observed elimination of neurosphere formation could be attributable to either 1) a decreased ability of NICs to initiate neurosphere formation or 2) a decreased ability of NIC-derived progenitors to expand neurosphere size. To differentiate between these possibilities, we performed delayed treatments with TGF-β1 or an inhibitor of the TGF-β1 receptor one kinase, Alk5. The addition of the Alk5 inhibitor to dissociated spinal cord cells did not by itself increase EGF/FGF2-mediated neurosphere formation, indicating that baseline NIC recruitment is not constitutively inhibited by TGF-β1 endogenously produced within primary cultures (**Fig. IV.5C**). While TGF-β1 treatment at day zero of cultures eliminated primary neurosphere formation (**Fig. IV.5B**), treatment beginning on day three and seven had partial and no effects respectively (**Fig. IV.5D, E**). Similarly, when TGF-β1 was added at day zero of cultures, the addition of the Alk5 inhibitor at day three but not day seven was able to rescue partially neurosphere formation (**Fig. IV.5F, G**).

These data show that TGF-β1 suppresses primary neurosphere formation by acting at the initial stages of neurosphere formation, and support the notion that TGF-β1 blocks EGF/FGF2-mediated expansion of NICs.

IV.5.6. TGF-β1 acts directly on neural precursors and promotes reversible quiescence of NICs

The TGF-β1-induced suppression of NICs following ICV infusion *in vivo* or during primary neurosphere formation *in vitro* could either result from a direct effect of TGF-β1 on ependymal cells themselves, or indirect effects via other cell types *in vivo* and within primary cultures. We therefore passaged twice primary neurospheres grown under standard conditions to generate purified cultures of tertiary neurospheres. Passaging of floating neurosphere cultures results in the elimination of differentiated (adherent) cells such as neurons, astrocytes and oligodendrocytes that are present within the primary cultures. We then asked whether the growth inhibitory effect was still present after TGF-β1 treatment on these purified cultures (**Fig. IV.6A**). Tertiary neurosphere formation was decreased by about 50% from established secondary neurosphere cultures with a 30ng/mL treatment (**Fig. IV.6B**). Given the low cell density in these clonal neurosphere assays, we further reduced TGF-β1 concentration to 5 ng/mL and observed identical effects (**Fig. IV.6B**). Furthermore, tertiary neurospheres grown in the presence of TGF-β1 showed a shift towards smaller neurosphere sizes (**Fig. IV.6C**).

Interestingly, TGF-β1-induced inhibition of neurosphere formation was substantially more pronounced when using primary dissociated spinal cord cells than from purified secondary neurosphere cultures. To test the possibility that a portion of TGF-β1 effects in primary cultures might still be mediated indirectly via other cell types, conditioned medium (CM) was collected and filtered from dissociated spinal cord cells treated with Vehicle or TGF-β1 and added in increasing concentrations during growth of secondary neurospheres (**Fig. IV.7A-C**). Regardless of whether grown in 25%, 50%, or 100% CM, CM from TGF-β1-treated primary cells still reduced secondary neurosphere formation by only 50-60%, arguing against the presence of indirect mediators of TGF-β1's effects in primary cultures. Consistent with this, when secondary neurospheres were grown in the presence of CM from TGF-β1-treated primary cells, the inhibitory effect of the CM was completely blocked by the addition of the inhibitor of the TGF-β1 receptor

one kinase, Alk5 (**Fig. IV.7D, E**). These results show that the entire growth-inhibitory effect of primary culture CM is attributable to residual TGF-β1.

Lastly, to gain insight into whether TGF-β1 treatment leads to a *loss* of NICs (i.e., via terminal differentiation or death) or to *NIC quiescence* (i.e., a reversible inhibition of proliferation), we performed a self-renewal assay. Primary neurospheres were grown in medium supplemented with Vehicle or TGF-β1, and the resulting secondary neurospheres were then dissociated and tested for their ability to grow into tertiary neurospheres under standard conditions (see Methods). This revealed that neurospheres grown in TGF-β1-supplemented medium, although smaller, contained an identical frequency of NICs (5.72% Vehicle, 5.31-5.86% TGF-β1). This result suggests that TGF-β1's inhibitory effect on neurosphere formation is not due to NIC loss, and can rather be attributed primarily to a direct effect on NIC quiescence (**Fig. IV.8 A-C**).

IV.5.7. Downstream effects: TGF-β promotes proliferation and astrocytic differentiation of NIC-derived progenitors

To study the impact of TGF-β1 on NIC-derived progenitors, we assessed TGF-β1-induced changes in proliferation, survival, and differentiation in high-density adherent cultures of spinal cord-derived neurosphere cells. Progenitor-enriched adherent cultures were established by first using EGF/FGF2 to grow clonally-derived neurospheres from the dissociated spinal cord, passaging these neurospheres to purify and expand their number, and then plating cells dissociated from secondary neurospheres (approximately 95% progenitors) at high-density for immunohistochemical, flow cytometry, and Western blotting experiments.

High-density adherent stem/progenitor cell cultures were plated in EGF/FGF2-containing medium supplemented with either Vehicle or TGF-β1, and then analyzed by immunostaining or flow cytometry (**Fig. IV.9A**). Immunofluorescence labeling at three days *in vitro* (DIV) revealed that TGF-β1 stimulated a 56% increase in Ki67+ proliferating cells ($p=0.0110$) and a 45% decrease in TUNEL+ apoptotic cells ($p=0.0938$, not significant) (**Fig. IV.9B-D**). To confirm that the significant increase in Ki67 immunoreactivity corresponded to an increase in cell proliferation we used a CFSE label retention assay. CFSE is a fluorescent dye that is passively incorporated into

cells and then serially diluted from their cytoplasm during each subsequent cell division. Flow cytometry analysis confirmed that when stem/progenitor cells were pre-labelled with CFSE, TGF-β1 caused a significant decrease in CFSE retention by three DIV (**Fig. IV.9E, F**), indicative of a proliferation-induced dilution of fluorescence. Cell cycle analysis by PI flow cytometry further showed that TGF-β1 treatment was associated with a small but significant increase (2.47%, p=0.0326) in the proportion of cells in G2-M phase (**Fig. IV.9G-I**). These data show that TGF-β1 promotes proliferation and survival in spinal cord stem/progenitor cell bulk cultures.

We also induced differentiation of high-density adherent stem/progenitor cell cultures by plating them in the absence of EGF/FGF2, to assess the effect of Vehicle versus TGF-β1 on the pattern of neural differentiation. Western blotting (**Fig. IV.9J-K**) and immunofluorescence labelling (**Fig. IV.9L, M**) at three DIV both showed a strong and selective TGF-β1-induced stimulation of the astrocyte marker GFAP. No significant changes in neuronal (βIII-tubulin) or oligodendrocyte lineage (Olig2, CNP) cells was detected, and TGF-β1 was not sufficient to maintain proliferation (Proliferating Cell Nuclear Antigen, PCNA) in the absence of EGF/FGF2.

These data reveal that TGF-β1 promotes cell proliferation in high-density spinal cord stem/progenitor cell cultures, and favours their subsequent differentiation to the astrocytic lineage.

IV.6. Discussion

TGF-β1 is a major injury-induced cytokine that is prominently involved in many facets of wound-healing across diverse tissues, including the injured spinal cord. In this study, we begin by asking a fundamental question concerning the biology of ependymal cells: how do they acquire stem cell properties (proliferative ability and multipotency) following SCI? To answer this question, we designed a strategy to isolate reactive ependymal cells and study their genome-wide transcriptomic changes in an unbiased manner. Biological replicates were prepared and each replicate represented a pool of eight mice, which provided a strong reproducibility between N (Fig. 1D). The RNA-Seq approach provided a wealth of data that was studied in greater detail to gain insights into the molecular programs involved in the acquisition of stem cell properties by these latent precursors. The bio-informatics analysis allowed us to identify TGF-β1 as a predicted

upstream regulator of reactive ependymal cell behaviour. Therefore, we then investigated its potential impact on the properties of adult spinal cord stem/progenitor cells. Our data support four main conclusions (summarized in **Fig. IV.10**). First, raising TGF-β1 levels increases proliferation of ependymal cells surrounding the spinal cord central canal *in vivo* and of spinal cord-derived stem/progenitors cells *in vitro*, favouring their astrocytic differentiation. Second, raising TGF-β1 levels decreases the ability of spinal cord-derived NICs to generate neurosphere colonies in response to EGF/FGF2. Third, the initial recruitment step of NICs from primary spinal cord cells is even more sensitive to TGF-β1's inhibitory effects than the subsequent *in vitro* expansion of these NICs. Thus, TGF-β1 has pleiotropic effects on the neural precursor lineage, suppressing expansion of upstream NICs while promoting proliferation of downstream progenitors and their differentiation into astrocytes. Given the strong upregulation of TGF-β1 following SCI, these data raise the possibility that TGF-β1 is a key mediator of the previously observed injury-induced proliferation and astrogenesis of adult spinal cord ependymal cells (Barnabe-Heider et al. 2010; Meletis et al. 2008). Interestingly, it might be possible that the implication of TGF-β1 in scar formation is partly mediated by the effect of this cytokine on astrocytic differentiation of ependymal cells (Kohta et al. 2009).

A limited number of studies have examined the effects of TGF-β1 on neural precursors of the brain, with both similarities and differences. At the level of NICs, Wachs and colleagues reported that TGF-β1 decreased neurosphere formation from the adult rat forebrain, suggesting a similar effect on the NICs of the brain and spinal cord (Wachs et al. 2006). However, our data showed that TGF-β1 increases proliferation in cultures of spinal cord-derived stem/progenitors *in vitro* (also reported by (Park et al. 2008) and in the spinal cord ependymal cell niche *in vivo*. This contrasts findings by Wachs and colleagues, who found that TGF-β1 both suppressed proliferation in cultures derived from the adult rat forebrain and inhibited proliferation in both the forebrain and hippocampal niches. Moreover, while our *in vitro* results showed that TGF-β1 strongly promotes an astrocytic fate, others found it had no effect on forebrain neural precursor differentiation (Wachs et al. 2006), and promoted survival of hippocampal neurons (Kandasamy et al. 2014). Such differences might be attributable to the apparent distinct cellular identity of the NICs in the brain (astrocytes) versus spinal cord (ependymal cells).

The relationships between NICs, endogenous NSCs, and endogenous proliferating cells remain only partially defined. NICs in the forebrain likely correspond to activated NSCs (Doetsch et al. 2002; Mich et al. 2014), and since these NSCs continuously give rise to highly proliferative progenitors within the forebrain niche, a coordinated TGF-β1-induced reduction in NICs and levels of proliferation in the forebrain niche is not surprising. In contrast, there is no evidence for endogenous NSC activity within the intact spinal cord, and the lineage relationship between spinal cord NICs and proliferating cells in the spinal cord EZ niche has yet to be established (Fig.10). One possibility is that NICs are the direct source of proliferating ependymal cells. In that case, TGF-β1 could reduce NICs through multiple mechanisms. For example, TGF-β1-induced proliferation of ependymal cells may result in depletion of the upstream NICs, or TGF-β1 may directly drive quiescence or terminal differentiation of NICs while simultaneously promoting ependymal cell proliferation (similar to bone morphogenetic protein in the forebrain (Joppé et al. 2015). Alternatively, NICs could represent a cellular reserve that is either more distantly related to the proliferating ependymal cells or a distinct lineage within the FoxJ1 population, in which case NICs and proliferating ependymal cells may be independently regulated. In this regard, since spinal cord ependymal cells are neither neurogenic nor multipotential under baseline conditions, a deeper understanding of the molecular mechanisms underlying the emergence of NICs from the FoxJ1 population is of considerable interest for spinal cord repair applications.

In the context of CNS injury, TGF-β1 is thought to be a significant promoter of the astrogliosis that ultimately results in glial scar formation (Rabchevsky et al. 1998), as TGF-β1 blocking antibodies impair glial scar formation (Kohta et al. 2009). Interestingly, it might be possible that this process is partly mediated by the effect of this cytokine on astrocytic differentiation. SCI-induced astrogliosis involves activation of pre-existing astrocytes, but recent work shows a significant contribution of spinal cord ependymal cells in this response, which undergo substantial proliferation and differentiation into astrocytes that integrate into the lesion site (Barnabe-Heider et al. 2010; Gregoire et al. 2015). Thus, the observed TGF-β1-induced proliferation and astrocytic differentiation of spinal cord-derived neural precursors suggests a role for TGF-β1 in the *in vivo* responses of ependymal cells to SCI. Moreover, our data suggests that modulating TGF-β1 signaling following SCI may impact the injury responses of ependymal cells and their progeny.

IV.7. Future directions

In this study, the main missing experiment is to address whether blocking TGF-β1 *in vivo* following injury leads to similar consequences on proliferation and astrocytic differentiation than our experiments conducted under normal conditions or *in vitro*. To accurately address this question, with the help of our collaborators (Dr. Steve Lacroix's laboratory, Quebec City), we will cross a FoxJ1-CreERT2 mouse with a TGF-β1-floxed mouse to obtain an inducible transgenic mouse model in which TGF-β1 can specifically be deleted in ependymal cells following recombination. We will first determine the ependymal cell proliferation and differentiation baseline in non-recombined mice that underwent SCI to know how it compares to our TGF-β1 infusions. Then, we will expose our recombined mice to injury, and observe whether TGF-β1 activity is necessary for the ependymal response to injury. We could also put into culture the injured spinal cords and observe the effect on neurosphere initiation once TGF-β1 activity is blocked.

Another interesting aspect is to determine the role played by other predicted upstream regulators. In Table 4, TNF-α, IL-6, and IL-1β were identified within the top ten of upstream regulators within the cytokine category. It was shown in the literature that these three cytokines are secreted shortly after SCI (Donnelly and Popovich 2008). IL-1β is the first one expressed after 5 minutes, then TNF-α appears 15 minutes post-SCI, and finally IL-6 is secreted between 3 hours and 4 days following injury (Pineau and Lacroix 2007).

We began testing the impact of these three factors on neurosphere formation, and found additive effects on suppressing neurosphere growth, suggesting that they are indeed likely to be endogenous regulators of ependymal cell behaviour as well. Dissociated spinal cord cells were grown in presence of EGF and FGF2 to generate neurospheres in media supplemented with the IL-6, IL-1β, and TNF-α added individually, in duo or in trio (**Fig. IV.11A**). We discovered that cells treated in presence of IL-6 showed a trend towards less primary neurospheres, but we the number of animals should be increased for this effect to become significant (**Fig. IV.11B**). This result suggests an effect on the NSCs. Moreover, primary neurospheres grown in the presence of

IL-6, TNF-α, of all duos and of the trio showed a shift towards smaller neurosphere sizes, suggesting an effect on the progenitors (**Fig. IV.11C**). This preliminary data demonstrates that the effect of these cytokines on purified cultures (secondary neurospheres), for example, should be investigated further.

IV.8. Figures and legends

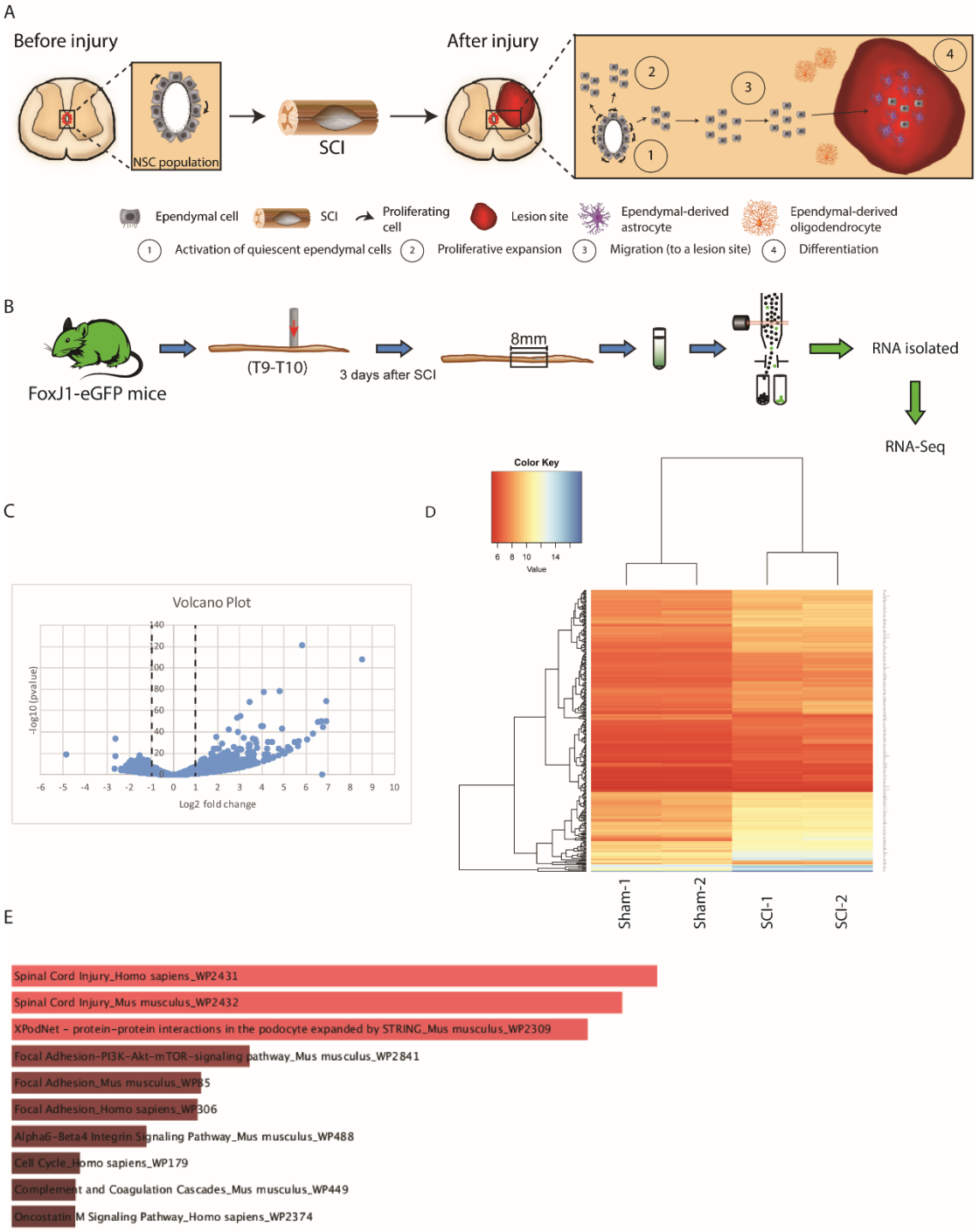


Figure IV.1. RNA-Seq analysis

Representative image of the major ependymal cell behaviour after SCI, including activation of quiescent ependymal cells, proliferative expansion, migration, and differentiation (**A**). Schematic

illustration of the steps leading to the isolation of ependymal NSCs from the injured or sham-operated spinal cord of FoxJ1-eGFP mice to perform RNA-Seq (see methods) (**B**). Volcano plot identifying changes in genes. A threshold of -1 > log2 fold > 1 and p(adj) < 0.05 was used to determine the most significant changes (**C**). Heatmap with the log read count values, showing that genes in sham and SCI samples are distinct (**D**). Main signaling pathways generated by WikiPathways (EnrichR) for the most significant changes after SCI. The brighter the colour, the more significant that term is (**E**).

Table IV.1. Top 25 of up-regulated genes

Ensembl gene ID	Gene name	Description	Log2 fold	Fold
ENSMUSG00000030905	Crym	crystallin, mu	9.293	627.332
ENSMUSG00000029816	Gpnmb	glycoprotein (transmembrane) nmb	8.526	368.595
ENSMUSG00000069516	Lyz2	lysozyme 2	6.903	119.711
ENSMUSG00000037428	Vgf	VGF nerve growth factor inducible	6.903	119.692
ENSMUSG00000020427	Igfbp3	insulin-like growth factor binding protein 3	6.749	107.524
ENSMUSG00000020932	Gfap	glial fibrillary acidic protein	6.713	104.901
ENSMUSG00000033788	Dysf	dysferlin	6.706	104.408
ENSMUSG00000007888	Crlf1	cytokine receptor-like factor 1	6.535	92.743
ENSMUSG00000027994	Ccdc109b	coiled-coil domain containing 109B	6.321	79.964
ENSMUSG00000053175	Bcl3	B cell leukemia/lymphoma 3	6.049	66.206
ENSMUSG00000023885	Thbs2	thrombospondin 2	5.821	56.529
ENSMUSG00000047261	Gap43	growth associated protein 43	5.672	50.989
ENSMUSG00000062593	Lilrb4	leukocyte immunoglobulin-like receptor, subfamily B, member 4A	5.623	49.276
ENSMUSG00000022382	Wnt7b	wingless-type MMTV integration site family, member 7B	5.474	44.458
ENSMUSG00000030748	Il4ra	interleukin 4 receptor, alpha	5.307	39.601
ENSMUSG00000045608	Dbx2	developing brain homeobox 2	5.267	38.512
ENSMUSG00000026535	Ifi202b	interferon activated gene 202B	5.217	37.187
ENSMUSG00000028364	Tnc	tenascin C	5.183	36.340
ENSMUSG00000039109	F13a1	coagulation factor XIII, A1 subunit	5.036	32.804
ENSMUSG00000022371	Col14a1	collagen, type XIV, alpha 1	4.967	31.270
ENSMUSG00000021508	Cxcl14	chemokine (C-X-C motif) ligand 14	4.949	30.894
ENSMUSG00000030077	Chl1	cell adhesion molecule L1-like	4.914	30.155
ENSMUSG00000039899	Fgl2	fibrinogen-like protein 2	4.814	28.121
ENSMUSG00000020000	Moxd1	monooxygenase, DBH-like 1	4.766	27.200
ENSMUSG00000024501	Dpysl3	dihydropyrimidinase-like 3	4.760	27.087

Using a threshold of $-1 >$ log2 fold > 1 and $p(adj) < 0.05$, we identified 1,241 SCI-induced and significantly changed genes. Within the 824 up-regulated genes, the table shows the top 25 genes, including *gfap* and *thrombospondin2* that are implicated in astrocytic fate. including 824 up-regulated and 417 down-regulated.

Table IV.2. Top 25 down-regulated genes

Ensembl gene ID	Gene name	Description	Log2 fold	Fold
ENSMUSG00000050368	Hoxd10	homeobox D10	-4.848	0.035
ENSMUSG00000048329	Mfsd6l	major facilitator superfamily domain containing 6-like	-2.645	0.160
ENSMUSG00000028222	Calb1	calbindin 1	-2.625	0.162
ENSMUSG00000041923	Nol4	nucleolar protein 4	-2.607	0.164
ENSMUSG00000091345	Col6a5	collagen, type VI, alpha 5	-2.348	0.196
ENSMUSG00000049555	Tmie	transmembrane inner ear	-2.347	0.197
ENSMUSG00000030228	Pik3c2g	phosphatidylinositol 3-kinase, C2 domain containing, gamma polypeptide	-2.345	0.197
ENSMUSG00000030443	Zfp583	zinc finger protein 583	-2.339	0.198
ENSMUSG00000031825	Crispld2	cysteine-rich secretory protein LCCL domain containing 2	-2.270	0.207
ENSMUSG00000038242	Aox4	aldehyde oxidase 4	-2.250	0.210
ENSMUSG00000030108	Slc6a13	solute carrier family 6 (neurotransmitter transporter, GABA), member 13	-2.220	0.215
ENSMUSG00000078639	Gm12695	predicted gene 12695	-2.218	0.215
	Gm10432	predicted gene 10432	-2.216	0.215
ENSMUSG00000042499	Hoxd11	homeobox D11	-2.168	0.222
ENSMUSG00000024292	Cyp4f14	cytochrome P450, family 4, subfamily f, polypeptide 14	-2.138	0.227
ENSMUSG00000068099	1500009C09Rik	RIKEN cDNA 1500009C09 gene	-2.085	0.236
ENSMUSG00000047496	Rnf152	ring finger protein 152	-2.050	0.242
ENSMUSG00000039099	Wdr93	WD repeat domain 93	-1.966	0.256
ENSMUSG00000041616	Nppa	natriuretic peptide type A	-1.966	0.256
ENSMUSG00000041237	Pklr	pyruvate kinase liver and red blood cell	-1.944	0.260
ENSMUSG00000027895	Kcnc4	potassium voltage gated channel, Shaw-related subfamily, member 4	-1.927	0.263
ENSMUSG00000043020	Wdr63	WD repeat domain 63	-1.898	0.268
ENSMUSG00000055102	Zfp819	zinc finger protein 819	-1.897	0.268
ENSMUSG00000042567	Nek10	NIMA (never in mitosis gene a)- related kinase 10	-1.896	0.269
ENSMUSG00000040452	Cdh12	cadherin 12	-1.894	0.269

Using a threshold of $-1 > \log2\ \text{fold} > 1$ and $p(adj) < 0.05$, we identified 1,241 SCI-induced and significantly changed genes. Within the 417 up-regulated genes, the table shows the top 25 genes.

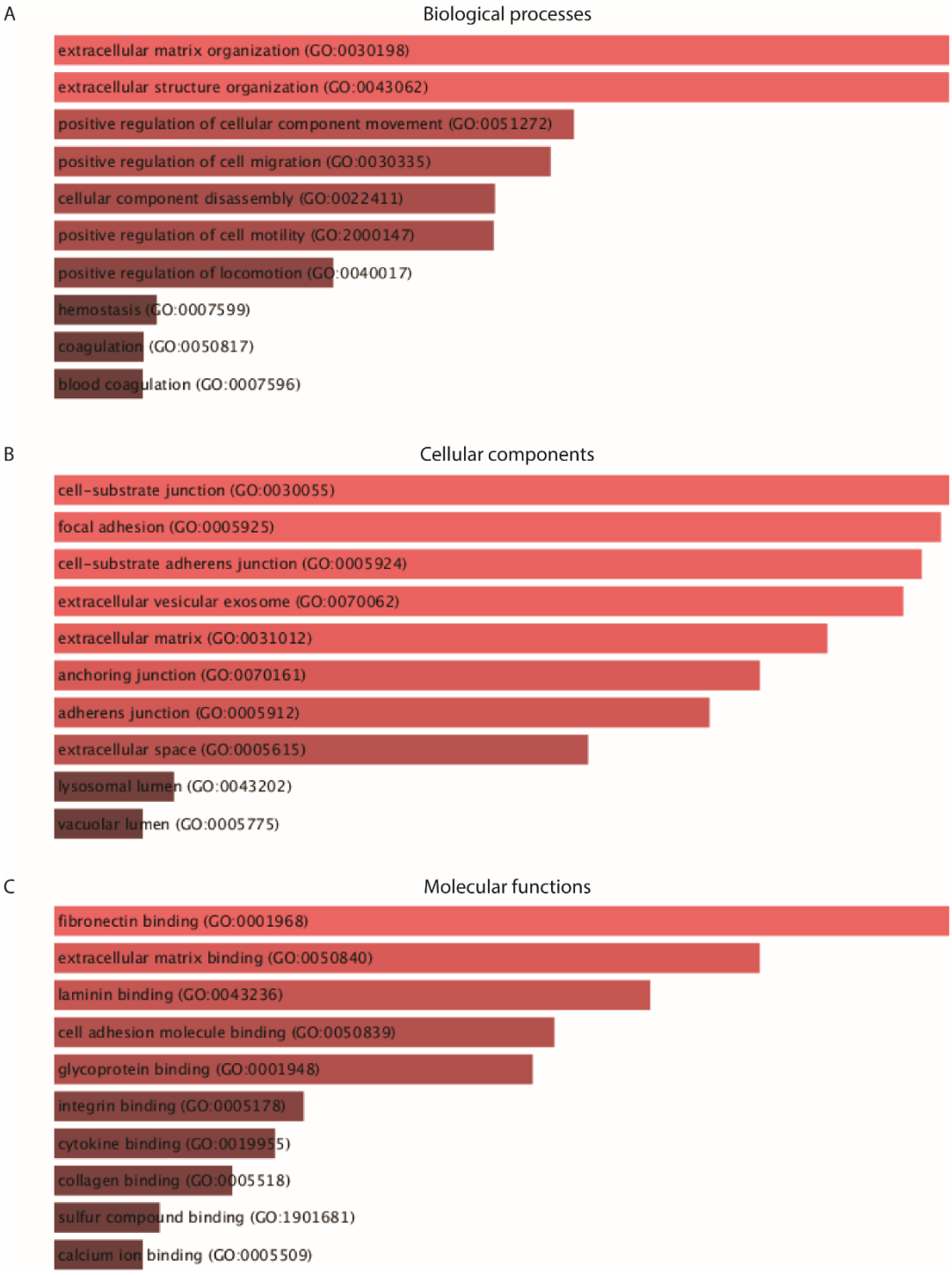


Figure IV.2. GO enrichment analysis

Differentially expressed genes were submitted to a GO enrichment analysis using EnrichR to look at the main biological processes (**A**), molecular functions (**B**), and cellular components (**C**) that were affected.

Table IV.3. Top 10 upstream regulators (growth factors)

Growth factors
TGFB1
HGF
IGF1
FGF2
AGT
EGF
FGF1
TGFB3
NGF
IGF2

Ingenuity Pathway Analysis was used to perform an Upstream Regulator Analysis of the differentially expressed genes. This table shows the top 10 of upstream regulators that are found within the growth factor category.

Table IV.4. Top 10 upstream regulators (cytokines)

Cytokines
TNF
IFNG
IL6
IL1B
CSF2
IL4
CSF3
IL13
OSM
TNFSF11

Ingenuity Pathway Analysis was used to perform an Upstream Regulator Analysis of the differentially expressed genes. This table shows the top 10 of upstream regulators that are found within the cytokine category.

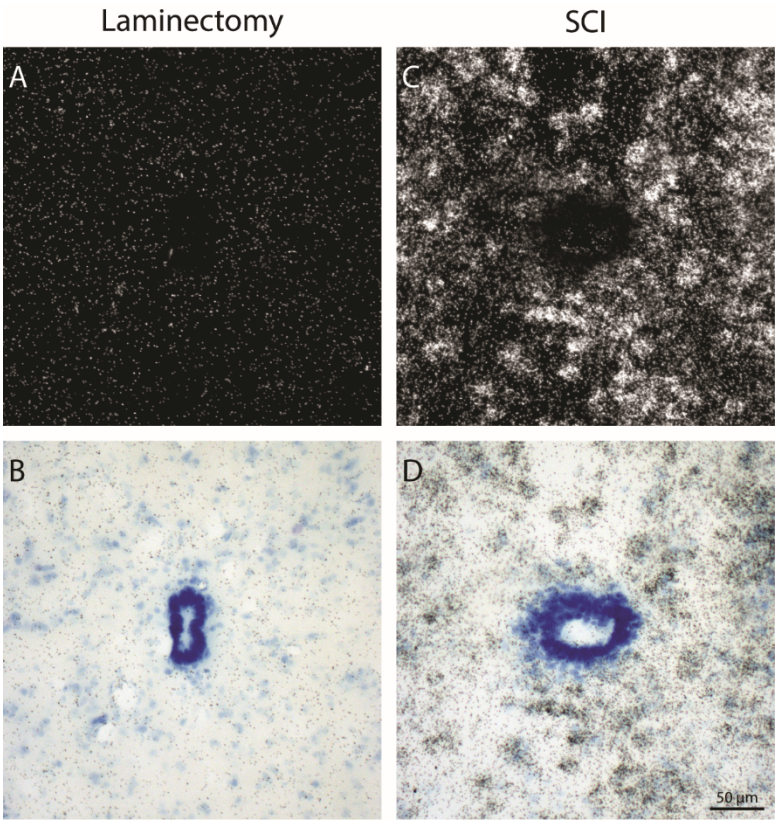


Figure IV.3. Spinal cord contusion in adult mice induces strong expression of TGF-β1 mRNA

Dark-field photomicrographs showing TGF-β1 mRNA expression surrounding the spinal cord central canal of a sham-operated (laminectomy) mouse (**A-B**) and SCI mouse (~0.5 mm caudal to the lesion epicenter, (**C-D**) at four days post-surgery. Bright-field photomicrographs showing the location of cells expressing TGF-β1 mRNA with regard to the ependymal layer of the central canal, identifiable by the thionin counterstaining. Scale bar = 50µm.

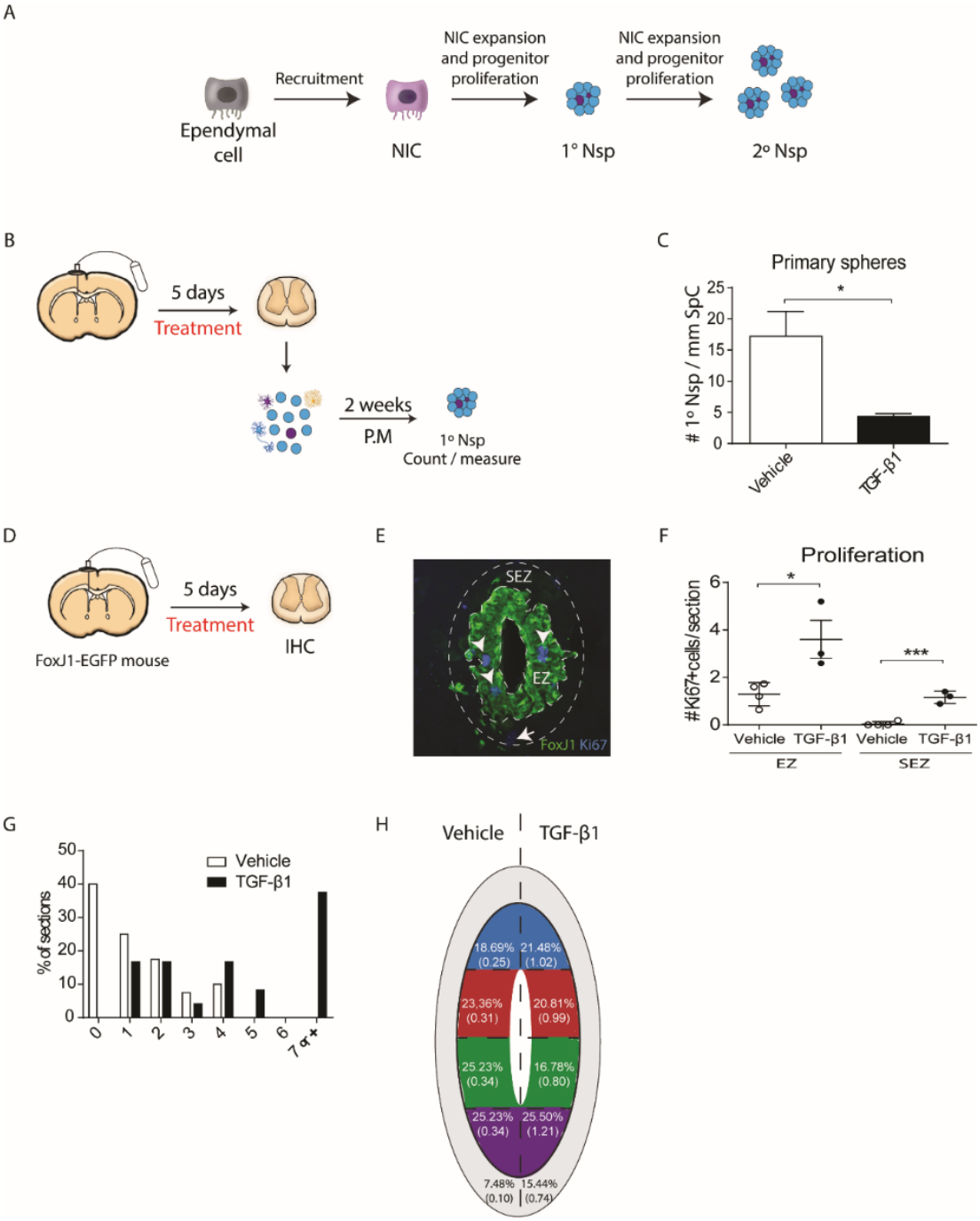


Figure IV.4. NIC recruitment and proliferation

Ependymal cells are recruited to become neurosphere-initiating cells (NICs). NICs will then expand along with progenitor proliferation to give rise to primary neurospheres and then secondary neurospheres (**A**). Experimental paradigm for growth of primary neurospheres from spinal cords of Vehicle or TGF-β1-infused mice (**B**). Quantification of average primary neurosphere number per mm of spinal cord. Unpaired t-test, *=p≤0.05 (**C**). Experimental paradigm for

immunohistochemical analyses (**D**). Image of the central canal in FoxJ1-EGFP mice following Ki67 immunostaining, showing definition of EZ and SEZ used in the present study (**E**). Quantification of Ki67+ cells in the EZ and SEZ following Vehicle or TGF-β1 infusion. Unpaired t-test, *=p≤0.05, ***=p≤0.001 (**F**). Frequency histogram of the mean number of Ki67+ cells per section (**G**). Dorso-ventral distribution of Ki67+ cells within the EZ and SEZ (**H**). (#) = absolute number of Ki67+ cells / section.

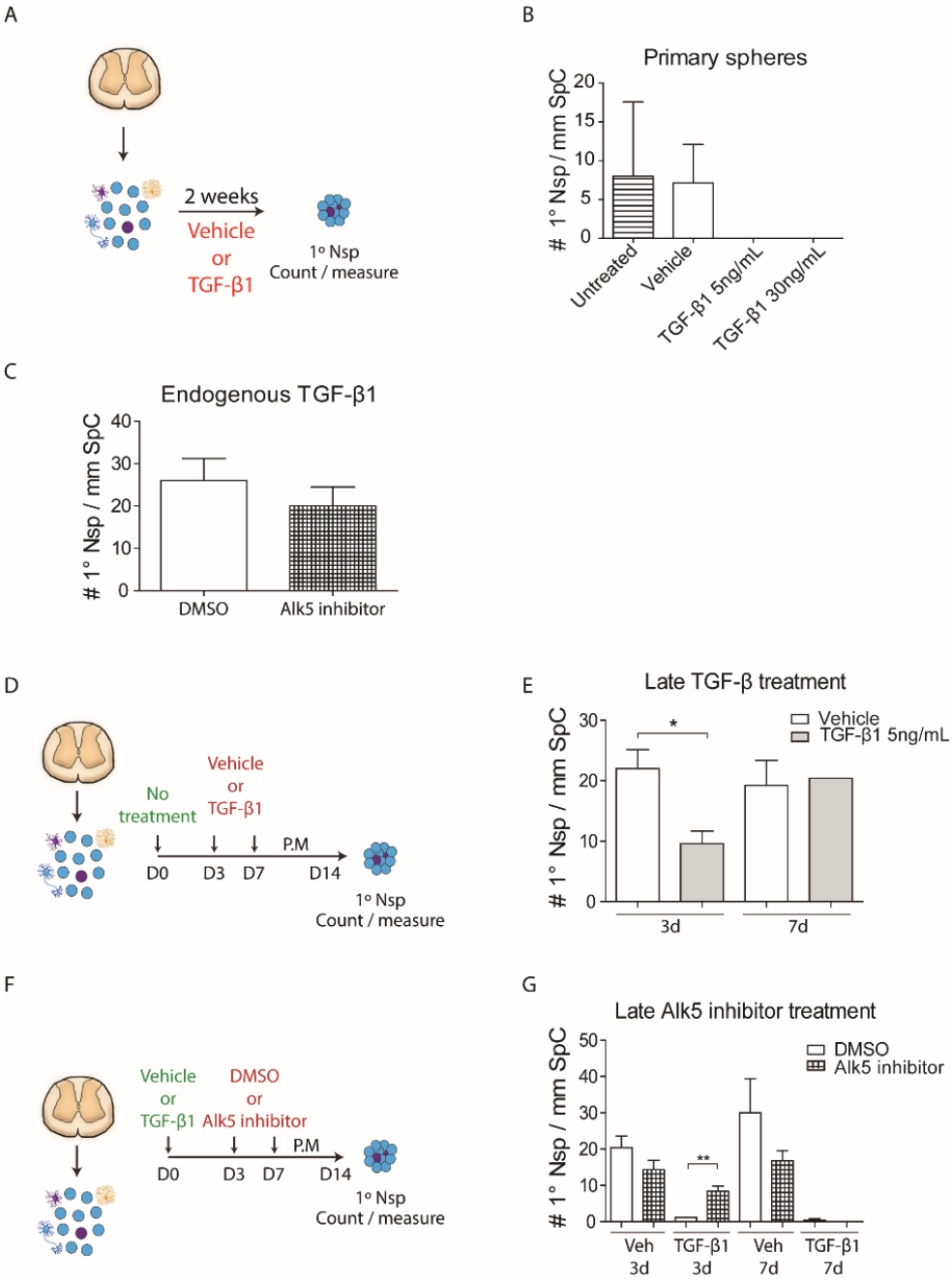


Figure IV.5. Effect of TGF-β1 on NIC expansion

Experimental treatment paradigm for assessing NIC expansion (**A**), and average number of primary spheres obtained per mm of dissociated spinal cord when cells are treatment with Vehicle or TGF-β1 (**B**). One way-ANOVA, Dunnett's multiple comparison tes. The Alk5 inhibitor does not increase numbers of primary neurospheres that can be grown from the dissociated spinal cord (**C**). Experimental paradigm for testing the effect of delaying TGF-β1 treatment until day three or

seven of primary neurosphere formation (**D**) Quantification of primary neurosphere numbers (**E**). Effect of delayed Alk5 inhibitor treatment on primary neurosphere formation. Experimental paradigm for blocking TGF-β1-induced signaling with the Alk5 inhibitor beginning at day three or seven (**F**). Quantification of primary neurosphere numbers (**G**). Unpaired t-test, *= $p \leq 0.05$, **= $p \leq 0.01$.

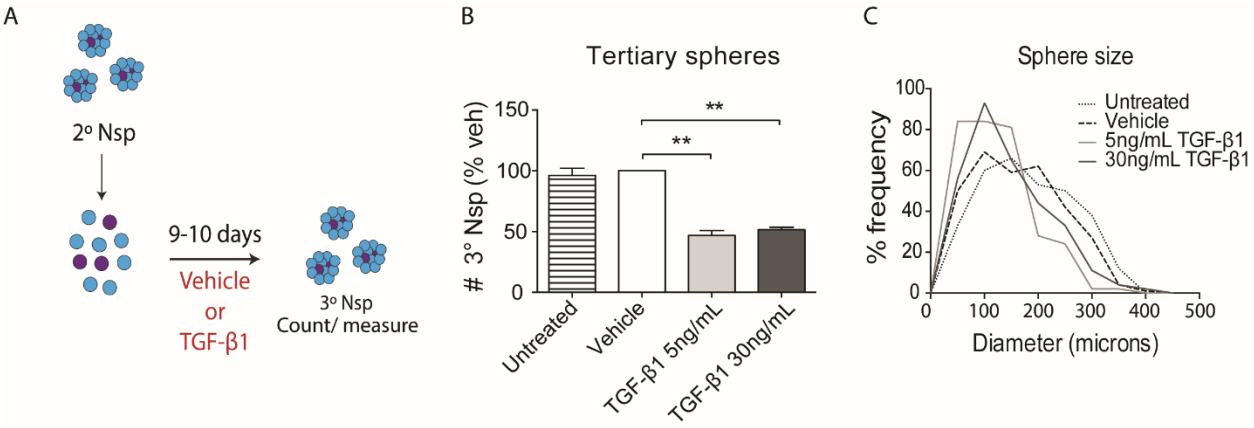


Figure IV.6. Effect of TGF-β1 on purified cultures

Experimental treatment paradigm for assessing *in vitro* expansion of spinal cord-derived NICs (**A**). Average number (**B**) and size distribution (**C**) of tertiary neurospheres generated from dissociated secondary neurospheres when grown in proliferation medium supplemented with Vehicle versus TGF-β1. One sample t-test (**D**), **= $p \leq 0.01$.

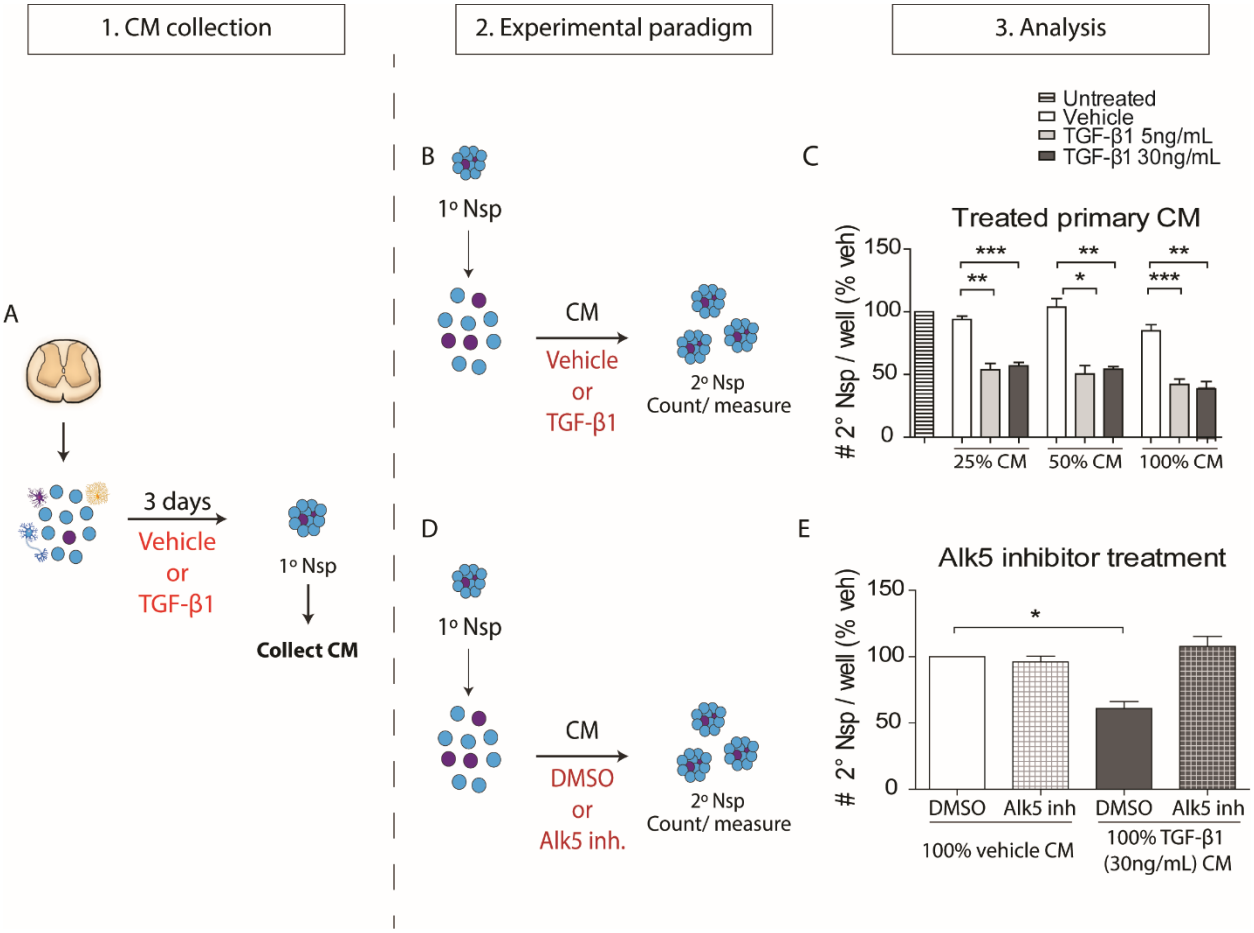


Figure IV.7. Neurosphere growth inhibition is TGF-β1-specific, not due to indirect effects

Experimental paradigm for obtaining conditioned-medium (CM) from dissociated spinal cords grown under neurosphere-forming conditions for three days in the presence of Vehicle or TGF-β1 (**A**). Effect of increasing concentrations of CM on growth of secondary neurospheres. Experimental paradigm (**B**) and quantification of the effects of 25%, 50% and 100% CM on numbers of secondary neurospheres (**C**). Effect of blocking TGF-β1 signaling during CM-induced neurosphere growth inhibition. Experimental paradigm (**D**) and quantification of average number of secondary neurospheres grown in the presence of 100% Vehicle CM or 100% TGF-β1 (30 ng/mL) CM (**E**).

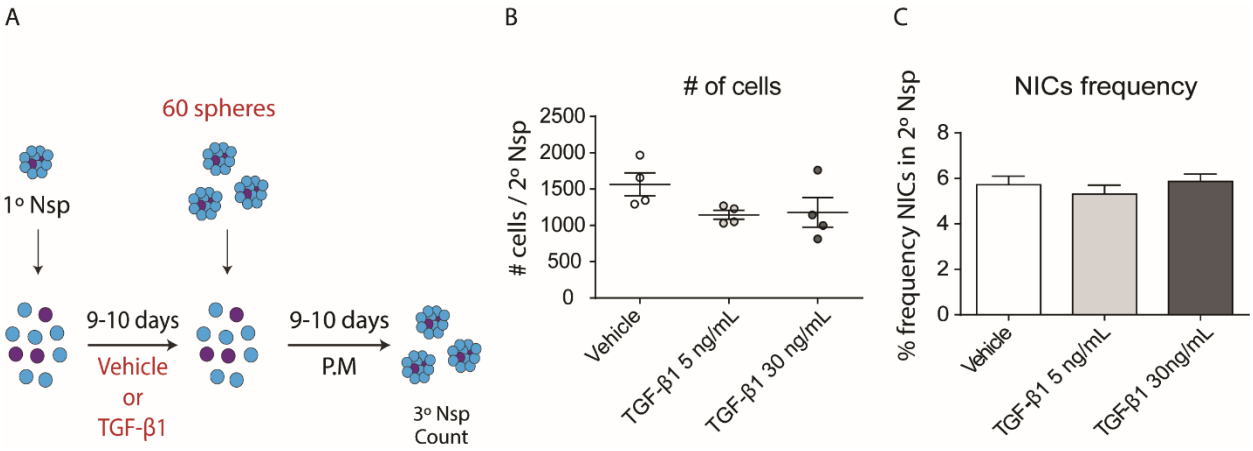


Figure IV.8. Self-renewal assay

Self-renewal assay. Experimental paradigm for retrospectively assessing number of NICs per secondary neurosphere (**A**). Counts of total number of cells per secondary neurosphere when grown in the presence of Vehicle versus TGF-β1 (**B**) and the percentage of secondary neurosphere cells that subsequently give rise to tertiary neurospheres when grown under normal proliferation conditions (**C**). One way-ANOVA, Dunnett's multiple comparison test.

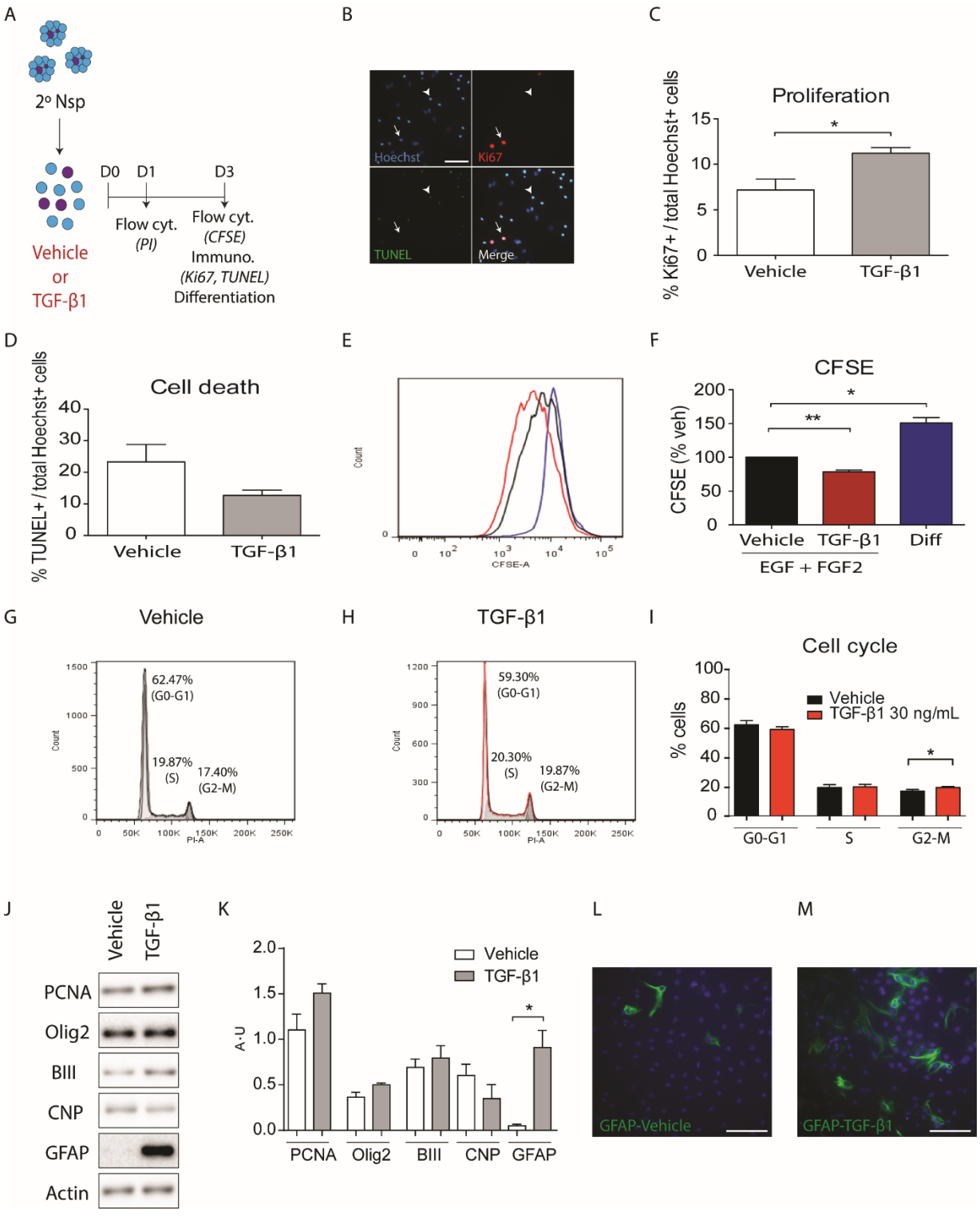


Figure IV.9. TGF-β1 promotes proliferation and astrocytic differentiation *in vitro*

Experimental paradigm of tests performed on dissociated secondary sphere cells (**A**). Individual representative images of Hoechst, Ki67, and TUNEL staining as well as the merge image. Arrow shows a

Ki67+ cell and the arrowhead shows a TUNEL+ cell (**B**), and quantification of the proportion of cells that are Ki67+ (**C**) or TUNEL+ (**D**). Unpaired t-test, *= $p \leq 0.05$. Curves (**E**) and mean fluoresence (**F**) for the CFSE cell division assay (See methods for details). Note the TGF-β1-induced decrease in fluorescence, indicative of increased proliferation. A differentiation (diff) condition was added as a negative control. Propidium iodide cell cycle analysis following treatment with Vehicle (**G**) or TGF-β1 (**H**), showing a small but significant increase in cells within the G2-M phase (quantified in **I**). One way-ANOVA, Dunnett's multiple comparison test (CFSE), and unpaired t-test (PI), *= $p \leq 0.05$, **= $p \leq 0.01$. Differentiation experiments. Western blots (**J**) and densitometric quantifications (**K**) following three days of Vehicle or TGF-β1 treatment. Immunocytochemistry for GFAP in Vehicle-treated (**L**) or TGF-β1-treated (**M**) cultures (400x magnification). Scale bar = 25µm. Unpaired t-test, *= $p \leq 0.05$.

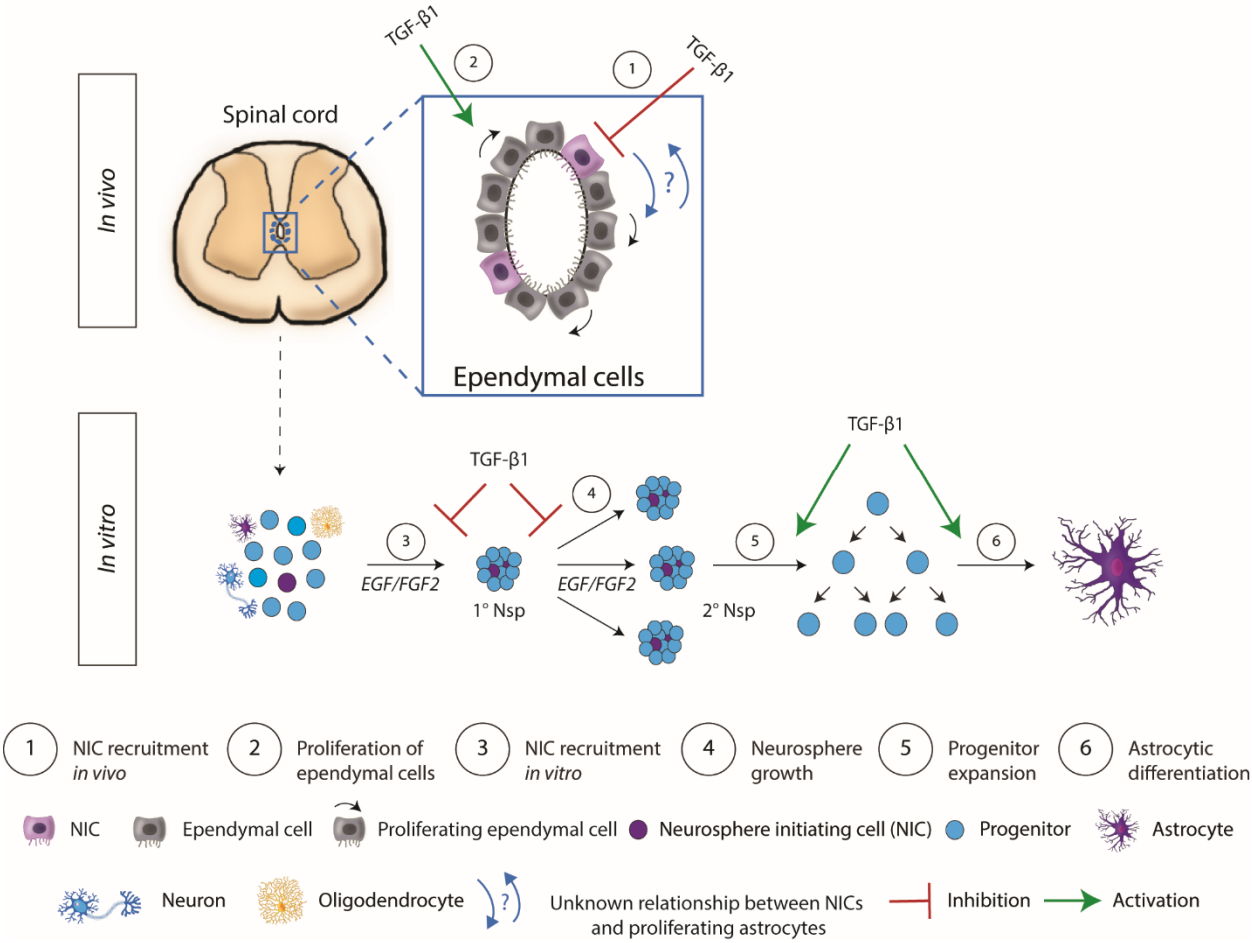


Figure IV.10. Summary

TGF-β1 infusion *in vivo* inhibits NIC recruitment (**1**) and stimulates proliferation of ependymal cells (**2**). TGF-β1 treatment *in vitro* suppresses initial NIC recruitment (**3**) and inhibits subsequent NIC expansion (**4**), while simultaneously increasing proliferation of a subpopulation of progenitors (**5**) and astrocytic differentiation (**6**). The relationship between the NIC and proliferative ependymal cells *in vivo* is not established.

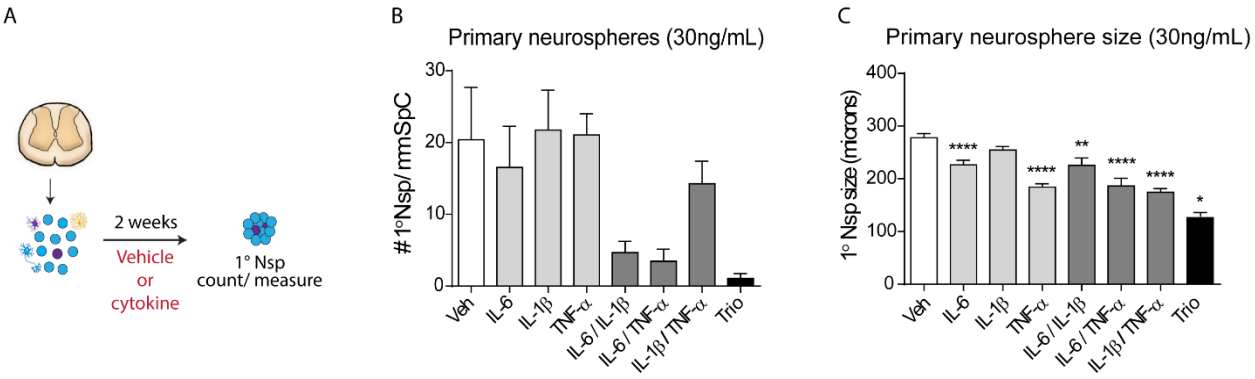


Figure IV.11. Preliminary data for IL-6, IL-1β1, and TNF-α

Experimental treatment paradigm for assessing NIC recruitment (**A**), and average number of primary spheres obtained per mm of dissociated spinal cord when cells are treated with individual, duo, or trio of cytokines (**B**), and average size (**C**) (One way-ANOVA, Dunnett's multiple comparison test.

Chapter V. Discussion

V.1. Discussion

The aim of this thesis work was to investigate the mechanisms underlying activation of neural stem cells in the adult central nervous system. My specific aims were to address this question using adult mice in two complementary models: 1) activation of hippocampal NSCs by environmental enrichment (chapters II and III), and 2) activation of spinal cord NSCs by injury-induced neuroinflammation (chapter IV). Moreover, 3) to gain new insights into the molecular mechanisms of these models, we performed transcriptomics studies to open new lines of investigation (chapters III and IV). This dissertation work was comprised of three studies and the related findings are discussed below.

V.1.1. Activation of hippocampal NSCs by environmental enrichment

The first model of NSC activation used in this dissertation is a physiological stimulation, known as EE, that can be useful to better understand how NSCs improve cognitive function in the brain. In my first study, we investigated the effect of individual components of EE on hippocampal neurogenesis at the cellular level (chapter II). This study allowed us to identify the key components of EE that are implicated in the activation of hippocampal NSCs. Then, in my second study, we confirmed that these key EE variables showed differences in cognitive function by doing behavioural tests and pursued with an unbiased RNA-Seq approach to unravel new cellular and molecular processes that could be implicated in the observed changes (chapter III).

V.1.1.1. Impact of individual components of EE

In my first study (chapter II), the main challenge was to develop a paradigm that allowed efficient separation of individual components found within an enriched environment. We came up with an "Alternating EE" paradigm allowing the stepwise addition or subtraction of EE variables. The outbred CD1 strain was chosen over the most commonly used inbred C57BL/6 mouse strain based on the results of one of our control experiments and on the literature (Clark et al. 2011b). A lower baseline proliferation was observed in CD1 mice when compared to C57BL/6, and a running-induced increase detectable with only four animals and we decided to pursue the study

with this mouse strain. However, this choice has its downsides mainly because a minority of EE studies are conducted in outbred strains, hence making it harder to compare results. Furthermore, the genetic background itself was shown to have an influence on hippocampal neurogenesis (Kempermann and Gage 2002; Kempermann et al. 1997a). However, the priority was to have a valid mouse model in which we could observe the effect of EE and finally our results were consistent with our previous studies (Bednarczyk et al. 2009; Bednarczyk et al. 2011).

We first asked whether these different environments had any impact on the main stages of neurogenesis (proliferation, neuroblasts, immature neurons, and survival) after a period of four weeks. We did not detect any significant difference between our impoverished and locked disc conditions, which contradicts previous findings from our laboratory. Bednarczyk and colleagues demonstrated that mice exposed to a locked disc environment showed an increase in proliferation similar to runners. Other stages were modulated as their control environment (impoverished), suggesting running independent and running dependent stages (Bednarczyk et al. 2011). The same mouse strain was used and we controlled for the difference between running discs and wheels as well as daily handling. The use of an antigen-retrieval to reveal Ki67+ proliferating cells for the current study might be responsible for this difference even though this change applies to all the groups and should be uniform. As expected, runners showed increased levels of all four stages when compared to animals within the locked disc group, regardless of the three-day or seven-day condition. In contrast, the complex environment (CE) had no significant effect. Interestingly, when running was added to the CE, the amount of neuroblasts and newly-born cells increased. However, the opposite did not lead to the potentiation of the effects. These results suggest that running is the main neurogenic component of EE, and not CE. There was also no sign of additive effect as suggested in the literature (Fabel et al. 2009). However, Fabel and colleagues exposed their mice to consecutive periods of EE, such as ten days of running followed by 35 days of EE, which differs considerably from our paradigm. Moreover, no details were provided in regards to the EE components. One possible caveat to this study is that our type of enrichment could be considered a mild form of cognitive stimulation, therefore explaining the lack of neurogenic effect from the CE group. Mice could have been exposed to learning-associated stimuli to increase cell survival, for example (Leuner et al. 2004).

We next asked whether the pattern of DG activation using a depolarization-induced immediate early gene, c-fos, exhibited differences among groups. We suggested that the social component within CE was positively modulates c-fos expression. The addition of a toys only group could have improved this study and allowed us to accurately say that social interactions are responsible for the increased neuronal activation observed in the CE group, unless the toys group would have shown similar results. Surprisingly, exercise decreased c-fos levels, which contradicts other studies (Clark et al. 2012; Clark et al. 2011a; Clark et al. 2009). Rhodes group observed increases in immediate early gene expression in paradigms ranging between five to seven weeks of running, which suggests that our four-week paradigm might have been too short too see this effect. We investigated a step further to see if stress could be involved in the running neurogenic effect or lack of effect from CE. All groups including a CE component showed low plasma corticosterone levels, suggesting that high stress is not responsible for the lack of neurogenic effect. Moreover, two of the CE components tested (locked disc and social interactions) did not show a reduced corticosterone expression, suggesting that the rotated tunnels could be mediating this effect. As mentioned previously, the presence of a toys only group would have been beneficial to confirm this hypothesis.

Together this work confirmed that 1) running is the neurogenic component of EE, regardless of the frequency (three or seven days a week), 2) showed that social interactions within the CE increase neuronal activation and that 3) social isolation, group housing, nor increased levels of plasma corticosterone had an impact on neurogenesis. Moreover, it allowed us to identify the running and social interactions as key EE components for further investigation.

V.1.1.2. Behavioural impact of exercise and social interactions

In my second study, the goal was to first investigate the functional outcome following exposure to the previously identified key components of EE, exercise and social interactions. Then use an unbiased method such as RNA-Seq to assess if these components have distinct cellular and molecular processes mediating their distinct consequences on the DG. We first established three

behavioural tests that would assess learning and memory: CPP (contextual learning and memory), RAM (spatial learning and memory), and NOR (object recognition memory), and the open-field that assesses locomotor activity. CPP and RAM were incentive-driven while NOR was driven by the mouse innate novelty preference. Unfortunately, technical difficulties during CPP led to inconclusive results that were excluded from the study, but mice were still exposed to this environment and can be considered as another type of enrichment. In fact, this current paradigm differs from the (Gregoire et al. 2014) paradigm in two aspects: 1) the length of the study (eight weeks versus four weeks) and 2) the addition of behavioural testing that could be considered as a learning-associated stimulus (cognitive stimulation). This type of stimulus was shown to increase cell survival (Leuner et al. 2004). A control experiment demonstrated that after eight weeks, neuroblast numbers were still increased by voluntary running and socially-housed mice were not different from locked disc controls, consistent with the four-week paradigm data (Gregoire et al. 2014). In addition, it would be interesting to test for the expression of immediate early genes, such as c-fos, zif268, or arc to see if we would detect an increase consistent with other studies (Clark et al. 2011a; Clark et al. 2009).

Behavioral studies confirmed our hypothesis that exposure to exercise and social interactions leads to different behavioral outcomes. First, we confirmed that changes between groups would not be due to differences in baseline locomotor activity as all groups showed similar travelled distances in the open-field. In the RAM, runners demonstrated a stronger short-term recall (more entries in the previously baited arm), whereas socially-housed mice had better cognitive flexibility (better arm accuracy). In the NOR test, a mouse normal behaviour consists in exploring more the novel object as it remembers the familiar object. Both the locked disc controls and socially-housed mice showed short-term object recognition memory. In contrast, runners had a preference ratio equal to chance after an hour, but showed a strong tendency for long-term memory object recognition memory. It would be interesting to test additional animals to see if can reach significant results. From several experiments conducted in the laboratory, we noticed that the CD1 outbred strain shows a lot of variability, and therefore more animals are needed in order to show conclusive effects.

The socially-enriched complex environment and exercise groups affected differently the cellular and genetic processes. Our previous published results on complex environment suggested the social interactions component was mediating the neuronal activation increase (Gregoire et al. 2014). However, this previous study did not assess the impact of the rotating tunnels on their own to clearly confirm this interpretation. Ideally, we would have performed this RNA-Seq with the social interactions only group. Our bioinformatics analysis revealed interesting data: 1) CE and running stimulate distinct genetic changes in the DG, and 2) running intensity differentially impacts DG gene expression. A more in-depth bioinformatics analysis will be needed to select genes of interest and verify their implication in NSC activation or neuronal function.

Together, this work demonstrated that key components of EE, exercise and social interactions, are responsible for changes not only at the cellular level, but also at the behavioral and genetic levels. The transcriptomics analysis will allow us to identify new targets that could be implicated in the mechanism underlying NSC activation or neuronal activation.

V.1.2. Activation of spinal cord NSCs by injury-induced neuroinflammation

The second approach to activate NSCs in this thesis is a pathological stimulation known as spinal cord injury (SCI). This stimulation can help us better understand how NSCs modulate their activity to promote repair of the CNS after a lesion. The neuroinflammation reaction after injury has been extensively studied as well as the spinal cord ependymal cell proliferation, but not the link between both events (Barnabe-Heider et al. 2010; Donnelly and Popovich 2008; Meletis et al. 2008; Popovich et al. 1997). We therefore decided to start by performing a RNA-Seq on a specific cell population (ependymal cells) following injury to target molecular changes that could be implicated in the activation of ependymal cells following SCI as little information is known in the literature.

V.1.2.1. TGF-β1 impact on ependymal cells

The goal of this study (Chapter IV) was to first identify a potential upstream regulator of the transcriptome modifications detected following ependymal cells activation by SCI. Second, to understand at what stage it is acting upon: activation of quiescent ependymal cells, proliferative expansion, migration or differentiation. Ependymal cells from FoxJ1-eGFP mouse spinal cords (sham or injured) were isolated through FACS and then RNA was isolated and prepared for RNA-Seq. A bioinformatics analysis of upstream regulators suggested that TGF-β1 could play an important role in the ependymal cell activation, and we thus confirmed the presence of TGF-β1 mRNA around the central canal of the spinal cord after SCI. TGF-β1 treatment decreased *in vivo* numbers of NICs, but increased proliferating ependymal-derived progenitors. We next confirmed that TGF-β1 suppresses completely *in vitro* growth factor-mediated formation of primary spheres. This was tested via the use of a TGF-β1 receptor 1 kinase inhibitor, Alk 5, on primary neurospheres from dissociated spinal cord cells at different time points after treating cells with Vehicle or TGF-β1. The experiment showed a partial rescue when Alk5 was added on day three and no rescue when added on day seven (decreasing effect through time), suggesting that TGF-β1 acts on early stages of neurosphere formation. A 50% decrease in purified tertiary neurosphere formation was also observed suggesting an effect on neural precursors. To determine if this effect was direct or indirect, conditioned medium from non-purified primary cultures was added to dissociated primary sphere cells. Results showed that the growth-inhibitory effect was attributable to residual TGF-β1 as the effect disappeared when cells were treated with Alk5. TGF-β1 also had downstream effects on progenitors as it increased their proliferation and promoted their astrocytic fate.

The main remaining question concerns the ependymal cell behaviour following injury when TGF-β1 is specifically blocked in these cells *in vivo* in order to confirm our model. Moreover, TGF-β1 is not the only upstream regulator of interest that showed up in our ingenuity pathway analysis. Furthermore, the link between NIC and proliferating cell is still unknown. Our work shows that TGF-β1 acts upon NIC at an early stage of neurosphere formation, but we do not know if proliferating ependymal cells after injury are the NIC or if NIC are the ones proliferating or if they may be two distinct ependymal cell population. Neuroinflammation following SCI is a very complex reaction and molecules that are secreted could have synergic, additive or inhibitory

effects on one another and studying the different factors within this reaction is crucial to fully understand what is happening. For example, IL-6, IL-1β, and TNF-α are within the first cytokines to be secreted following SCI and they were part of the top 10 of upstream regulators in the cytokines category (Donnelly and Popovich 2008). We started investigating the impact of these cytokines on ependymal cells and preliminary data suggested that they should be investigated further as they show a decrease in neurosphere growth formation.

Together, these results show three main conclusions: 1) the initial recruitment step of NICs from primary cultures is more sensitive to TGF-β1 than subsequent expansion of NICs (primary versus tertiary neurospheres). 2) Raising TGF-β1 levels decreases the ability of spinal-cord derived NICs to generate neurosphere colonies in response to EGF/FGF2. Finally, raising TGF-β1 levels increases proliferation and favours astrocytic differentiation of downstream progenitors.

V.1.3. Insights from both models

The DG, a neurogenic niche, responds differently from the central canal, a quiescent niche, following a stimulus. This is why we chose to stimulate these niches using two different methods: physiological (EE) and pathological (SCI). Although the two complementary models of NSC activation differ considerably, it could be possible to translate the notions learned from one model to the other.

Under normal conditions, hippocampal neurogenesis occurs in the brain and was shown to be increased by different types of EE (Kempermann et al. 1997b; van Praag et al. 1999b). Exercise was identified as the pro-neurogenic EE component (Gregoire et al. 2014). It is well established that exercise increases systemic growth factors such as FGF2, IGF1, VEGF, and BDNF (Aberg et al. 2000; Fabel et al. 2003; Jin et al. 2002; Rossi et al. 2006; Wagner et al. 1999). In our RNA-Seq, we unravelled novel potential targets that may be mediating the hippocampal neurogenesis increase observed following exercise. There are possibilities that systemic factors might also affect the spinal cord. A study showed that seven days of exercise was sufficient to increase the number of oligodendrocyte precursor NG2+ cells, while 14 days led to significant increases in Nestin+

neural precursor cells, GFAP+ astrocytes and BIII-tubulin+ neurons in the intact spinal cord (Krityakiarana et al. 2010). Moreover, Villeda and colleagues demonstrated using parabiosis experiments that the systemic milieu could induce or block age-related impairments in both hippocampal neurogenesis and cognitive function (Villeda et al. 2011; Villeda et al. 2014). These results show the importance of systemic changes that could influence both quiescent and neurogenic niches.

Neuroinflammation is a process that does not only occur in the spinal cord following SCI, but also after traumatic brain injury (Chiu et al. 2016). It is possible that some insights gained from our RNA-Seq could be applied to the brain. However, in contrast to the RNA-Seq performed in the DG, this one was performed on ependymal cells found in the central canal specifically. This may limit the transferable findings. Moreover, several studies showed that the inflammatory response is greater in the spinal cord than in the brain, which could mean different individual and synergic effects (Batchelor et al. 2008; Schnell et al. 1999).

V.2. Perspectives

The main findings of this dissertation lead to other questions that remain unanswered to better understand what are the key mechanisms underlying the activation of NSCs in the CNS. This section will look into the mouse model, follow-up experiments as well as other avenues that could be worth exploring based on these new findings.

V.2.1. Mouse model – possibilities and limitations

The mouse model was chosen for this thesis work mainly because it allows the study of NSCs in both brain and spinal cord using invasive methods that would go against human ethical principles. It was demonstrated recently that hippocampal neurogenesis occurs in both rodents and humans, further confirming the validity of this model (Ernst and Frisen 2015; Spalding et al. 2013). Similarly, the presence of NICs was shown in the human spinal cord (Dromard et al. 2008; Mothe et al. 2011). The mouse offers a vast variety of possibilities such as transgenic models, *in vivo* experiments including electroporation and osmotic pumps, and *in vitro* cultures (Barnabe-Heider et al. 2008; Dhaliwal and Lagace 2011; Martens et al. 2002; Weiss et al. 1996). The ability to cut brain and spinal cord sections to perform immunohistochemistry or the use of microdissections of specific regions to perform RNA-Seq or proteomics is of great importance to discover new cellular and molecular mechanisms that could have applications in humans.

Unfortunately, each model has its limitations. First, in our case, most transgenic mouse lines have a C57BL/6 background and this could cause complications for any future investigations (refer to section V.1.1.1.). Second, and most importantly, most drugs tested in rodents fail to work in humans, a concept called attrition (Garner 2014; Mak et al. 2014). A recent review on the topic goes against the prevalent idea that animals are too different from humans to predict human outcomes by suggesting that we should rather advocate a fundamental shift (Garner 2014). A shift to human-based biomarker and personalized medicine is observed and less interest is directed towards animal-based genomic and phenotyping because the value of animal research is recently put into question. One example to demonstrate this argument is the concern about how a MWM can model the human subtle changes in cognition, while in humans there is an entire

neuropsychology discipline that studies it. Garner suggests that researchers should eliminate false positive in animal models by 1) using the same clinical parameters in animals than in humans (avoid the -like word), 2) having a strong specific null hypothesis (one test per hypothesis to avoid multiplicity), 3) modeling the development of disease itself in a wild-type population (not the veracity of a tool, such as a scalpel or knock-out approaches), and 4) using false positives as validation controls (Garner 2014). Interestingly, Garner states that "a good model will be one where genetics confers risk, not certainty, and the modulators of that risk can be studied" (Garner 2014). These are important points to keep in mind for any future experiments discussed below.

V.2.2. Follow-up experiments

V.2.2.1. EE project

In the EE project, the RNA-Seq experiment opened multiple avenues of research as different cellular and molecular changes were identified for each condition. Low Runners showed changes in multi-system synaptic changes, extracellular matrix and growth factor and calcium signalling. In contrast, High Runners exhibited changes associated with stress responses and negative regulation of synaptic activity. The key difference between both running intensities is the regulation of synaptic activity. How are Low and High Runners DG niche differently regulated? To address this question, we would first confirm the RNA-Seq data by assessing the expression of specific changes by immunohistochemistry such as SST and NPY (interneuron markers) in Low Runners and prostanglandin-endoperoxide synthase 2 (also known as COX-2) in High Runners. The DG receives several synaptic inputs: dopaminergic (ventral tegmental area), cholinergic (septum), glutamatergic (entorhinal cortex), and gabaergic (local interneurons) (Kempermann et al. 2015a). All of which were shown to be significantly modulated in Low Runners, and probably inhibited in High Runners. It would be interesting to discover exactly what inputs are inhibited in High Runners by either inhibiting/activating the specific neurons using optogenetics approaches which would be more specific than lesions or other inhibition techniques that were used in the past (Ho et al. 2009; Park and Enikolopov 2010; Song et al. 2012). Moreover, these loss and gain-of-function experiments could be helpful to determine the exact differences in the niche regulation of each synaptic input in Low and High Runners. As we can identify specific genes that were

modulated in these processes (from the RNA-Seq), we could pinpoint genes responsible for the observed changes in the DG niche (neurogenesis and functional outcome).

The complex environment condition, in contrast, primarily affected the extracellular matrix. We would first confirm these changes by evaluating the expression of thrombospondin 1 by immunohistochemistry. Then, we could investigate further the role of extracellular matrix genes in neuronal activation. As it is only a small percentage of newly-born cells that are activated, it would be advantageous for us to use another method than immediate-early gene labeling, such as electrophysiology (hippocampal slice cultures) to confirm their activation in a specific model (loss or gain-of-function).

In these experiments, transgenic mouse models are needed but they take time to create. Unfortunately, the use of hippocampal neurosphere cultures is not reliable in our hands (preliminary data based on a modified (Babu et al. 2007) protocol), and cannot be used to perform transfections or simple drug testing (inhibitors and/or activators). However, other experiments could be conducted such as *in vivo* electroporation of cell-specific plasmids, which would be a faster method than generating new transgenic mouse lines (Barnabe-Heider et al. 2008). We could therefore knockout or overexpress genes of interest (to be determined by more in-depth bioinformatics analyses). For each gene, we would first confirm their presence in humans, if it is not already known, using either post-mortem tissues, blood or CSF samples before investigating further. In all cases, the functional outcome of these models would be assessed by electrophysiology and behavioural tests.

V.2.2.2. Spinal cord project

Understanding NSC activation within the spinal cord can have important beneficial consequences as we know that the spinal cord is mostly quiescent under normal conditions. NSCs have the potential to play an important role in neuronal repair after SCI. Similar to the context of neurodegenerative diseases, once the cells are activated it is important to make sure they differentiate into the right cell type and survive in the injured environment. The importance of

unraveling what mediates the acquisition of stem cell properties by ependymal cells after injury remains crucial. However, the complexity of the neuroinflammatory response following injury makes it harder to select one of the significantly modulated genes as our target. This is why our approach has been to look at upstream regulators of these modified downstream genes as it is more likely that multiple changes are responsible in the ependymal cell behaviour changes. The next step was to look at the regulators that were secreted first following the microglia activation. We first selected TGF-β1, then the next step will be to study IL-6, IL-1β, and TNF-α, which are all secreted within minutes after injury (Bastien and Lacroix 2014) and found within our top 10 upstream regulators. As these factors are secreted around the same time, it is worth asking if they affect ependymal cell activation (behaviour) in a synergic manner. Hence, we would test their individual, duo, and trio effect on proliferation, differentiation, survival by using spinal cord-derived cultures as we did for TGF-β1. As TGF-β1 blocked NIC recruitment, it would be interesting to see if these other cytokines have a different effect by promoting the recruitment of ependymal cells. It will also be important to determine their *in vivo* effect by either blocking their secretion, their receptors or using a Cre-Lox approach after spinal cord injury. It is important to keep in mind the importance of what these activated ependymal cells are becoming, each cytokine could influence a different cell fate that could, in turn, influence glial scar formation (Gregoire et al. 2015).

V.2.3. Other avenues

This section covers different avenues that could be covered in the long-term and may not be direct follow-ups of the current projects.

V.2.3.1. Reward system

Could intense running negatively influence neurogenesis and cognitive function due to mesolimbic system dysregulation? Exercise was demonstrated to be rewarding, but the impact of different running intensities on the mesolimbic circuitry (reward pathway that connects the ventral tegmental area to the nucleus accumbens) remains unclear (Greenwood et al. 2011). It was previously shown that dysregulation of the dopamine system can lead to compulsive behaviour

such as excessive running (Mathes et al. 2010). Could excessive running lead to dopaminergic dysregulation as well? Inoue and colleagues recently showed that intense running activity in rats leads to higher stress levels, whereas mild exercise increases neurogenesis (Inoue et al. 2015). Moreover, heavy physical workload leads to cognitive function impairments in humans (Mekari et al. 2015). However, we showed that mice that ran either ran three or seven days a week reached the same levels of neurogenesis (Gregoire et al. 2014). This was also demonstrated in mice that ran 80% more, in average, on a running disc than their counterparts with access to a running wheel. Moreover, we did not detect any difference in corticosterone levels (Gregoire et al. 2014). This discrepancy could be due to the use of rats versus mice and treadmill versus voluntary running. However, we did observe important differences between Low and High Runners in our RNA-Seq that suggested that synaptic activity could negatively regulated in High Runners. Interestingly, in our RNA-Seq, enkaphilin, an endogenous ligand for opioid receptors expressed in the mesolimbic system, was only expressed in high runners (Garzon and Pickel 2002). It would be interesting to investigate further this question to discover if what is called the runner's high can lead to detrimental effects on cognitive function.

V.2.3.2. Exercise pill

To fully understand the mechanisms underlying the activation of NSCs in the DG niche, it implicates to carefully screen our transcriptomics results to test different possibilities and look at the functional relevance of each potential target. A current infatuation is to develop exercise pills to counter sedentary lifestyles and inactivity from people with disabilities or chronic diseases. Understanding how (the mechanism) exercise increases neurogenesis and improves learning and memory could complement the knowledge about the known molecular pathways in whole organ systems that are activated following exercise (Li and Laher 2015). For example, an exercise pill, called compound B6, was developed using the knowledge that PGC-1α is induced by exercise in the muscles (refer to discussion chapter III) to look at its downstream effect. However, there is still a lot of controversy around exercise pills as most studies are conducted in mice and the relevance in humans remains to be shown (Li and Laher 2015). On the other hand, exercise and cognitive stimulation can also be used as non pharmaceutical approaches in aged patients to prevent cognitive decline if we understand better their mechanism of action (Bherer 2015).

V.2.3.3. Proliferating ependymal cells versus NIC

Another avenue of research could be to investigate further the link between the proliferating ependymal cells and NIC that is currently unknown (Gregoire et al. 2015). Ependymal cells around the central canal proliferate more following injury (Barnabe-Heider et al. 2010). However, it is not clear if it is the proliferating population that acquires NSC properties to become NICs or if they are two distinct populations. A better understanding of the homogenous or heterogeneous ependymal cell population could help targeting a more specific population and learn more from its characteristics (and potential) for therapeutic purposes.

V.3. Conclusions

In summary, these studies allowed us to gain some insights into potential cellular and genetic processes that mediate NSC activation following two different types of stimulation: physiological (EE) and pathological (neuroinflammation). My thesis work demonstrated distinct cellular and molecular changes within the DG niche following exposure to different running intensities and social interactions that could lead to improved cognitive function. Moreover, TGF-β1 was identified as an important player in the acquisition of stem cell properties by spinal cord ependymal cells and other potential targets were identified.

VI. Bibliography

Abbas AK, Lichtman AH. 2006. Basic immunology - Functions and disorders of the immune system: Saunders Elsevier. 324 p.

Aberg MA, Aberg ND, Hedbacker H, Oscarsson J, Eriksson PS. 2000. Peripheral infusion of IGF-I selectively induces neurogenesis in the adult rat hippocampus. J Neurosci 20:2896-903.

Aberg MA, Pedersen NL, Toren K, Svartengren M, Backstrand B, Johnsson T, Cooper-Kuhn CM, Aberg ND, Nilsson M, Kuhn HG. 2009. Cardiovascular fitness is associated with cognition in young adulthood. Proc Natl Acad Sci U S A 106:20906-11.

Abrous DN, Koehl M, Le Moal M. 2005. Adult neurogenesis: from precursors to network and physiology. Physiol Rev 85:523-69.

Aimone JB. 2016. Computational Modeling of Adult Neurogenesis. Cold Spring Harb Perspect Biol 8.

Aimone JB, Deng W, Gage FH. 2011. Resolving new memories: a critical look at the dentate gyrus, adult neurogenesis, and pattern separation. Neuron 70:589-96.

Aimone JB, Li Y, Lee SW, Clemenson GD, Deng W, Gage FH. 2014. Regulation and function of adult neurogenesis: from genes to cognition. Physiol Rev 94:991-1026.

Aimone JB, Wiles J, Gage FH. 2009. Computational influence of adult neurogenesis on memory encoding. Neuron 61:187-202.

Akers KG, Martinez-Canabal A, Restivo L, Yiu AP, De Cristofaro A, Hsiang HL, Wheeler AL, Guskjolen A, Niibori Y, Shoji H and others. 2014. Hippocampal neurogenesis regulates forgetting during adulthood and infancy. Science 344:598-602.

Alfaro-Cervello C, Soriano-Navarro M, Mirzadeh Z, Alvarez-Buylla A, Garcia-Verdugo JM. 2012. Biciliated ependymal cell proliferation contributes to spinal cord growth. J Comp Neurol 520:3528-52.

Altman J, Das GD. 1965. Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. J Comp Neurol 124:319-35.

Alvarez-Buylla A, Garcia-Verdugo JM, Tramontin AD. 2001. A unified hypothesis on the lineage of neural stem cells. Nat Rev Neurosci 2:287-93.

Alvarez-Buylla A, Kirn JR. 1997. Birth, migration, incorporation, and death of vocal control neurons in adult songbirds. J Neurobiol 33:585-601.

Alvarez-Buylla A, Theelen M, Nottebohm F. 1988. Birth of projection neurons in the higher vocal center of the canary forebrain before, during, and after song learning. Proc Natl Acad Sci U S A 85:8722-6.

Alvarez-Buylla A, Theelen M, Nottebohm F. 1990. Proliferation "hot spots" in adult avian ventricular zone reveal radial cell division. Neuron 5:101-9.

Ambrogini P, Orsini L, Mancini C, Ferri P, Barbanti I, Cuppini R. 2002. Persistently high corticosterone levels but not normal circadian fluctuations of the hormone affect cell proliferation in the adult rat dentate gyrus. Neuroendocrinology 76:366-72.

Anderluh A, Klotzsch E, Ries J, Reismann AW, Weber S, Folser M, Koban F, Freissmuth M, Sitte HH, Schutz GJ. 2014. Tracking single serotonin transporter molecules at the endoplasmic reticulum and plasma membrane. Biophys J 106:L33-5.

Anders S, Pyl PT, Huber W. 2015. HTSeq--a Python framework to work with high-throughput sequencing data. Bioinformatics 31:166-9.

Anderson BJ, Rapp DN, Baek DH, McCloskey DP, Coburn-Litvak PS, Robinson JK. 2000. Exercise influences spatial learning in the radial arm maze. Physiol Behav 70:425-9.
Anderson P, Morris R, Amaral D, Bliss T, O'Keefe J. 2007. The Hippocampus Book: Oxford University Press. 832 p.
Antunes M, Biala G. 2012. The novel object recognition memory: neurobiology, test procedure, and its modifications. Cogn Process 13:93-110.
Arruda-Carvalho M, Sakaguchi M, Akers KG, Josselyn SA, Frankland PW. 2011. Posttraining ablation of adult-generated neurons degrades previously acquired memories. J Neurosci 31:15113-27.
Augustinack JC, van der Kouwe AJ, Salat DH, Benner T, Stevens AA, Annese J, Fischl B, Frosch MP, Corkin S. 2014. H.M.'s contributions to neuroscience: a review and autopsy studies. Hippocampus 24:1267-86.
Babu H, Cheung G, Kettenmann H, Palmer TD, Kempermann G. 2007. Enriched monolayer precursor cell cultures from micro-dissected adult mouse dentate gyrus yield functional granule cell-like neurons. PLoS One 2:e388.
Bakker A, Kirwan CB, Miller M, Stark CE. 2008. Pattern separation in the human hippocampal CA3 and dentate gyrus. Science 319:1640-2.
Bannai H, Niwa F, Sherwood MW, Shrivastava AN, Arizono M, Miyamoto A, Sugiura K, Levi S, Triller A, Mikoshiba K. 2015. Bidirectional Control of Synaptic GABAAR Clustering by Glutamate and Calcium. Cell Rep 13:2768-80.
Bannerman DM, Grubb M, Deacon RM, Yee BK, Feldon J, Rawlins JN. 2003. Ventral hippocampal lesions affect anxiety but not spatial learning. Behav Brain Res 139:197-213.
Barnabe-Heider F, Goritz C, Sabelstrom H, Takebayashi H, Pfrieger FW, Meletis K, Frisen J. 2010. Origin of new glial cells in intact and injured adult spinal cord. Cell Stem Cell 7:470-82.
Barnabe-Heider F, Meletis K, Eriksson M, Bergmann O, Sabelstrom H, Harvey MA, Mikkers H, Frisen J. 2008. Genetic manipulation of adult mouse neurogenic niches by in vivo electroporation. Nat Methods 5:189-96.
Barnea A, Nottebohm F. 1994. Seasonal recruitment of hippocampal neurons in adult free-ranging black-capped chickadees. Proc Natl Acad Sci U S A 91:11217-21.
Barnea A, Pravosudov V. 2011. Birds as a model to study adult neurogenesis: bridging evolutionary, comparative and neuroethological approaches. Eur J Neurosci 34:884-907.
Bartanusz V, Jezova D, Alajajian B, Digicaylioglu M. 2011. The blood-spinal cord barrier: morphology and clinical implications. Ann Neurol 70:194-206.
Bartsch U, Bandtlow CE, Schnell L, Bartsch S, Spillmann AA, Rubin BP, Hillenbrand R, Montag D, Schwab ME, Schachner M. 1995. Lack of evidence that myelin-associated glycoprotein is a major inhibitor of axonal regeneration in the CNS. Neuron 15:1375-81.
Bastien D, Lacroix S. 2014. Cytokine pathways regulating glial and leukocyte function after spinal cord and peripheral nerve injury. Exp Neurol 258:62-77.
Batchelor PE, Tan S, Wills TE, Porritt MJ, Howells DW. 2008. Comparison of inflammation in the brain and spinal cord following mechanical injury. J Neurotrauma 25:1217-25.
Bednarczyk MR, Aumont A, Decary S, Bergeron R, Fernandes KJ. 2009. Prolonged voluntary wheel-running stimulates neural precursors in the hippocampus and forebrain of adult CD1 mice. Hippocampus 19:913-27.

Bednarczyk MR, Hacker LC, Fortin-Nunez S, Aumont A, Bergeron R, Fernandes KJ. 2011. Distinct stages of adult hippocampal neurogenesis are regulated by running and the running environment. Hippocampus 21:1334-47.

Belarbi K, Rosi S. 2013. Modulation of adult-born neurons in the inflamed hippocampus. Front Cell Neurosci 7:145.

Ben-Ari Y. 2002. Excitatory actions of gaba during development: the nature of the nurture. Nat Rev Neurosci 3:728-39.

Benner EJ, Luciano D, Jo R, Abdi K, Paez-Gonzalez P, Sheng H, Warner DS, Liu C, Eroglu C, Kuo CT. 2013. Protective astrogenesis from the SVZ niche after injury is controlled by Notch modulator Thbs4. Nature 497:369-73.

Bennett EL, Rosenzweig MR, Diamond MC. 1969. Rat brain: effects of environmental enrichment on wet and dry weights. Science 163:825-6.

Bergmann O, Liebl J, Bernard S, Alkass K, Yeung MS, Steier P, Kutschera W, Johnson L, Landen M, Druid H and others. 2012. The age of olfactory bulb neurons in humans. Neuron 74:634-9.

Bherer L. 2015. Cognitive plasticity in older adults: effects of cognitive training and physical exercise. Ann N Y Acad Sci 1337:1-6.

Bishop KM, Rubenstein JL, O'Leary DD. 2002. Distinct actions of Emx1, Emx2, and Pax6 in regulating the specification of areas in the developing neocortex. J Neurosci 22:7627-38.

Boldrini M, Underwood MD, Hen R, Rosoklija GB, Dwork AJ, John Mann J, Arango V. 2009. Antidepressants increase neural progenitor cells in the human hippocampus. Neuropsychopharmacology 34:2376-89.

Bonaguidi MA, Peng CY, McGuire T, Falciglia G, Gobeske KT, Czeisler C, Kessler JA. 2008. Noggin expands neural stem cells in the adult hippocampus. J Neurosci 28:9194-204.

Bonaguidi MA, Song J, Ming GL, Song H. 2012. A unifying hypothesis on mammalian neural stem cell properties in the adult hippocampus. Curr Opin Neurobiol 22:754-61.

Bonaguidi MA, Wheeler MA, Shapiro JS, Stadel RP, Sun GJ, Ming GL, Song H. 2011. In vivo clonal analysis reveals self-renewing and multipotent adult neural stem cell characteristics. Cell 145:1142-55.

Bond AM, Ming GL, Song H. 2015. Adult Mammalian Neural Stem Cells and Neurogenesis: Five Decades Later. Cell Stem Cell 17:385-95.

Bond AM, Peng CY, Meyers EA, McGuire T, Ewaleifoh O, Kessler JA. 2014. BMP signaling regulates the tempo of adult hippocampal progenitor maturation at multiple stages of the lineage. Stem Cells 32:2201-14.

Bonfanti L. 2011. From hydra regeneration to human brain structural plasticity: a long trip through narrowing roads. ScientificWorldJournal 11:1270-99.

Bonfanti L, Peretto P. 2011. Adult neurogenesis in mammals--a theme with many variations. Eur J Neurosci 34:930-50.

Brandt MD, Jessberger S, Steiner B, Kronenberg G, Reuter K, Bick-Sander A, von der Behrens W, Kempermann G. 2003. Transient calretinin expression defines early postmitotic step of neuronal differentiation in adult hippocampal neurogenesis of mice. Mol Cell Neurosci 24:603-13.

Bregman BS, Kunkel-Bagden E, Schnell L, Dai HN, Gao D, Schwab ME. 1995. Recovery from spinal cord injury mediated by antibodies to neurite growth inhibitors. Nature 378:498-501.

Breton-Provencher V, Lemasson M, Peralta MR, 3rd, Saghatelyan A. 2009. Interneurons produced in adulthood are required for the normal functioning of the olfactory bulb network and for the execution of selected olfactory behaviors. J Neurosci 29:15245-57.
Buss A, Pech K, Kakulas BA, Martin D, Schoenen J, Noth J, Brook GA. 2008. TGF-beta1 and TGF-beta2 expression after traumatic human spinal cord injury. Spinal Cord 46:364-71.
Cacioppo JT, Hawkley LC, Norman GJ, Berntson GG. 2011. Social isolation. Ann N Y Acad Sci 1231:17-22.
Cafferty WB, Duffy P, Huebner E, Strittmatter SM. 2010. MAG and OMgp synergize with Nogo-A to restrict axonal growth and neurological recovery after spinal cord trauma. J Neurosci 30:6825-37.
Cameron HA, McKay RD. 1999. Restoring production of hippocampal neurons in old age. Nat Neurosci 2:894-7.
Cameron HA, McKay RD. 2001. Adult neurogenesis produces a large pool of new granule cells in the dentate gyrus. J Comp Neurol 435:406-17.
Cao L, Jiao X, Zuzga DS, Liu Y, Fong DM, Young D, During MJ. 2004. VEGF links hippocampal activity with neurogenesis, learning and memory. Nat Genet 36:827-35.
Chaker Z, George C, Petrovska M, Caron JB, Lacube P, Caille I, Holzenberger M. 2016. Hypothalamic neurogenesis persists in the aging brain and is controlled by energy-sensing IGF-I pathway. Neurobiol Aging 41:64-72.
Chiu CC, Liao YE, Yang LY, Wang JY, Tweedie D, Karnati HK, Greig NH, Wang JY. 2016. Neuroinflammation in animal models of traumatic brain injury. J Neurosci Methods.
Choe Y, Pleasure SJ, Mira H. 2016. Control of Adult Neurogenesis by Short-Range Morphogenic-Signaling Molecules. Cold Spring Harb Perspect Biol 8:a018887.
Choi SH, Li Y, Parada LF, Sisodia SS. 2009. Regulation of hippocampal progenitor cell survival, proliferation and dendritic development by BDNF. Mol Neurodegener 4:52.
Christian KM, Song H, Ming GL. 2014. Functions and dysfunctions of adult hippocampal neurogenesis. Annu Rev Neurosci 37:243-62.
Clark PJ, Bhattacharya TK, Miller DS, Kohman RA, DeYoung EK, Rhodes JS. 2012. New neurons generated from running are broadly recruited into neuronal activation associated with three different hippocampus-involved tasks. Hippocampus 22:1860-7.
Clark PJ, Bhattacharya TK, Miller DS, Rhodes JS. 2011a. Induction of c-Fos, Zif268, and Arc from acute bouts of voluntary wheel running in new and pre-existing adult mouse hippocampal granule neurons. Neuroscience 184:16-27.
Clark PJ, Brzezinska WJ, Puchalski EK, Krone DA, Rhodes JS. 2009. Functional analysis of neurovascular adaptations to exercise in the dentate gyrus of young adult mice associated with cognitive gain. Hippocampus 19:937-50.
Clark PJ, Kohman RA, Miller DS, Bhattacharya TK, Brzezinska WJ, Rhodes JS. 2011b. Genetic influences on exercise-induced adult hippocampal neurogenesis across 12 divergent mouse strains. Genes Brain Behav 10:345-53.
Clelland CD, Choi M, Romberg C, Clemenson GD, Jr., Fragniere A, Tyers P, Jessberger S, Saksida LM, Barker RA, Gage FH and others. 2009. A functional role for adult hippocampal neurogenesis in spatial pattern separation. Science 325:210-3.
Codega P, Silva-Vargas V, Paul A, Maldonado-Soto AR, Deleo AM, Pastrana E, Doetsch F. 2014. Prospective identification and purification of quiescent adult neural stem cells from their in vivo niche. Neuron 82:545-59.

Cohen SJ, Stackman RW, Jr. 2015. Assessing rodent hippocampal involvement in the novel object recognition task. A review. Behav Brain Res 285:105-17.
Cooper-Kuhn CM, Kuhn HG. 2002. Is it all DNA repair? Methodological considerations for detecting neurogenesis in the adult brain. Brain Res Dev Brain Res 134:13-21.
Covacu R, Brundin L. 2015. Effects of Neuroinflammation on Neural Stem Cells. Neuroscientist.
Creer DJ, Romberg C, Saksida LM, van Praag H, Bussey TJ. 2010. Running enhances spatial pattern separation in mice. Proc Natl Acad Sci U S A 107:2367-72.
Curtis MA, Kam M, Nannmark U, Anderson MF, Axell MZ, Wikkelso C, Holtas S, van Roon-Mom WM, Bjork-Eriksson T, Nordborg C and others. 2007. Human neuroblasts migrate to the olfactory bulb via a lateral ventricular extension. Science 315:1243-9.
Czeh B, Michaelis T, Watanabe T, Frahm J, de Biurrun G, van Kampen M, Bartolomucci A, Fuchs E. 2001. Stress-induced changes in cerebral metabolites, hippocampal volume, and cell proliferation are prevented by antidepressant treatment with tianeptine. Proc Natl Acad Sci U S A 98:12796-801.
Czeh B, Muller-Keuker JI, Rygula R, Abumaria N, Hiemke C, Domenici E, Fuchs E. 2007. Chronic social stress inhibits cell proliferation in the adult medial prefrontal cortex: hemispheric asymmetry and reversal by fluoxetine treatment. Neuropsychopharmacology 32:1490-503.
Czeh B, Welt T, Fischer AK, Erhardt A, Schmitt W, Muller MB, Toschi N, Fuchs E, Keck ME. 2002. Chronic psychosocial stress and concomitant repetitive transcranial magnetic stimulation: effects on stress hormone levels and adult hippocampal neurogenesis. Biol Psychiatry 52:1057-65.
David S, Kroner A. 2011. Repertoire of microglial and macrophage responses after spinal cord injury. Nat Rev Neurosci 12:388-99.
de Quervain DJ, Roozendaal B, Nitsch RM, McGaugh JL, Hock C. 2000. Acute cortisone administration impairs retrieval of long-term declarative memory in humans. Nat Neurosci 3:313-4.
DeCarolis NA, Mechanic M, Petrik D, Carlton A, Ables JL, Malhotra S, Bachoo R, Gotz M, Lagace DC, Eisch AJ. 2013. In vivo contribution of nestin- and GLAST-lineage cells to adult hippocampal neurogenesis. Hippocampus 23:708-19.
Deng W, Aimone JB, Gage FH. 2010. New neurons and new memories: how does adult hippocampal neurogenesis affect learning and memory? Nat Rev Neurosci 11:339-50.
Deng W, Mayford M, Gage FH. 2013. Selection of distinct populations of dentate granule cells in response to inputs as a mechanism for pattern separation in mice. Elife 2:e00312.
Deng W, Saxe MD, Gallina IS, Gage FH. 2009. Adult-born hippocampal dentate granule cells undergoing maturation modulate learning and memory in the brain. J Neurosci 29:13532-42.
Dhaliwal J, Lagace DC. 2011. Visualization and genetic manipulation of adult neurogenesis using transgenic mice. Eur J Neurosci 33:1025-36.
Dhaliwal J, Xi Y, Bruel-Jungerman E, Germain J, Francis F, Lagace DC. 2015. Doublecortin (DCX) is not Essential for Survival and Differentiation of Newborn Neurons in the Adult Mouse Dentate Gyrus. Front Neurosci 9:494.
Ding Q, Vaynman S, Souda P, Whitelegge JP, Gomez-Pinilla F. 2006. Exercise affects energy metabolism and neural plasticity-related proteins in the hippocampus as revealed by proteomic analysis. Eur J Neurosci 24:1265-76.

Doetsch F, Caille I, Lim DA, Garcia-Verdugo JM, Alvarez-Buylla A. 1999a. Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. Cell 97:703-16.
Doetsch F, Garcia-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.
Doetsch F, Petreanu L, Caille I, Garcia-Verdugo JM, Alvarez-Buylla A. 2002. EGF converts transit-amplifying neurogenic precursors in the adult brain into multipotent stem cells. Neuron 36:1021-34.
Donnelly DJ, Popovich PG. 2008. Inflammation and its role in neuroprotection, axonal regeneration and functional recovery after spinal cord injury. Exp Neurol 209:378-88.
Dranovsky A, Picchini AM, Moadel T, Sisti AC, Yamada A, Kimura S, Leonardo ED, Hen R. 2011. Experience dictates stem cell fate in the adult hippocampus. Neuron 70:908-23.
Dromard C, Guillon H, Rigau V, Ripoll C, Sabourin JC, Perrin FE, Scamps F, Bozza S, Sabatier P, Lonjon N and others. 2008. Adult human spinal cord harbors neural precursor cells that generate neurons and glial cells in vitro. J Neurosci Res 86:1916-26.
Eisch AJ, Petrik D. 2012. Depression and hippocampal neurogenesis: a road to remission? Science 338:72-5.
Ekdahl CT, Kokaia Z, Lindvall O. 2009. Brain inflammation and adult neurogenesis: the dual role of microglia. Neuroscience 158:1021-9.
Encinas JM, Michurina TV, Peunova N, Park JH, Tordo J, Peterson DA, Fishell G, Koulakov A, Enikolopov G. 2011. Division-coupled astrocytic differentiation and age-related depletion of neural stem cells in the adult hippocampus. Cell Stem Cell 8:566-79.
Epp JR, Silva Mera R, Kohler S, Josselyn SA, Frankland PW. 2016. Neurogenesis-mediated forgetting minimizes proactive interference. Nat Commun 7:10838.
Erickson KI, Voss MW, Prakash RS, Basak C, Szabo A, Chaddock L, Kim JS, Heo S, Alves H, White SM and others. 2011. Exercise training increases size of hippocampus and improves memory. Proc Natl Acad Sci U S A 108:3017-22.
Eriksson PS, Perfilieva E, Bjork-Eriksson T, Alborn AM, Nordborg C, Peterson DA, Gage FH. 1998. Neurogenesis in the adult human hippocampus. Nat Med 4:1313-7.
Ernst A, Alkass K, Bernard S, Salehpour M, Perl S, Tisdale J, Possnert G, Druid H, Frisen J. 2014. Neurogenesis in the striatum of the adult human brain. Cell 156:1072-83.
Ernst A, Frisen J. 2015. Adult neurogenesis in humans- common and unique traits in mammals. PLoS Biol 13:e1002045.
Fabel K, Fabel K, Tam B, Kaufer D, Baiker A, Simmons N, Kuo CJ, Palmer TD. 2003. VEGF is necessary for exercise-induced adult hippocampal neurogenesis. Eur J Neurosci 18:2803-12.
Fabel K, Kempermann G. 2008. Physical activity and the regulation of neurogenesis in the adult and aging brain. Neuromolecular Med 10:59-66.
Fabel K, Wolf SA, Ehninger D, Babu H, Leal-Galicia P, Kempermann G. 2009. Additive effects of physical exercise and environmental enrichment on adult hippocampal neurogenesis in mice. Front Neurosci 3:50.
Faiz M, Sachewsky N, Gascon S, Bang KW, Morshead CM, Nagy A. 2015. Adult Neural Stem Cells from the Subventricular Zone Give Rise to Reactive Astrocytes in the Cortex after Stroke. Cell Stem Cell 17:624-34.
Feng L, Allen NS, Simo S, Cooper JA. 2007. Cullin 5 regulates Dab1 protein levels and neuron positioning during cortical development. Genes Dev 21:2717-30.

Ferreira LM, Floriddia EM, Quadrato G, Di Giovanni S. 2012. Neural regeneration: lessons from regenerating and non-regenerating systems. Mol Neurobiol 46:227-41.
Fiala BA, Joyce JN, Greenough WT. 1978. Environmental complexity modulates growth of granule cell dendrites in developing but not adult hippocampus of rats. Exp Neurol 59:372-83.
Fitzsimons CP, Herbert J, Schouten M, Meijer OC, Lucassen PJ, Lightman S. 2016. Circadian and ultradian glucocorticoid rhythmicity: Implications for the effects of glucocorticoids on neural stem cells and adult hippocampal neurogenesis. Front Neuroendocrinol.
Frankland PW, Cestari V, Filipkowski RK, McDonald RJ, Silva AJ. 1998. The dorsal hippocampus is essential for context discrimination but not for contextual conditioning. Behav Neurosci 112:863-74.
Frisen J, Johansson CB, Torok C, Risling M, Lendahl U. 1995. Rapid, widespread, and longlasting induction of nestin contributes to the generation of glial scar tissue after CNS injury. J Cell Biol 131:453-64.
Fuentealba LC, Obernier K, Alvarez-Buylla A. 2012. Adult neural stem cells bridge their niche. Cell Stem Cell 10:698-708.
Fuentealba LC, Rompani SB, Parraguez JI, Obernier K, Romero R, Cepko CL, Alvarez-Buylla A. 2015. Embryonic Origin of Postnatal Neural Stem Cells. Cell 161:1644-55.
Gaete M, Munoz R, Sanchez N, Tampe R, Moreno M, Contreras EG, Lee-Liu D, Larrain J. 2012. Spinal cord regeneration in Xenopus tadpoles proceeds through activation of Sox2-positive cells. Neural Dev 7:13.
Gage FH. 2000. Mammalian neural stem cells. Science 287:1433-8.
Garcion E, Halilagic A, Faissner A, ffrench-Constant C. 2004. Generation of an environmental niche for neural stem cell development by the extracellular matrix molecule tenascin C. Development 131:3423-32.
Garner JP. 2014. The significance of meaning: why do over 90% of behavioral neuroscience results fail to translate to humans, and what can we do to fix it? ILAR J 55:438-56.
Garthe A, Roeder I, Kempermann G. 2016. Mice in an enriched environment learn more flexibly because of adult hippocampal neurogenesis. Hippocampus 26:261-71.
Garzon M, Pickel VM. 2002. Ultrastructural localization of enkephalin and mu-opioid receptors in the rat ventral tegmental area. Neuroscience 114:461-74.
Ge S, Goh EL, Sailor KA, Kitabatake Y, Ming GL, Song H. 2006. GABA regulates synaptic integration of newly generated neurons in the adult brain. Nature 439:589-93.
Gemma C, Bachstetter AD, Cole MJ, Fister M, Hudson C, Bickford PC. 2007. Blockade of caspase-1 increases neurogenesis in the aged hippocampus. Eur J Neurosci 26:2795-803.
Gilbert SF. 2006. Developmental Biology: Sinauer Associates Inc. 817 p.
Glasper ER, Schoenfeld TJ, Gould E. 2012. Adult neurogenesis: optimizing hippocampal function to suit the environment. Behav Brain Res 227:380-3.
Gobeske KT, Das S, Bonaguidi MA, Weiss C, Radulovic J, Disterhoft JF, Kessler JA. 2009. BMP signaling mediates effects of exercise on hippocampal neurogenesis and cognition in mice. PLoS One 4:e7506.
Goldman SA, Nottebohm F. 1983. Neuronal production, migration, and differentiation in a vocal control nucleus of the adult female canary brain. Proc Natl Acad Sci U S A 80:2390-4.
Goodman T, Hajihosseini MK. 2015. Hypothalamic tanycytes-masters and servants of metabolic, neuroendocrine, and neurogenic functions. Front Neurosci 9:387.

Gould E, Cameron HA, Daniels DC, Woolley CS, McEwen BS. 1992. Adrenal hormones suppress cell division in the adult rat dentate gyrus. J Neurosci 12:3642-50.
Gould E, McEwen BS, Tanapat P, Galea LA, Fuchs E. 1997. Neurogenesis in the dentate gyrus of the adult tree shrew is regulated by psychosocial stress and NMDA receptor activation. J Neurosci 17:2492-8.
Gould E, Tanapat P. 1999. Stress and hippocampal neurogenesis. Biol Psychiatry 46:1472-9.
Gould E, Tanapat P, McEwen BS, Flugge G, Fuchs E. 1998. Proliferation of granule cell precursors in the dentate gyrus of adult monkeys is diminished by stress. Proc Natl Acad Sci U S A 95:3168-71.
Green EJ, Greenough WT. 1986. Altered synaptic transmission in dentate gyrus of rats reared in complex environments: evidence from hippocampal slices maintained in vitro. J Neurophysiol 55:739-50.
Greenwood BN, Foley TE, Le TV, Strong PV, Loughridge AB, Day HE, Fleshner M. 2011. Long-term voluntary wheel running is rewarding and produces plasticity in the mesolimbic reward pathway. Behav Brain Res 217:354-62.
Gregoire CA, Bonenfant D, Le Nguyen A, Aumont A, Fernandes KJ. 2014. Untangling the influences of voluntary running, environmental complexity, social housing and stress on adult hippocampal neurogenesis. PLoS One 9:e86237.
Gregoire CA, Goldenstein BL, Floriddia EM, Barnabe-Heider F, Fernandes KJ. 2015. Endogenous neural stem cell responses to stroke and spinal cord injury. Glia 63:1469-82.
Grossman SD, Rosenberg LJ, Wrathall JR. 2001. Temporal-spatial pattern of acute neuronal and glial loss after spinal cord contusion. Exp Neurol 168:273-82.
Gu Y, Arruda-Carvalho M, Wang J, Janoschka SR, Josselyn SA, Frankland PW, Ge S. 2012. Optical controlling reveals time-dependent roles for adult-born dentate granule cells. Nat Neurosci 15:1700-6.
Hamilton LK, Dufresne M, Joppe SE, Petryszyn S, Aumont A, Calon F, Barnabe-Heider F, Furtos A, Parent M, Chaurand P and others. 2015. Aberrant Lipid Metabolism in the Forebrain Niche Suppresses Adult Neural Stem Cell Proliferation in an Animal Model of Alzheimer's Disease. Cell Stem Cell 17:397-411.
Hamilton LK, Truong MK, Bednarczyk MR, Aumont A, Fernandes KJ. 2009. Cellular organization of the central canal ependymal zone, a niche of latent neural stem cells in the adult mammalian spinal cord. Neuroscience 164:1044-56.
Hammond RS, Tull LE, Stackman RW. 2004. On the delay-dependent involvement of the hippocampus in object recognition memory. Neurobiol Learn Mem 82:26-34.
Harris JA. 1998. Using c-fos as a neural marker of pain. Brain Res Bull 45:1-8.
Hebb D. 1947. The effects of early experience on problem solving at maturity. American Psychologist 2:306-307.
Herrera DG, Robertson HA. 1996. Activation of c-fos in the brain. Prog Neurobiol 50:83-107.
Herting MM, Nagel BJ. 2012. Aerobic fitness relates to learning on a virtual Morris Water Task and hippocampal volume in adolescents. Behav Brain Res 233:517-25.
Ho NF, Han SP, Dawe GS. 2009. Effect of voluntary running on adult hippocampal neurogenesis in cholinergic lesioned mice. BMC Neurosci 10:57.
Hodge RD, Kowalczyk TD, Wolf SA, Encinas JM, Rippey C, Enikolopov G, Kempermann G, Hevner RF. 2008. Intermediate progenitors in adult hippocampal neurogenesis: Tbr2 expression and coordinate regulation of neuronal output. J Neurosci 28:3707-17.

Horky LL, Galimi F, Gage FH, Horner PJ. 2006. Fate of endogenous stem/progenitor cells following spinal cord injury. J Comp Neurol 498:525-38.

Horner PJ, Power AE, Kempermann G, Kuhn HG, Palmer TD, Winkler J, Thal LJ, Gage FH. 2000. Proliferation and differentiation of progenitor cells throughout the intact adult rat spinal cord. J Neurosci 20:2218-28.

Hubel DH, Wiesel TN. 1970. The period of susceptibility to the physiological effects of unilateral eye closure in kittens. J Physiol 206:419-36.

Hugnot JP, Franzen R. 2011. The spinal cord ependymal region: a stem cell niche in the caudal central nervous system. Front Biosci (Landmark Ed) 16:1044-59.

Hulsen T, de Vlieg J, Alkema W. 2008. BioVenn - a web application for the comparison and visualization of biological lists using area-proportional Venn diagrams. BMC Genomics 9:488.

Ickes BR, Pham TM, Sanders LA, Albeck DS, Mohammed AH, Granholm AC. 2000. Long-term environmental enrichment leads to regional increases in neurotrophin levels in rat brain. Exp Neurol 164:45-52.

Ihrie RA, Alvarez-Buylla A. 2011. Lake-front property: a unique germinal niche by the lateral ventricles of the adult brain. Neuron 70:674-86.

Inoue K, Okamoto M, Shibato J, Lee MC, Matsui T, Rakwal R, Soya H. 2015. Long-Term Mild, rather than Intense, Exercise Enhances Adult Hippocampal Neurogenesis and Greatly Changes the Transcriptomic Profile of the Hippocampus. PLoS One 10:e0128720.

Iosif RE, Ekdahl CT, Ahlenius H, Pronk CJ, Bonde S, Kokaia Z, Jacobsen SE, Lindvall O. 2006. Tumor necrosis factor receptor 1 is a negative regulator of progenitor proliferation in adult hippocampal neurogenesis. J Neurosci 26:9703-12.

Jacobs BL, van Praag H, Gage FH. 2000. Adult brain neurogenesis and psychiatry: a novel theory of depression. Mol Psychiatry 5:262-9.

Jessberger S, Kempermann G. 2003. Adult-born hippocampal neurons mature into activity-dependent responsiveness. Eur J Neurosci 18:2707-12.

Jin K, Zhu Y, Sun Y, Mao XO, Xie L, Greenberg DA. 2002. Vascular endothelial growth factor (VEGF) stimulates neurogenesis in vitro and in vivo. Proc Natl Acad Sci U S A 99:11946-50.

Johansson CB, Momma S, Clarke DL, Risling M, Lendahl U, Frisen J. 1999. Identification of a neural stem cell in the adult mammalian central nervous system. Cell 96:25-34.

Johnston ST, Shtrahman M, Parylak S, Goncalves JT, Gage FH. 2016. Paradox of pattern separation and adult neurogenesis: A dual role for new neurons balancing memory resolution and robustness. Neurobiol Learn Mem 129:60-8.

Jones TB, McDaniel EE, Popovich PG. 2005. Inflammatory-mediated injury and repair in the traumatically injured spinal cord. Curr Pharm Des 11:1223-36.

Joppé SE, Hamilton LK, Cochard LM, Levros L-C, Aumont A, Barnabé-Heider F, Fernandes K. 2015. Bone morphogenetic protein dominantly suppresses epidermal growth factor-induced proliferative expansion of adult forebrain neural precursors. Frontiers in Neuroscience 9.

Jullienne A, Badaut J. 2013. Molecular contributions to neurovascular unit dysfunctions after brain injuries: lessons for target-specific drug development. Future Neurol 8:677-689.

Kandasamy M, Lehner B, Kraus S, Sander PR, Marschallinger J, Rivera FJ, Trumbach D, Ueberham U, Reitsamer HA, Strauss O and others. 2014. TGF-beta signalling in the

adult neurogenic niche promotes stem cell quiescence as well as generation of new neurons. J Cell Mol Med 18:1444-59.
Kandel ER, Schwartz JH, Jessell TM. 2000. Principles of neural science: McGraw-Hill Companies Inc. . 1414 p.
Kannangara TS, Webber A, Gil-Mohapel J, Christie BR. 2009. Stress differentially regulates the effects of voluntary exercise on cell proliferation in the dentate gyrus of mice. Hippocampus 19:889-97.
Kaplan MS, Hinds JW. 1977. Neurogenesis in the adult rat: electron microscopic analysis of light radioautographs. Science 197:1092-4.
Kempermann G. 2002. Regulation of adult hippocampal neurogenesis - implications for novel theories of major depression. Bipolar Disord 4:17-33.
Kempermann G. 2011. Adult neurogenesis 2: Oxford University Press, Inc. 587 p.
Kempermann G, Fabel K, Ehninger D, Babu H, Leal-Galicia P, Garthe A, Wolf SA. 2010. Why and how physical activity promotes experience-induced brain plasticity. Front Neurosci 4:189.
Kempermann G, Gage FH. 2002. Genetic influence on phenotypic differentiation in adult hippocampal neurogenesis. Brain Res Dev Brain Res 134:1-12.
Kempermann G, Kuhn HG, Gage FH. 1997a. Genetic influence on neurogenesis in the dentate gyrus of adult mice. Proc Natl Acad Sci U S A 94:10409-14.
Kempermann G, Kuhn HG, Gage FH. 1997b. More hippocampal neurons in adult mice living in an enriched environment. Nature 386:493-5.
Kempermann G, Kuhn HG, Gage FH. 1998. Experience-induced neurogenesis in the senescent dentate gyrus. J Neurosci 18:3206-12.
Kempermann G, Song H, Gage FH. 2015a. Neurogenesis in the Adult Hippocampus. Cold Spring Harb Perspect Med 5:a018812.
Kempermann G, Song H, Gage FH. 2015b. Neurogenesis in the Adult Hippocampus. Cold Spring Harb Perspect Biol 7:a018812.
Kempermann G, Wiskott L, Gage FH. 2004. Functional significance of adult neurogenesis. Curr Opin Neurobiol 14:186-91.
Kheirbek MA, Drew LJ, Burghardt NS, Costantini DO, Tannenholz L, Ahmari SE, Zeng H, Fenton AA, Hen R. 2013. Differential control of learning and anxiety along the dorsoventral axis of the dentate gyrus. Neuron 77:955-68.
Kim JS, Jung J, Lee HJ, Kim JC, Wang H, Kim SH, Shin T, Moon C. 2009. Differences in immunoreactivities of Ki-67 and doublecortin in the adult hippocampus in three strains of mice. Acta Histochem 111:150-6.
Kirkham M, Hameed LS, Berg DA, Wang H, Simon A. 2014. Progenitor cell dynamics in the Newt Telencephalon during homeostasis and neuronal regeneration. Stem Cell Reports 2:507-19.
Kirn J, O'Loughlin B, Kasparian S, Nottebohm F. 1994. Cell death and neuronal recruitment in the high vocal center of adult male canaries are temporally related to changes in song. Proc Natl Acad Sci U S A 91:7844-8.
Kitamura T, Saitoh Y, Takashima N, Murayama A, Niibori Y, Ageta H, Sekiguchi M, Sugiyama H, Inokuchi K. 2009. Adult neurogenesis modulates the hippocampus-dependent period of associative fear memory. Cell 139:814-27.

Kleiter I, Pedre X, Mueller AM, Poeschl P, Couillard-Despres S, Spruss T, Bogdahn U, Giegerich G, Steinbrecher A. 2007. Inhibition of Smad7, a negative regulator of TGF-beta signaling, suppresses autoimmune encephalomyelitis. J Neuroimmunol 187:61-73.

Kobilo T, Liu QR, Gandhi K, Mughal M, Shaham Y, van Praag H. 2011. Running is the neurogenic and neurotrophic stimulus in environmental enrichment. Learn Mem 18:605-9.

Kohta M, Kohmura E, Yamashita T. 2009. Inhibition of TGF-beta1 promotes functional recovery after spinal cord injury. Neurosci Res 65:393-401.

Kokoeva MV, Yin H, Flier JS. 2005. Neurogenesis in the hypothalamus of adult mice: potential role in energy balance. Science 310:679-83.

Kokoeva MV, Yin H, Flier JS. 2007. Evidence for constitutive neural cell proliferation in the adult murine hypothalamus. J Comp Neurol 505:209-20.

Komitova M, Zhao LR, Gido G, Johansson BB, Eriksson P. 2005. Postischemic exercise attenuates whereas enriched environment has certain enhancing effects on lesion-induced subventricular zone activation in the adult rat. Eur J Neurosci 21:2397-405.

Kozorovitskiy Y, Gould E. 2004. Dominance hierarchy influences adult neurogenesis in the dentate gyrus. J Neurosci 24:6755-9.

Kramer AF, Hahn S, Cohen NJ, Banich MT, McAuley E, Harrison CR, Chason J, Vakil E, Bardell L, Boileau RA and others. 1999. Ageing, fitness and neurocognitive function. Nature 400:418-9.

Kriegstein A, Alvarez-Buylla A. 2009. The glial nature of embryonic and adult neural stem cells. Annu Rev Neurosci 32:149-84.

Krityakiarana W, Espinosa-Jeffrey A, Ghiani CA, Zhao PM, Topaldjikian N, Gomez-Pinilla F, Yamaguchi M, Kotchabhakdi N, de Vellis J. 2010. Voluntary exercise increases oligodendrogenesis in spinal cord. Int J Neurosci 120:280-90.

Kronenberg G, Bick-Sander A, Bunk E, Wolf C, Ehninger D, Kempermann G. 2006. Physical exercise prevents age-related decline in precursor cell activity in the mouse dentate gyrus. Neurobiol Aging 27:1505-13.

Kronenberg G, Reuter K, Steiner B, Brandt MD, Jessberger S, Yamaguchi M, Kempermann G. 2003. Subpopulations of proliferating cells of the adult hippocampus respond differently to physiologic neurogenic stimuli. J Comp Neurol 467:455-63.

Kuan CY, Schloemer AJ, Lu A, Burns KA, Weng WL, Williams MT, Strauss KI, Vorhees CV, Flavell RA, Davis RJ and others. 2004. Hypoxia-ischemia induces DNA synthesis without cell proliferation in dying neurons in adult rodent brain. J Neurosci 24:10763-72.

Kuhn HG, Dickinson-Anson H, Gage FH. 1996. Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. J Neurosci 16:2027-33.

La Torre JC. 1968. Effect of differential environmental enrichment on brain weight and on acetylcholinesterase and cholinesterase activities in mice. Exp Neurol 22:493-503.

Lacroix S, Hamilton LK, Vaugeois A, Beaudoin S, Breault-Dugas C, Pineau I, Levesque SA, Gregoire CA, Fernandes KJ. 2014. Central canal ependymal cells proliferate extensively in response to traumatic spinal cord injury but not demyelinating lesions. PLoS One 9:e85916.

Lagace DC, Donovan MH, DeCarolis NA, Farnbauch LA, Malhotra S, Berton O, Nestler EJ, Krishnan V, Eisch AJ. 2010. Adult hippocampal neurogenesis is functionally important for stress-induced social avoidance. Proc Natl Acad Sci U S A 107:4436-41.

Lagace DC, Whitman MC, Noonan MA, Ables JL, DeCarolis NA, Arguello AA, Donovan MH, Fischer SJ, Farnbauch LA, Beech RD and others. 2007. Dynamic contribution of nestin-expressing stem cells to adult neurogenesis. J Neurosci 27:12623-9.

Langdon KD, Corbett D. 2012. Improved working memory following novel combinations of physical and cognitive activity. Neurorehabil Neural Repair 26:523-32.

Lazarini F, Lledo PM. 2011. Is adult neurogenesis essential for olfaction? Trends Neurosci 34:20-30.

Leasure JL, Decker L. 2009. Social isolation prevents exercise-induced proliferation of hippocampal progenitor cells in female rats. Hippocampus 19:907-12.

Lee-Liu D, Edwards-Faret G, Tapia VS, Larrain J. 2013. Spinal cord regeneration: lessons for mammals from non-mammalian vertebrates. Genesis 51:529-44.

Lee DA, Bedont JL, Pak T, Wang H, Song J, Miranda-Angulo A, Takiar V, Charubhumi V, Balordi F, Takebayashi H and others. 2012. Tanycytes of the hypothalamic median eminence form a diet-responsive neurogenic niche. Nat Neurosci 15:700-2.

Leggio MG, Mandolesi L, Federico F, Spirito F, Ricci B, Gelfo F, Petrosini L. 2005. Environmental enrichment promotes improved spatial abilities and enhanced dendritic growth in the rat. Behav Brain Res 163:78-90.

Leranth C, Hajszan T. 2007. Extrinsic afferent systems to the dentate gyrus. Prog Brain Res 163:63-84.

Leuner B, Glasper ER, Gould E. 2010a. Parenting and plasticity. Trends Neurosci 33:465-73.

Leuner B, Glasper ER, Gould E. 2010b. Sexual experience promotes adult neurogenesis in the hippocampus despite an initial elevation in stress hormones. PLoS One 5:e11597.

Leuner B, Gould E, Shors TJ. 2006. Is there a link between adult neurogenesis and learning? Hippocampus 16:216-24.

Leuner B, Mendolia-Loffredo S, Kozorovitskiy Y, Samburg D, Gould E, Shors TJ. 2004. Learning enhances the survival of new neurons beyond the time when the hippocampus is required for memory. J Neurosci 24:7477-81.

Leuner B, Mirescu C, Noiman L, Gould E. 2007. Maternal experience inhibits the production of immature neurons in the hippocampus during the postpartum period through elevations in adrenal steroids. Hippocampus 17:434-42.

Li G, Fang L, Fernandez G, Pleasure SJ. 2013. The ventral hippocampus is the embryonic origin for adult neural stem cells in the dentate gyrus. Neuron 78:658-72.

Li J, Tang Y, Cai D. 2012. IKKbeta/NF-kappaB disrupts adult hypothalamic neural stem cells to mediate a neurodegenerative mechanism of dietary obesity and pre-diabetes. Nat Cell Biol 14:999-1012.

Li L, Clevers H. 2010. Coexistence of quiescent and active adult stem cells in mammals. Science 327:542-5.

Li S, Laher I. 2015. Exercise Pills: At the Starting Line. Trends Pharmacol Sci 36:906-17.

Liauw J, Hoang S, Choi M, Eroglu C, Choi M, Sun GH, Percy M, Wildman-Tobriner B, Bliss T, Guzman RG and others. 2008. Thrombospondins 1 and 2 are necessary for synaptic plasticity and functional recovery after stroke. J Cereb Blood Flow Metab 28:1722-32.

Lieberwirth C, Wang Z. 2012. The social environment and neurogenesis in the adult Mammalian brain. Front Hum Neurosci 6:118.

Lillien L, Raphael H. 2000. BMP and FGF regulate the development of EGF-responsive neural progenitor cells. Development 127:4993-5005.

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, Garcia-Verdugo JM, Alvarez-Buylla A. 2000. Noggin antagonizes BMP signaling to create a niche for adult neurogenesis. Neuron 28:713-26.
Lindsey BW, Tropepe V. 2006. A comparative framework for understanding the biological principles of adult neurogenesis. Prog Neurobiol 80:281-307.
Little JP, Safdar A, Cermak N, Tarnopolsky MA, Gibala MJ. 2010. Acute endurance exercise increases the nuclear abundance of PGC-1alpha in trained human skeletal muscle. Am J Physiol Regul Integr Comp Physiol 298:R912-7.
Liu N, He S, Yu X. 2012. Early natural stimulation through environmental enrichment accelerates neuronal development in the mouse dentate gyrus. PLoS One 7:e30803.
Love MI, Huber W, Anders S. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 15:550.
Lucassen PJ, Naninck EF, van Goudoever JB, Fitzsimons C, Joels M, Korosi A. 2013. Perinatal programming of adult hippocampal structure and function; emerging roles of stress, nutrition and epigenetics. Trends Neurosci 36:621-31.
Luo J, Shook BA, Daniels SB, Conover JC. 2008. Subventricular zone-mediated ependyma repair in the adult mammalian brain. J Neurosci 28:3804-13.
Mak GK, Weiss S. 2010. Paternal recognition of adult offspring mediated by newly generated CNS neurons. Nat Neurosci 13:753-8.
Mak IW, Evaniew N, Ghert M. 2014. Lost in translation: animal models and clinical trials in cancer treatment. Am J Transl Res 6:114-8.
Malberg JE, Eisch AJ, Nestler EJ, Duman RS. 2000. Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. J Neurosci 20:9104-10.
Manosevitz M, Joel U. 1973. Behavioral effects of environmental enrichment in randomly bred mice. J Comp Physiol Psychol 85:373-82.
Markakis EA, Palmer TD, Randolph-Moore L, Rakic P, Gage FH. 2004. Novel neuronal phenotypes from neural progenitor cells. J Neurosci 24:2886-97.
Martens DJ, Seaberg RM, van der Kooy D. 2002. In vivo infusions of exogenous growth factors into the fourth ventricle of the adult mouse brain increase the proliferation of neural progenitors around the fourth ventricle and the central canal of the spinal cord. Eur J Neurosci 16:1045-57.
Mathes WF, Nehrenberg DL, Gordon R, Hua K, Garland T, Jr., Pomp D. 2010. Dopaminergic dysregulation in mice selectively bred for excessive exercise or obesity. Behav Brain Res 210:155-63.
Mathews EA, Morgenstern NA, Piatti VC, Zhao C, Jessberger S, Schinder AF, Gage FH. 2010. A distinctive layering pattern of mouse dentate granule cells is generated by developmental and adult neurogenesis. J Comp Neurol 518:4479-90.
McAvoy K, Besnard A, Sahay A. 2015. Adult hippocampal neurogenesis and pattern separation in DG: a role for feedback inhibition in modulating sparseness to govern population-based coding. Front Syst Neurosci 9:120.
McCartney-Francis NL, Wahl SM. 1994. Transforming growth factor beta: a matter of life and death. J Leukoc Biol 55:401-9.

McCormick CM, Thomas CM, Sheridan CS, Nixon F, Flynn JA, Mathews IZ. 2012. Social instability stress in adolescent male rats alters hippocampal neurogenesis and produces deficits in spatial location memory in adulthood. Hippocampus 22:1300-12.

McHugh TJ, Jones MW, Quinn JJ, Balthasar N, Coppari R, Elmquist JK, Lowell BB, Fanselow MS, Wilson MA, Tonegawa S. 2007. Dentate gyrus NMDA receptors mediate rapid pattern separation in the hippocampal network. Science 317:94-9.

McTigue DM, Popovich PG, Morgan TE, Stokes BT. 2000. Localization of transforming growth factor-beta1 and receptor mRNA after experimental spinal cord injury. Exp Neurol 163:220-30.

Mekari S, Fraser S, Bosquet L, Bonnery C, Labelle V, Pouliot P, Lesage F, Bherer L. 2015. The relationship between exercise intensity, cerebral oxygenation and cognitive performance in young adults. Eur J Appl Physiol 115:2189-97.

Meletis K, Barnabe-Heider F, Carlen M, Evergren E, Tomilin N, Shupliakov O, Frisen J. 2008. Spinal cord injury reveals multilineage differentiation of ependymal cells. PLoS Biol 6:e182.

Menn B, Garcia-Verdugo JM, Yaschine C, Gonzalez-Perez O, Rowitch D, Alvarez-Buylla A. 2006. Origin of oligodendrocytes in the subventricular zone of the adult brain. J Neurosci 26:7907-18.

Mich JK, Signer RA, Nakada D, Pineda A, Burgess RJ, Vue TY, Johnson JE, Morrison SJ. 2014. Prospective identification of functionally distinct stem cells and neurosphere-initiating cells in adult mouse forebrain. Elife 3:e02669.

Ming GL, Song H. 2005. Adult neurogenesis in the mammalian central nervous system. Annu Rev Neurosci 28:223-50.

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.

Mitra R, Sundlass K, Parker KJ, Schatzberg AF, Lyons DM. 2006. Social stress-related behavior affects hippocampal cell proliferation in mice. Physiol Behav 89:123-7.

Monje ML, Mizumatsu S, Fike JR, Palmer TD. 2002. Irradiation induces neural precursor-cell dysfunction. Nat Med 8:955-62.

Monje ML, Toda H, Palmer TD. 2003. Inflammatory blockade restores adult hippocampal neurogenesis. Science 302:1760-5.

Moreno MM, Linster C, Escanilla O, Sacquet J, Didier A, Mandairon N. 2009. Olfactory perceptual learning requires adult neurogenesis. Proc Natl Acad Sci U S A 106:17980-5.

Morris RG, Garrud P, Rawlins JN, O'Keefe J. 1982. Place navigation impaired in rats with hippocampal lesions. Nature 297:681-3.

Mothe AJ, Zahir T, Santaguida C, Cook D, Tator CH. 2011. Neural stem/progenitor cells from the adult human spinal cord are multipotent and self-renewing and differentiate after transplantation. PLoS One 6:e27079.

Mu Y, Lee SW, Gage FH. 2010. Signaling in adult neurogenesis. Curr Opin Neurobiol 20:416-23.

Mustroph ML, Chen S, Desai SC, Cay EB, DeYoung EK, Rhodes JS. 2012a. Aerobic exercise is the critical variable in an enriched environment that increases hippocampal neurogenesis and water maze learning in male C57BL/6J mice. Neuroscience 219:62-71.

Mustroph ML, Chen S, Desai SC, Cay EB, Deyoung EK, Rhodes JS. 2012b. Aerobic exercise is the critical variable in an enriched environment that increases hippocampal neurogenesis and water maze learning in male C57BL/6J mice. Neuroscience.

Nakashiba T, Cushman JD, Pelkey KA, Renaudineau S, Buhl DL, McHugh TJ, Rodriguez Barrera V, Chittajallu R, Iwamoto KS, McBain CJ and others. 2012. Young dentate granule cells mediate pattern separation, whereas old granule cells facilitate pattern completion. Cell 149:188-201.

Nithianantharajah J, Hannan AJ. 2006. Enriched environments, experience-dependent plasticity and disorders of the nervous system. Nat Rev Neurosci 7:697-709.

Olson AK, Eadie BD, Ernst C, Christie BR. 2006. Environmental enrichment and voluntary exercise massively increase neurogenesis in the adult hippocampus via dissociable pathways. Hippocampus 16:250-60.

Ozsolak F, Milos PM. 2011. RNA sequencing: advances, challenges and opportunities. Nat Rev Genet 12:87-98.

Palmer TD, Takahashi J, Gage FH. 1997. The adult rat hippocampus contains primordial neural stem cells. Mol Cell Neurosci 8:389-404.

Pang TY, Hannan AJ. 2013. Enhancement of cognitive function in models of brain disease through environmental enrichment and physical activity. Neuropharmacology 64:515-28.

Park JH, Enikolopov G. 2010. Transient elevation of adult hippocampal neurogenesis after dopamine depletion. Exp Neurol 222:267-76.

Park SM, Jung JS, Jang MS, Kang KS, Kang SK. 2008. Transforming growth factor-beta1 regulates the fate of cultured spinal cord-derived neural progenitor cells. Cell Prolif 41:248-64.

Pierce AA, Xu AW. 2010. De novo neurogenesis in adult hypothalamus as a compensatory mechanism to regulate energy balance. J Neurosci 30:723-30.

Pineau I, Lacroix S. 2007. Proinflammatory cytokine synthesis in the injured mouse spinal cord: multiphasic expression pattern and identification of the cell types involved. J Comp Neurol 500:267-85.

Pinkel D, Segraves R, Sudar D, Clark S, Poole I, Kowbel D, Collins C, Kuo WL, Chen C, Zhai Y and others. 1998. High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. Nat Genet 20:207-11.

Popovich PG, Wei P, Stokes BT. 1997. Cellular inflammatory response after spinal cord injury in Sprague-Dawley and Lewis rats. J Comp Neurol 377:443-64.

Proulx MJ, Brown DJ, Pasqualotto A, Meijer P. 2014. Multisensory perceptual learning and sensory substitution. Neurosci Biobehav Rev 41:16-25.

Rabchevsky AG, Weinitz JM, Coulpier M, Fages C, Tinel M, Junier MP. 1998. A role for transforming growth factor alpha as an inducer of astrogliosis. J Neurosci 18:10541-52.

Ramón y Cajal S. 1928. Degeneration and Regeneration of the Nervous System: London: Oxford University Press.

Reeve RL, Yammine SZ, DeVeale B, van der Kooy D. 2016. Targeted activation of primitive neural stem cells in the mouse brain. Eur J Neurosci 43:1474-85.

Reger ML, Hovda DA, Giza CC. 2009. Ontogeny of Rat Recognition Memory measured by the novel object recognition task. Dev Psychobiol 51:672-8.

Reynolds BA, Weiss S. 1992. Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. Science 255:1707-10.

Rhodes JS, Garland T, Jr., Gammie SC. 2003. Patterns of brain activity associated with variation in voluntary wheel-running behavior. Behav Neurosci 117:1243-56.
Riquelme PA, Drapeau E, Doetsch F. 2008. Brain micro-ecologies: neural stem cell niches in the adult mammalian brain. Philos Trans R Soc Lond B Biol Sci 363:123-37.
Risedal A, Mattsson B, Dahlqvist P, Nordborg C, Olsson T, Johansson BB. 2002. Environmental influences on functional outcome after a cortical infarct in the rat. Brain Res Bull 58:315-21.
Robins SC, Stewart I, McNay DE, Taylor V, Giachino C, Goetz M, Ninkovic J, Briancon N, Maratos-Flier E, Flier JS and others. 2013. alpha-Tanycytes of the adult hypothalamic third ventricle include distinct populations of FGF-responsive neural progenitors. Nat Commun 4:2049.
Rodriguez-Jimenez FJ, Alastrue-Agudo A, Stojkovic M, Erceg S, Moreno-Manzano V. 2015. Connexin 50 Expression in Ependymal Stem Progenitor Cells after Spinal Cord Injury Activation. Int J Mol Sci 16:26608-18.
Rodriguez EM, Blazquez JL, Pastor FE, Pelaez B, Pena P, Peruzzo B, Amat P. 2005. Hypothalamic tanycytes: a key component of brain-endocrine interaction. Int Rev Cytol 247:89-164.
Rolls A, Shechter R, Schwartz M. 2009. The bright side of the glial scar in CNS repair. Nat Rev Neurosci 10:235-41.
Roozendaal B, Okuda S, de Quervain DJ, McGaugh JL. 2006. Glucocorticoids interact with emotion-induced noradrenergic activation in influencing different memory functions. Neuroscience 138:901-10.
Rosenzweig MR, Krech D, Bennett EL, Diamond MC. 1962. Effects of environmental complexity and training on brain chemistry and anatomy: a replication and extension. J Comp Physiol Psychol 55:429-37.
Rossi C, Angelucci A, Costantin L, Braschi C, Mazzantini M, Babbini F, Fabbri ME, Tessarollo L, Maffei L, Berardi N and others. 2006. Brain-derived neurotrophic factor (BDNF) is required for the enhancement of hippocampal neurogenesis following environmental enrichment. Eur J Neurosci 24:1850-6.
Sachewsky N, Leeder R, Xu W, Rose KL, Yu F, van der Kooy D, Morshead CM. 2014. Primitive neural stem cells in the adult mammalian brain give rise to GFAP-expressing neural stem cells. Stem Cell Reports 2:810-24.
Sagar SM, Sharp FR, Curran T. 1988. Expression of c-fos protein in brain: metabolic mapping at the cellular level. Science 240:1328-31.
Sahay A, Hen R. 2007. Adult hippocampal neurogenesis in depression. Nat Neurosci 10:1110-5.
Sahay A, Scobie KN, Hill AS, O'Carroll CM, Kheirbek MA, Burghardt NS, Fenton AA, Dranovsky A, Hen R. 2011a. Increasing adult hippocampal neurogenesis is sufficient to improve pattern separation. Nature 472:466-70.
Sahay A, Wilson DA, Hen R. 2011b. Pattern separation: a common function for new neurons in hippocampus and olfactory bulb. Neuron 70:582-8.
Sakamoto M, Kageyama R, Imayoshi I. 2014. The functional significance of newly born neurons integrated into olfactory bulb circuits. Front Neurosci 8:121.
Sanes DH, Reh TA, Hams WA. 2012. Development of the nervous system: Elsevier. 340 p.
Sanger F, Nicklen S, Coulson AR. 1977. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci U S A 74:5463-7.

Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, Weisstaub N, Lee J, Duman R, Arancio O and others. 2003. Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science 301:805-9.

Saxe MD, Battaglia F, Wang JW, Malleret G, David DJ, Monckton JE, Garcia AD, Sofroniew MV, Kandel ER, Santarelli L and others. 2006. Ablation of hippocampal neurogenesis impairs contextual fear conditioning and synaptic plasticity in the dentate gyrus. Proc Natl Acad Sci U S A 103:17501-6.

Schloesser RJ, Manji HK, Martinowich K. 2009. Suppression of adult neurogenesis leads to an increased hypothalamo-pituitary-adrenal axis response. Neuroreport 20:553-7.

Schnell L, Fearn S, Klassen H, Schwab ME, Perry VH. 1999. Acute inflammatory responses to mechanical lesions in the CNS: differences between brain and spinal cord. Eur J Neurosci 11:3648-58.

Schoenfeld TJ, Gould E. 2012. Stress, stress hormones, and adult neurogenesis. Exp Neurol 233:12-21.

Scholzen T, Gerdes J. 2000. The Ki-67 protein: from the known and the unknown. J Cell Physiol 182:311-22.

Schwabe L, Wolf OT. 2012. Stress modulates the engagement of multiple memory systems in classification learning. J Neurosci 32:11042-9.

Scoville WB, Milner B. 1957. Loss of recent memory after bilateral hippocampal lesions. J Neurol Neurosurg Psychiatry 20:11-21.

Seidenfaden R, Desoeuvre A, Bosio A, Virard I, Cremer H. 2006. Glial conversion of SVZ-derived committed neuronal precursors after ectopic grafting into the adult brain. Mol Cell Neurosci 32:187-98.

Seki T. 2002. Expression patterns of immature neuronal markers PSA-NCAM, CRMP-4 and NeuroD in the hippocampus of young adult and aged rodents. J Neurosci Res 70:327-34.

Seri B, Garcia-Verdugo JM, Collado-Morente L, McEwen BS, Alvarez-Buylla A. 2004. Cell types, lineage, and architecture of the germinal zone in the adult dentate gyrus. J Comp Neurol 478:359-78.

Seri B, Garcia-Verdugo JM, McEwen BS, Alvarez-Buylla A. 2001. Astrocytes give rise to new neurons in the adult mammalian hippocampus. J Neurosci 21:7153-60.

Sheline YI, Wang PW, Gado MH, Csernansky JG, Vannier MW. 1996. Hippocampal atrophy in recurrent major depression. Proc Natl Acad Sci U S A 93:3908-13.

Shihabuddin LS, Horner PJ, Ray J, Gage FH. 2000. Adult spinal cord stem cells generate neurons after transplantation in the adult dentate gyrus. J Neurosci 20:8727-35.

Sierra A, Encinas JM, Deudero JJ, Chancey JH, Enikolopov G, Overstreet-Wadiche LS, Tsirka SE, Maletic-Savatic M. 2010. Microglia shape adult hippocampal neurogenesis through apoptosis-coupled phagocytosis. Cell Stem Cell 7:483-95.

Silva CF, Duarte FS, Lima TC, de Oliveira CL. 2011. Effects of social isolation and enriched environment on behavior of adult Swiss mice do not require hippocampal neurogenesis. Behav Brain Res 225:85-90.

Silver J, Miller JH. 2004. Regeneration beyond the glial scar. Nat Rev Neurosci 5:146-56.

Simon M, Czeh B, Fuchs E. 2005. Age-dependent susceptibility of adult hippocampal cell proliferation to chronic psychosocial stress. Brain Res 1049:244-8.

Snyder JS, Hong NS, McDonald RJ, Wojtowicz JM. 2005. A role for adult neurogenesis in spatial long-term memory. Neuroscience 130:843-52.

Snyder JS, Radik R, Wojtowicz JM, Cameron HA. 2009. Anatomical gradients of adult neurogenesis and activity: young neurons in the ventral dentate gyrus are activated by water maze training. Hippocampus 19:360-70.
Snyder JS, Soumier A, Brewer M, Pickel J, Cameron HA. 2011. Adult hippocampal neurogenesis buffers stress responses and depressive behaviour. Nature 476:458-61.
Song J, Olsen RH, Sun J, Ming GL, Song H. 2016. Neuronal Circuitry Mechanisms Regulating Adult Mammalian Neurogenesis. Cold Spring Harb Perspect Biol.
Song J, Sun J, Moss J, Wen Z, Sun GJ, Hsu D, Zhong C, Davoudi H, Christian KM, Toni N and others. 2013. Parvalbumin interneurons mediate neuronal circuitry-neurogenesis coupling in the adult hippocampus. Nat Neurosci 16:1728-30.
Song J, Zhong C, Bonaguidi MA, Sun GJ, Hsu D, Gu Y, Meletis K, Huang ZJ, Ge S, Enikolopov G and others. 2012. Neuronal circuitry mechanism regulating adult quiescent neural stem-cell fate decision. Nature 489:150-4.
Soya H, Mukai A, Deocaris CC, Ohiwa N, Chang H, Nishijima T, Fujikawa T, Togashi K, Saito T. 2007. Threshold-like pattern of neuronal activation in the hypothalamus during treadmill running: establishment of a minimum running stress (MRS) rat model. Neurosci Res 58:341-8.
Spalding KL, Bergmann O, Alkass K, Bernard S, Salehpour M, Huttner HB, Bostrom E, Westerlund I, Vial C, Buchholz BA and others. 2013. Dynamics of hippocampal neurogenesis in adult humans. Cell 153:1219-27.
Spalding KL, Bhardwaj RD, Buchholz BA, Druid H, Frisen J. 2005. Retrospective birth dating of cells in humans. Cell 122:133-43.
Stranahan AM, Khalil D, Gould E. 2006. Social isolation delays the positive effects of running on adult neurogenesis. Nat Neurosci 9:526-33.
Suh H, Consiglio A, Ray J, Sawai T, D'Amour KA, Gage FH. 2007. In vivo fate analysis reveals the multipotent and self-renewal capacities of Sox2+ neural stem cells in the adult hippocampus. Cell Stem Cell 1:515-28.
Suh H, Deng W, Gage FH. 2009. Signaling in adult neurogenesis. Annu Rev Cell Dev Biol 25:253-75.
Svenson KL, Gatti DM, Valdar W, Welsh CE, Cheng R, Chesler EJ, Palmer AA, McMillan L, Churchill GA. 2012. High-resolution genetic mapping using the Mouse Diversity outbred population. Genetics 190:437-47.
Swan AA, Clutton JE, Chary PK, Cook SG, Liu GG, Drew MR. 2014. Characterization of the role of adult neurogenesis in touch-screen discrimination learning. Hippocampus 24:1581-91.
Tanaka EM, Ferretti P. 2009. Considering the evolution of regeneration in the central nervous system. Nat Rev Neurosci 10:713-23.
Tannenholz L, Jimenez JC, Kheirbek MA. 2014. Local and regional heterogeneity underlying hippocampal modulation of cognition and mood. Front Behav Neurosci 8:147.
Tashiro A, Makino H, Gage FH. 2007. Experience-specific functional modification of the dentate gyrus through adult neurogenesis: a critical period during an immature stage. J Neurosci 27:3252-9.
Taupin P. 2007. BrdU immunohistochemistry for studying adult neurogenesis: paradigms, pitfalls, limitations, and validation. Brain Res Rev 53:198-214.
Taylor AW. 2009. Review of the activation of TGF-beta in immunity. J Leukoc Biol 85:29-33.

Thomas RM, Urban JH, Peterson DA. 2006. Acute exposure to predator odor elicits a robust increase in corticosterone and a decrease in activity without altering proliferation in the adult rat hippocampus. Exp Neurol 201:308-15.
Tian W, Sawyer A, Kocaoglu FB, Kyriakides TR. 2011. Astrocyte-derived thrombospondin-2 is critical for the repair of the blood-brain barrier. Am J Pathol 179:860-8.
Todkar K, Scotti AL, Schwaller B. 2012. Absence of the calcium-binding protein calretinin, not of calbindin D-28k, causes a permanent impairment of murine adult hippocampal neurogenesis. Front Mol Neurosci 5:56.
Trapnell C, Pachter L, Salzberg SL. 2009. TopHat: discovering splice junctions with RNA-Seq. Bioinformatics 25:1105-11.
Treves A, Rolls ET. 1992. Computational constraints suggest the need for two distinct input systems to the hippocampal CA3 network. Hippocampus 2:189-99.
Trivino-Paredes J, Patten AR, Gil-Mohapel J, Christie BR. 2016. The effects of hormones and physical exercise on hippocampal structural plasticity. Front Neuroendocrinol.
Tyor WR, Avgeropoulos N, Ohlandt G, Hogan EL. 2002. Treatment of spinal cord impact injury in the rat with transforming growth factor-beta. J Neurol Sci 200:33-41.
Valley MT, Mullen TR, Schultz LC, Sagdullaev BT, Firestein S. 2009. Ablation of mouse adult neurogenesis alters olfactory bulb structure and olfactory fear conditioning. Front Neurosci 3:51.
Vallieres L, Campbell IL, Gage FH, Sawchenko PE. 2002. Reduced hippocampal neurogenesis in adult transgenic mice with chronic astrocytic production of interleukin-6. J Neurosci 22:486-92.
Van Bokhoven P, Oomen CA, Hoogendijk WJ, Smit AB, Lucassen PJ, Spijker S. 2011. Reduction in hippocampal neurogenesis after social defeat is long-lasting and responsive to late antidepressant treatment. Eur J Neurosci 33:1833-40.
Van Eekelen JA, Jiang W, De Kloet ER, Bohn MC. 1988. Distribution of the mineralocorticoid and the glucocorticoid receptor mRNAs in the rat hippocampus. J Neurosci Res 21:88-94.
van Praag H, Christie BR, Sejnowski TJ, Gage FH. 1999a. Running enhances neurogenesis, learning, and long-term potentiation in mice. Proc Natl Acad Sci U S A 96:13427-31.
van Praag H, Fleshner M, Schwartz MW, Mattson MP. 2014. Exercise, energy intake, glucose homeostasis, and the brain. J Neurosci 34:15139-49.
van Praag H, Kempermann G, Gage FH. 1999b. Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. Nat Neurosci 2:266-70.
van Praag H, Kempermann G, Gage FH. 2000. Neural consequences of environmental enrichment. Nat Rev Neurosci 1:191-8.
van Praag H, Schinder AF, Christie BR, Toni N, Palmer TD, Gage FH. 2002. Functional neurogenesis in the adult hippocampus. Nature 415:1030-4.
Villeda SA, Luo J, Mosher KI, Zou B, Britschgi M, Bieri G, Stan TM, Fainberg N, Ding Z, Eggel A and others. 2011. The ageing systemic milieu negatively regulates neurogenesis and cognitive function. Nature 477:90-4.
Villeda SA, Plambeck KE, Middeldorp J, Castellano JM, Mosher KI, Luo J, Smith LK, Bieri G, Lin K, Berdnik D and others. 2014. Young blood reverses age-related impairments in cognitive function and synaptic plasticity in mice. Nat Med 20:659-63.
Voigt T. 1989. Development of glial cells in the cerebral wall of ferrets: direct tracing of their transformation from radial glia into astrocytes. J Comp Neurol 289:74-88.

Volkmar FR, Greenough WT. 1972. Rearing complexity affects branching of dendrites in the visual cortex of the rat. Science 176:1445-7.
Vukovic J, Borlikova GG, Ruitenberg MJ, Robinson GJ, Sullivan RK, Walker TL, Bartlett PF. 2013. Immature doublecortin-positive hippocampal neurons are important for learning but not for remembering. J Neurosci 33:6603-13.
Wachs FP, Winner B, Couillard-Despres S, Schiller T, Aigner R, Winkler J, Bogdahn U, Aigner L. 2006. Transforming growth factor-beta1 is a negative modulator of adult neurogenesis. J Neuropathol Exp Neurol 65:358-70.
Wagner JP, Black IB, DiCicco-Bloom E. 1999. Stimulation of neonatal and adult brain neurogenesis by subcutaneous injection of basic fibroblast growth factor. J Neurosci 19:6006-16.
Walder S, Zhang F, Ferretti P. 2003. Up-regulation of neural stem cell markers suggests the occurrence of dedifferentiation in regenerating spinal cord. Dev Genes Evol 213:625-30.
Wang Z, Gerstein M, Snyder M. 2009. RNA-Seq: a revolutionary tool for transcriptomics. Nat Rev Genet 10:57-63.
Weiss S, Dunne C, Hewson J, Wohl C, Wheatley M, Peterson AC, Reynolds BA. 1996. Multipotent CNS stem cells are present in the adult mammalian spinal cord and ventricular neuroaxis. J Neurosci 16:7599-609.
Weisz VI, Argibay PF. 2012. Neurogenesis interferes with the retrieval of remote memories: forgetting in neurocomputational terms. Cognition 125:13-25.
Welch BL, Brown DG, Welch AS, Lin DC. 1974. Isolation, restrictive confinement or crowding of rats for one year. I. Weight, nucleic acids and protein of brain regions. Brain Res 75:71-84.
Wiesel TN, Hubel DH. 1965. Extent of recovery from the effects of visual deprivation in kittens. J Neurophysiol 28:1060-72.
Wilhelm BT, Landry JR. 2009. RNA-Seq-quantitative measurement of expression through massively parallel RNA-sequencing. Methods 48:249-57.
Wilson DA, Sullivan RM. 1995. The D2 antagonist spiperone mimics the effects of olfactory deprivation on mitral/tufted cell odor response patterns. J Neurosci 15:5574-81.
Woollett K, Maguire EA. 2011. Acquiring "the Knowledge" of London's layout drives structural brain changes. Curr Biol 21:2109-14.
Wosiski-Kuhn M, Stranahan AM. 2012. Opposing effects of positive and negative stress on hippocampal plasticity over the lifespan. Ageing Res Rev 11:399-403.
Yasuda M, Johnson-Venkatesh EM, Zhang H, Parent JM, Sutton MA, Umemori H. 2011. Multiple forms of activity-dependent competition refine hippocampal circuits in vivo. Neuron 70:1128-42.
Yu K, McGlynn S, Matise MP. 2013. Floor plate-derived sonic hedgehog regulates glial and ependymal cell fates in the developing spinal cord. Development 140:1594-604.
Zhang J, Ji F, Liu Y, Lei X, Li H, Ji G, Yuan Z, Jiao J. 2014. Ezh2 regulates adult hippocampal neurogenesis and memory. J Neurosci 34:5184-99.
Zhang RL, Zhang ZG, Chopp M. 2008. Ischemic stroke and neurogenesis in the subventricular zone. Neuropharmacology 55:345-52.
Zhao C, Deng W, Gage FH. 2008. Mechanisms and functional implications of adult neurogenesis. Cell 132:645-60.

VII. Appendix: Endogenous neural stem cell responses to stroke and spinal cord injury (review)

Endogenous Neural Stem Cell Responses to Stroke and Spinal Cord Injury

Catherine-Alexandra Grégoire,[1,2,3] Brianna L. Goldenstein,[1,2,4] Elisa M. Floriddia,[5] Fanie Barnabé-Heider,[5] and Karl J. L. Fernandes[1,2,4]

Stroke and spinal cord injury (SCI) are among the most frequent causes of central nervous system (CNS) dysfunction, affecting millions of people worldwide each year. The personal and financial costs for affected individuals, their families, and the broader communities are enormous. Although the mammalian CNS exhibits little spontaneous regeneration and self-repair, recent discoveries have revealed that subpopulations of glial cells in the adult forebrain subventricular zone and the spinal cord ependymal zone possess neural stem cell properties. These endogenous neural stem cells react to stroke and SCI by contributing a significant number of new neural cells to formation of the glial scar. These findings have raised hopes that new therapeutic strategies can be designed based on appropriate modulation of endogenous neural stem cell responses to CNS injury. Here, we review the responses of forebrain and spinal cord neural stem cells to stroke and SCI, the role of these responses in restricting injury-induced tissue loss, and the possibility of directing these responses to promote anatomical and functional repair of the CNS.

GLIA 2015;63:1469–1482

Key words: endogenous stem cells, stroke, spinal cord injury, ependymal cells

Introduction

Spinal cord injury (SCI) and stroke are debilitating, costly, and common pathological conditions affecting the central nervous system (CNS). Stroke affects 15 million people worldwide annually (World Health Organization, 2002); of these, 5 million will die and another 5 million will be permanently disabled. SCI, whose principal causes are motor vehicles accidents, falls, violence, sports, and work-related injuries, is a problem that is particularly frequent in developed countries. In Western Europe, the incidence of SCI is between 280 and 316 new cases per million people each year (Lee et al., 2014), while in the United States of America, approximately 273,000 people (about 80% of which are male) live with a SCI (The National Spinal Cord Injury Statistical Center, 2013). SCI typically occurs during the most active years in people's lives, and results in impairment of locomotor and sexual functions, neuropathic pain, spasticity, and incontinence (Westgren and Levi, 1998). Financial costs are likewise staggering: in 2013, the average costs within the first year of injury ranged from $340,000 to over $1 million per patient, depending on age and anatomical level of the injury (The National Spinal Cord Injury Statistical Center, 2013).

The relatively recent discovery of primitive neural precursors, i.e., neural stem cells (NSCs), within the adult CNS has raised hopes for improving the limited recovery that occurs following stroke and SCI. In this article, we review key advances made in uncovering the responses of endogenous neural stem cells to CNS injury, their role in the tissue repair process, and the potential for recruiting these cells to regenerate injured brain and spinal cord tissue.

Distinct Responses of Vertebrate Neural Stem Cells to CNS Injury

The regenerative ability of the CNS following lesions differs markedly across the vertebrate subphylum, providing

View this article online at wileyonlinelibrary.com. DOI: 10.1002/glia.22851

Published online April 29, 2015 in Wiley Online Library (wileyonlinelibrary.com). Received Dec 23, 2014, Accepted for publication Apr 13, 2015.

From the [1]Research Center of the University of Montreal Hospital (CRCHUM), Quebec, Canada; [2]CNS Research Group (GRSNC), University of Montreal, Quebec, Canada; [3]Department of Pathology and Cell Biology, Faculty of Medicine, Université De Montréal, Quebec, Canada; [4]Department of Neurosciences, Faculty of Medicine, Université De Montréal, Quebec, Canada; [5]Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden

fascinating insights into potential mechanisms of CNS regeneration (Bonfanti and Peretto, 2011; Ferreira et al., 2012).

Regenerating Vertebrates

In the three oldest vertebrate classes (fish, amphibians, and reptiles), many species exhibit continuous cellular production and growth throughout their lives ("indeterminate growth"; Diaz Quiroz and Echeverri, 2013; Lee-Liu et al., 2013; Tanaka and Ferretti, 2009). In such continuously growing species, embryonic NSCs (now recognized to be the embryonic radial glial cells) maintain a high basal activity even after maturity is reached, adding new neurons and glial cells continuously during adulthood. It is perhaps not surprising, then, that such species are capable of impressive feats of adult CNS regeneration. Notably, however, the oldest vertebrate classes also consist of species (such as the zebrafish, Danio rerio) that eventually achieve a mature size, but that nevertheless retain the capacity for scarless CNS regeneration. Moreover, even within regenerating species, hotspots of endogenous proliferation and neurogenesis do not necessarily correlate with regions of enhanced regenerative potential; rather, tissue injury seems to allow the reactivation of quiescent "ependymoglial" cells that subsequently re-establish zones of regenerative proliferation and neurogenesis (Kirkham et al., 2014). In urodele amphibians, for instance, the ependymoglial cells lining the spinal cord central canal maintain processes that contact the pial membrane, similar to radial glial cells. After spinal cord injury, these ependymoglial cells transiently lose their glial fibrillary protein (GFAP) expression, express NSC markers such as Nestin, and participate in the repair of the neuroepithelial tube that will eventually rebuild the spinal cord (Walder et al., 2003).

Transplantation and genetic studies reveal a central role for NSCs during spinal regeneration. Transplantation studies using green fluorescent protein-expressing transgenic axolotl have shown that, following tail amputation, clonally derived spinal cord neurospheres can regenerate both the spinal cord and its associated peripheral ganglia (McHedlishvili et al., 2012). Studies in Xenopus tadpoles (Gaete et al., 2012), adult zebrafish (Goldshmit et al., 2012), and axolotl (Fei et al., 2014) have also provided genetic evidence of the role of neural precursors in spinal regeneration. Gaete and colleagues observed a significant increase in $Sox2^{+}$ cells (undifferentiated neural precursors) 4 days after amputation of tadpole tails, where 20–60% of these cells also incorporated 5-bromo-2-deoxyuridine (BrdU, a synthetic nucleotide that is incorporated into dividing cells). Importantly, a dominant negative construct that inhibits Sox2 translocation to the nucleus prevented about half of the tadpoles from regenerating their tails (Gaete et al., 2012). Similarly, a clustered regularly interspaced short palindromic repeats (CRISPR)-mediated knockout of Sox2 in axolotl prevented NSC proliferation and regeneration of the amputated spinal cord, while regeneration of surrounding mesodermal tissues was unaffected (Fei et al., 2014). Fibroblast growth factor (FGF) is a known mitogenic factor for NSCs and plays an important role in coordinating spinal repair in adult zebrafish, as inhibition of FGF signaling prevents formation of a glial bridge between proximal and distal stumps of the transected spinal cord (Goldshmit et al., 2012). Collectively, these studies reveal that NSCs in regenerating fish, amphibians, and reptiles are either maintained postdevelopment and/or rapidly reactivated from more quiescent cellular phenotypes, and these NSCs are capable of driving the anatomical and functional regeneration of the spinal cord.

Mammals

Mammals (as well as birds) exhibit *determinate* growth: following embryogenesis, they begin undergoing a generalized decline in tissue growth and stem cell activity that continues into adulthood and old age. Mammals are capable of at least partial CNS regeneration when injury occurs during developmental periods, but exhibit regenerative failure following adult CNS lesions. However, remarkable studies conducted over the past 20 years have established that adult mammals (including humans) do retain discrete and regionally distinct populations of cells with NSC potential (Bonfanti and Peretto, 2011). During the early 1990s, Weiss and colleagues reported the isolation of NSCs from the adult rodent CNS (Reynolds and Weiss, 1992). These cells were isolated and expanded *in vitro* as free-floating, clonally derived colonies termed neurospheres, and revealed that a small subpopulation of cells intrinsic to the adult mammalian CNS display the cardinal stem cell characteristics of extensive self-renewal and multipotency (Reynolds and Weiss, 1992). Specifically, undifferentiated neurospheres can be serially passaged over many generations, with single cells retaining the ability to spontaneously differentiate into neurons and glial cells upon mitogen withdrawal. Subsequent studies went on to demonstrate that neurospheres could be grown from periventricular areas along the entire rostrocaudal extent of the rodent neuraxis (Weiss et al., 1996).

Unexpectedly, the neurosphere-initiating cells from the brain and spinal cord have been found to correspond to different types of glial cells. Neurospheres in the forebrain originate from GFAP-expressing astrocytes (Doetsch et al., 1999). These astrocytes are developmentally derived from radial glia and have retracted their pial processes, persisting as subependymal astrocytes within the subventricular zone (SVZ; Kriegstein and Alvarez-Buylla, 2009; Voigt, 1989). *In vivo*, SVZ astrocytes are neurogenic, continually generating neuroblasts that, in most adult mammals, migrate long-distances before

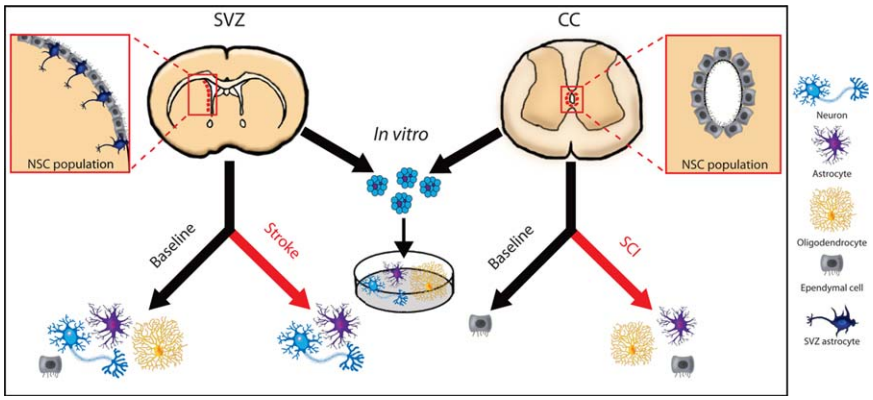


FIGURE 1: Comparison of the *in vitro* and *in vivo* multipotency of SVZ astrocytes and central canal ependymal cells. Neural stem cells in the adult forebrain are GFAP + SVZ astrocytes. Under normal baseline conditions, SVZ astrocytes give rise to olfactory bulb neurons, white matter oligodendrocytes, SVZ astrocytes, and SVZ ependymal cells (black arrow, baseline). However, after injury such as stroke (red arrow), they produce neuroblasts and astrocytes that migrate toward the site of tissue damage (see also Fig. 2). In the spinal cord, FoxJ1+ ependymal cells that line the central canal have latent neural stem cell potential. Under normal baseline conditions, central canal ependymal cells undergo symmetric self-renewing divisions that maintain the ependymal cell population (black arrow, baseline). Following SCI (red arrow), ependymal cells expand their pool and exhibit multipotency, producing new ependymal cells as well as astrocytes and oligodendrocytes that are found at the lesion site (see also Fig. 3). Interestingly, despite these differences in their *in vivo* phenotype, baseline activity, and injury-induced responses, both SVZ astrocytes and central canal ependymal cells can be cultured as multipotent and self-renewing neurospheres. These neurospheres differentiate into neurons, astrocytes, and oligodendrocytes *in vitro*. SVZ: subventricular zone; NSC: neural stem cell; GFAP: glial fibrillary acidic protein; SCI: spinal cord injury; CC: central canal.

differentiating into fully functional interneurons within the olfactory bulbs (Gheusi et al., 2013). They also produce glial cells, including oligodendrocytes that integrate into the adjacent white matter tracts (Gonzalez-Perez et al., 2009; Menn et al., 2006; but see Ortega et al., 2013) and ependymal cells that incorporate into the ventricular walls (Luo et al., 2008).

In the spinal cord, neurospheres grow solely from the central canal area and originate from FoxJ1-expressing ependymal cells (Barnabé-Heider et al., 2010; Martens et al., 2002; Meletis et al., 2008; Sabelström et al., 2013b). Unlike SVZ astrocytes, central canal ependymal cells are non-neurogenic *in vivo*, exhibiting a low level of constitutive proliferation that serves to maintain the ependymal cell population (Barnabé-Heider et al., 2010; Hamilton et al., 2009; Horky et al., 2006). Surprisingly, despite the distinct phenotype and endogenous behavior of SVZ astrocytes and spinal cord ependymal cells, these two glial cell types can both give rise to clonally derived neurospheres that are capable of self-renewing and of multipotency (ability to generate neurons, astrocytes, and oligodendrocytes *in vitro*). This suggests that different pools of glial cells are capable of expressing NSC properties under the appropriate conditions (Fig. 1).

The anatomy of the SVZ and central canal in humans differs somewhat from those of rodents. In the rodent SVZ, astrocytic stem cells are integrated in ependymal pinwheels within the lateral ventricle walls (Mirzadeh et al., 2008), while in humans, the ependymal layer is separated from a dense subependymal astrocytic "ribbon" by an acellular gap (Barbaro et al., 2004; Quiñones-Hinojosa et al., 2006). In the spinal cord, the human central canal is often occluded and the ependymal layer disorganized, frequently showing rosettes or microcanals (Hugnot and Franzen, 2011). Despite these anatomical variations, it remains highly relevant to study neural stem cell responses in rodent models, as multipotent neurospheres can also be expanded from the human brain (Barbaro et al., 2004) and spinal cord (Dromard et al., 2008; Mothe et al., 2011).

Responses of SVZ Neural Stem Cells to Brain Ischemia

Occluded arteries in the brain (stroke) or heart (heart attack) cause focal or global ischemia of the brain, respectively. Ischemia results in rapid and irreversible necrotic death of neural cells at the core of the deprived area and, in the case of focal ischemia, a surrounding penumbra where homeostasis may be partially restored. Over the past 15 years, there has been considerable interest in the possibility of stimulating endogenous SVZ neural stem cells to repair the irreversible cell loss caused by ischemic brain insults.

Experimental Models of Ischemic Brain Injury

The brain's large size compared with the spinal cord means that ischemic insults are often distant from the SVZ niche. Consequently, the cellular events following stroke are somewhat more variable than those occurring after SCI. Many different types of stroke models have been used to mimic ischemic insults in the brain, and four of these models are commonly used for investigating NSC responses. Middle

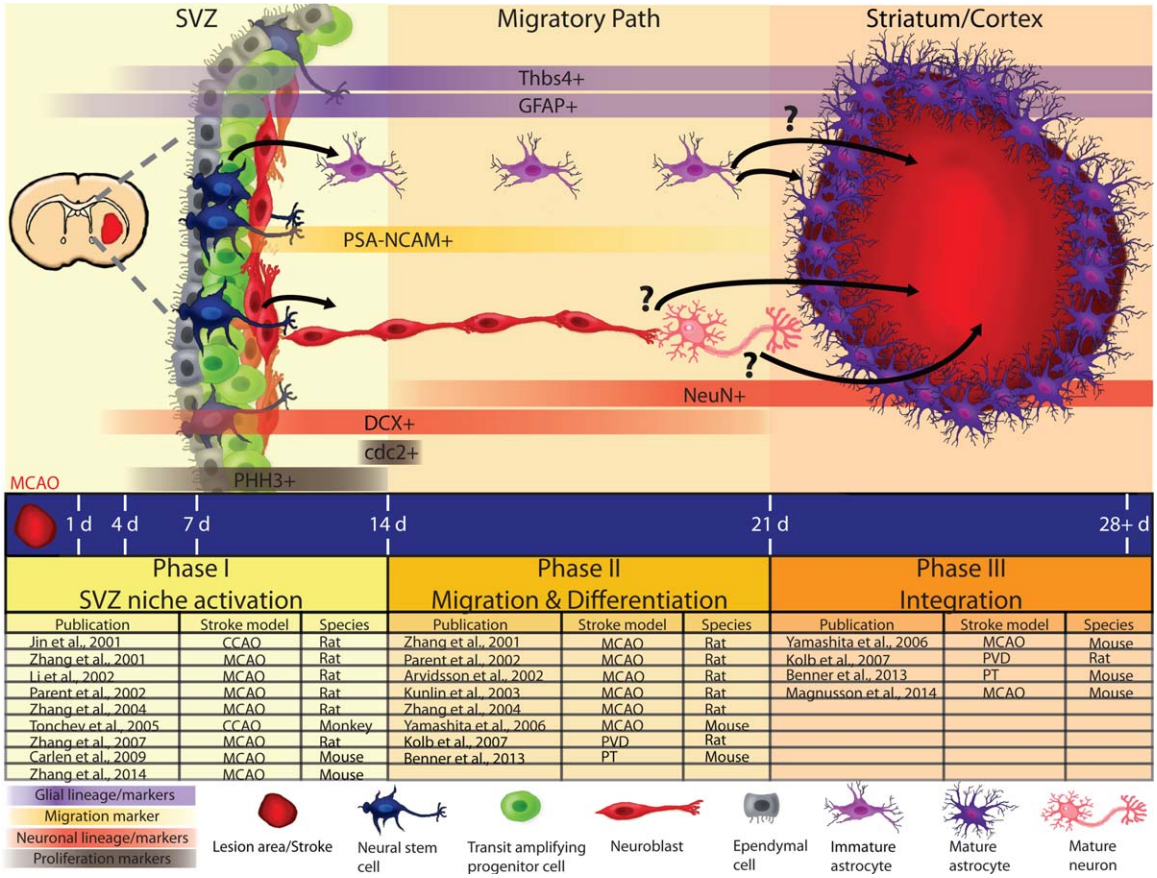


FIGURE 2: SVZ neural stem cell responses to stroke. The responses of SVZ neural stem cells to ischemic stroke injury in the brain (represented by the red lesion area) can be separated into three distinct phases: (1) SVZ niche activation (neural stem cells produce new cells to attend to the injury), (2) migration and differentiation (new cells travel to and develop into lineage-specific cell types), and (3) integration (new cells incorporate anatomically into the lesion site tissue). Post-MCAO, increases in proliferation markers (shown in gray) appear in the SVZ at 4, 7, and 14 days (PHH3) and peak at Day 14 for the G2/M cell cycle regulator, cdc2. Migrating neural cells (PSA-NCAM, shown in orange) from the SVZ expressing neuronal lineage markers (shown in red) for neuroblasts (DCX) appear as early as 3 days after stroke, with peak expression at 14–18 days post-MCAO. Neuroblasts differentiate into mature neurons (NeuN) around Day 14 post-MCAO. Whether or not SVZ-derived neurons integrate functionally at the injury site remains unclear (question mark). Astrocyte lineage markers (shown in purple) appear in SVZ neural stem cells inherently (GFAP) and in newly born SVZ astrocytes (Thbs4) as early as 3 days post-MCAO. Both markers are continually expressed as newborn astrocytes migrate and differentiate into mature astrocytes. Once arriving at the ischemic lesion site, SVZ-derived astrocytes integrate into the developing glial scar. It has not been established whether SVZ-derived astrocytes differentially contribute to the scar boundary and core, as described for ependymal-derived astrocytes following SCI. Publication information, stroke model, and species used are highlighted below each phase of the SVZ neural stem cell response to stroke (note that most publications are informative to multiple phases of neural stem cell responses, but are cited only once). CCAO: common carotid artery occlusion; MCAO: middle cerebral artery occlusion; PVD: pial vessel disruption; PT: photothrombosis; PHH3: phosphohistone H3; cdc2: cell division cycle protein-2, also known as cyclin-dependent kinase-1; DCX: doublecortin; PSA-NCAM: polysialylated-neural cell adhesion molecule; NeuN: neuronal nuclei; GFAP: glial fibrillary acidic protein; Thbs4: thrombospondin-4. SVZ: subventricular zone; d: day.

cerebral artery occlusion (MCAO; Longa et al., 1989) causes a striatal infarct that can extend to the cortex if occlusion time is long enough and most closely resembles human stroke. Common carotid artery occlusion (CCAO; Tone et al., 1987), also known as transient global ischemia, deprives the entire brain of its blood flow and is surgically simpler but a less accurate method for reproducing human stroke. Devascularization by pial vessel disruption (PVD; Sofroniew et al., 1983) causes local cortical ischemic damage but can be used to target specific motor or sensory areas and is an excellent model for testing functional recovery. Photothrombosis (Wester et al., 1995), like PVD, causes only focal cortical damage but uses an argon-ion laser and a photosensitive dye that enable highly accurate stroke placement without the need for a craniotomy.

Neural Stem Cell Responses to Stroke

Injuries to the CNS, including both stroke and SCI, result in the formation of a glial scar. The glial scar seals the lesion site during the weeks following injury and thereby limits

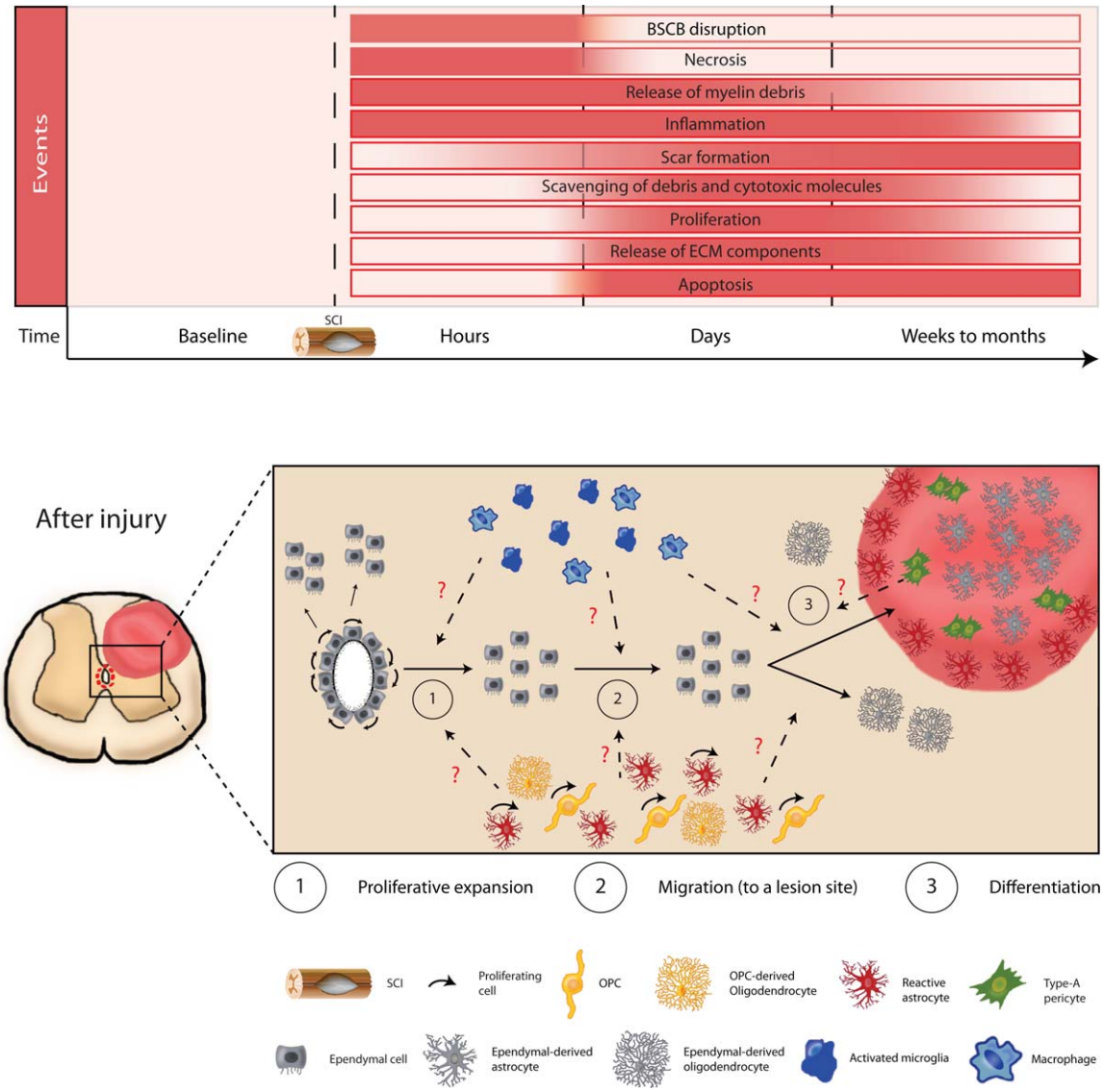


FIGURE 3: Overview of SCI-induced changes within the microenvironment of central canal ependymal cells. SCI triggers complex cascades of global, cellular, and molecular events. The effects of these events on the responses of endogenous spinal cord stem cells remain largely unknown. (Top) A simplified overview and timeline of some of these major changes (see text for details). (Bottom) The three main types of proliferating neural cells at baseline [oligodendrocyte progenitor cells (OPCs), astrocytes, and ependymal cells] all increase their proliferation following SCI. OPC numbers gradually recover, and partially regenerate the depleted oligodendrocyte population. Astrocytes proliferate, become reactive, and contribute heavily to formation of the glial scar. Neurosphere-initiating stem cell activity resides within ependymal cells, 90% of whom become activated to proliferate within a few days after SCI. Only ependymal cells show multi-lineage differentiation, giving rise to astrocytes (~90%) and oligodendrocytes (~4%) at the site of injury. Ependymal-derived astrocytes and reactive astrocytes are found in roughly equal numbers within the glial scar at 4 months post-SCI, but preferentially localize to the lesion core and boundary, respectively. Type-A pericytes also contribute prominently to the glial scar; these represent only a tenth of the astrocyte population in the uninjured spinal cord, but have increased up to 25-fold by 9 days postinjury. Ependymal cell responses can be divided into three stages: (1) proliferative expansion, (2) migration, and (3) differentiation. The environmental factors responsible for directing these responses remain poorly defined, but likely include molecules secreted by microglia and peripherally derived immune cells, reactive astrocytes, OPCs, and scar-associated cells such as pericytes (dotted arrows) (see text for details). SCI: spinal cord injury; BSCB: brain–spinal cord barrier; ECM: extracellular matrix.

expansion of cytotoxic cells and molecules into the surrounding CNS tissue. We discuss scar formation in greater detail in Responses of central canal ependymal cells to SCI section, since the scar-forming process has been most studied in the context of SCI; however, it should be noted that similar events occur at brain sites of ischemic injury. In the following sections, we focus on the responses of SVZ neural stem cells to stroke. We have subdivided these NSC responses into three phases: (1) activation of the SVZ niche, (2) migration and differentiation, and (3) integration. While the mechanisms of

migration and differentiation are distinct, these processes are typically studied in parallel and are therefore categorized together (see Fig. 2). Phases 1 and 2 have been the most investigated, while elucidation of Phase 3 is still in its infancy. We focus specifically on the SVZ niche; although stroke-induced neurogenesis and gliogenesis also occur in the brain's second main neurogenic niche, the hippocampal dentate gyrus, there is little evidence that these new cells can contribute to other brain areas (Kernie and Parent, 2010).

Activation of the SVZ niche. Studies of proliferative responses to stroke have been ongoing and progressive in their methods since the late 90s. There is now a significant body of work demonstrating that ischemia increases SVZ proliferation, and early studies used BrdU to track these proliferative changes. Following MCAO, increases in BrdU+ cells within the SVZ are detected after 2 h (Zhang et al., 2001), peak at 7–14 days (Li et al., 2002; Zhang et al., 2001, 2004b), and return to control levels by 28 days (Zhang et al., 2001). Such increases are further supported by increases in cdc2+ cells (a G2-M cell cycle marker) at 14 days after stroke (Parent et al., 2002) and increases in phosphorylated histone H3 marker (PHH3) at 4, 7, and 14 days postischemia (Zhang et al., 2004b). Increases in proliferation levels are also observed in the SVZ following CCAO in rats (Jin et al., 2001) and primates (Tonchev et al., 2005).

Stroke-induced increases in SVZ proliferation are likely to involve activation at the neural stem cell level of the neurogenic lineage. Zhang et al. (2014) analyzed the pinwheel architecture of the SVZ niche and found that the number of pinwheel-associated SVZ neural stem cells (positive for γ-tubulin and GFAP) was significantly increased 25 days after MCAO in mice. It has also been recently reported that MCAO stimulates proliferation of GFAP+/Nestin+/Sox2+ neural stem cells in ventricular zones caudal to the lateral ventricles, including the third and fourth ventricles (Lin et al., 2014). Intriguingly, this correlated with particularly permeable blood–brain barriers at these sites. These studies highlight that neural stem cell activity in the SVZ is sensitive to diffusible proliferation-inducing factors released upon brain ischemia.

Interestingly, besides GFAP + SVZ astrocytes, SVZ ependymal cells represent an additional, but temporary, neurogenic reservoir after stroke. SVZ ependymal cells normally do not proliferate, but transiently increase their proliferation following ischemia (Zhang et al., 2007b). These reactive SVZ ependymal cells temporarily regain neurogenic competence to produce new neurons following stroke (Carlén et al., 2009); importantly, however, they lack the self-renewal capacity of SVZ astrocytes and are rapidly depleted.

Migration and Differentiation. An important difference between the spinal cord and brain is that the SVZ is a highly neurogenic stem cell niche. Initial studies of migration and differentiation of SVZ-derived cells following MCAO in rats used double-labeling, typically combining BrdU with markers of migrating neuroblasts, like polysialylated-neural cell adhesion molecule (PSA-NCAM) or doublecortin (DCX). These first studies found exciting evidence that chains of migrating neuroblasts were rerouted from the SVZ or rostral migratory stream into the ischemic zone (Arvidsson et al., 2002; Jin et al., 2003; Parent et al., 2002; Zhang et al., 2001, 2004a). However, while suggestive, BrdU-based methods could not definitively establish whether BrdU+ cells originated from local cortical tissue or the SVZ (Magnusson et al., 2014).

This obstacle was first overcome by Yamashita et al. (2006), who used an adenovirus-based approach to show that SVZ astrocytes contribute DCX+ neuroblasts to the ischemic striatum following MCAO. Similarly, Kolb et al. (2007) used a combination of BrdU and intracerebroventricular injection of retrovirus to prelabel SVZ-born cells, and found that SVZ-derived cells were present in the cortex following PVD. Remarkably, these authors treated rats with epidermal growth factor (EGF, promoting proliferation and differentiation), and erythropoietin (EPO, promoting neurogenesis), and found complete regeneration of the ischemia-induced cortical cavity. The tissue-plug in the cavity contained BrdU+ cells co-labeled with NeuN (neurons) and GFAP (astrocytes). Furthermore, removal of the regenerated tissue in EGF- and EPO-treated poststroke animals resulted in the loss of previously observed motor improvements.

Recently, Benner et al. (2013) used a transgenic approach where SVZ-derived cells were specifically tracked in a photothrombosis model of cortical ischemia. Interestingly, these authors found extensive production and migration of SVZ-derived astrocytes that homed to the ischemic cortex. Unexpectedly, SVZ-derived astrocytes expressed much higher levels of Thrombospondin-4, which binds Notch1, than surrounding cortical astrocytes. When Notch signaling was blocked in the SVZ, there was a shift from astrocyte to neuroblast generation, leading to defective glial scar formation and enhanced microvascular hemorrhaging. This is the first evidence directly demonstrating a role for brain NSCs in glial scar formation (Benner et al., 2013). Thus, while considerable emphasis has been placed on the potential of SVZ neural stem cells for neuronal replacement, it is important to note that they have an important endogenous role in producing protective scar-contributing astrocytes.

Integration. The least investigated phase of newly generated SVZ-derived cells following brain ischemia is functional integration. Chains of neuroblasts migrating to the ischemic striatum or cortex (Arvidsson et al., 2002; Li et al., 2010; Parent

et al., 2002; Zhang et al., 2004a, 2007a), together with observations of presynaptic vesicles and synapses between SVZ-derived neuroblasts and neighboring cells (Yamashita et al., 2006), have fuelled hopes that regeneration of lost neurons is possible. Moreover, loss of motor improvements following removal of newly generated cells from a cortical stroke cavity suggest that SVZ-derived cells participate in functional improvements (Kolb et al., 2007). But how is such functional recovery actually mediated? Considering the protective role of NSC-derived astrocytes in scar formation in both the brain (Benner et al., 2013) (above), and the spinal cord (Barnabé-Heider et al., 2010; Sabelström et al., 2013a; Responses of central canal ependymal cells to SCI section), it will be essential to clearly establish whether SVZ-derived neurons can truly integrate into neuronal circuitry to yield functional improvements in behavioral parameters following brain ischemia. Mechanistically, a question to address is whether neuroblasts originally destined for the olfactory bulb are capable of being respecified to generate new target-appropriate neural phenotypes. Moreover, since ischemic brain insults typically occur at older ages, the endogenous neural stem cell response is likely less robust than observed in the young adult animal models that are typically used. The answers to these questions will impact therapeutic possibilities of harnessing SVZ neural stem cells in the context of stroke (Fig. 2).

Responses of Central Canal Ependymal Cells to SCI

Experimental Models of SCI

The small diameter of the spinal cord (<15 mm at the cervical enlargement in humans) means that spinal cord injuries inevitably perturb the central canal niche of ependymal cells. The majority of SCI cases in humans are caused by vertebral dislocation that compresses the spinal cord. A variety of experimental approaches have been used to model human SCI, and these have individual advantages and disadvantages. Most of these models were developed in rats but have been modified to apply to mice as well. The majority of injuries are performed at the thoracic level, but more recently, cervical injuries have also been tested. SCI models include contusion, compression, distraction, dislocation, and transection. The reproducibility of these methods varies considerably. The gold standard is the contusion model, including model spinal cord injury system (MSCIS) and Infinite Horizon, which generate highly reproducible transient and acute injuries and best mimics the most common spinal cord traumas of humans. The compression model consists of a prolonged spinal cord compression performed with clips and forceps, and also shows consistency. On the contrary, distraction (stretches the cord), dislocation (replicates human vertebral displacement), and transection (complete or partial cuts of the spinal cord) models yield more variable results (Cheriyan et al., 2014). Notably, while contusion and compression models result in reproducible damage to the central canal NSC niche, partial transaction models can leave the central canal intact, a difference that is likely to impact on the observed responses of ependymal cells to SCI. Based on the vast amounts of information gained from the analysis of these models, in the following sections, we summarize what we have learned about central canal ependymal cell responses in the injured spinal cord.

Ependymal Cell Proliferation and Multipotency Following SCI

Moderate and severe injuries to the CNS initiate a number of broad pathophysiological processes that take place over the acute (minutes) to chronic (years) time-periods. At the site of injury, there is immediate damage to the blood–spinal cord barrier, extensive necrotic cell death, axon degeneration and demyelination, and initiation of an immune response. These acute events trigger widespread chronic events including inflammation, apoptotic death, secondary demyelination, cyst formation (in rat but not mice), and, eventually, the formation of a prominent and permanent glial scar (Rolls et al., 2009a; Silver and Miller, 2004; Fig. 3). What is the impact of such dramatic cellular and biochemical changes in cell proliferation and neural stem cell activity in the spinal cord?

In the noninjured spinal cord, oligodendrocyte progenitors (NG2+/olig2+), astrocytes (GFAP+/Cx30+/Sox9+), and ependymal cells (FoxJ1+) constitute the 3 main types of dividing cells, accounting for approximately 80%, <5%, and <5% of proliferating cells, respectively (Barnabé-Heider et al., 2010; Horner et al., 2000; Meletis et al., 2008). Of these, lineage tracing experiments have shown that it is solely the ependymal cell population that is capable of generating neurospheres *in vitro*, and this hallmark of NSC potential remains restricted to the ependymal population following SCI (Barnabé-Heider et al., 2010). Ependymal cells are defined as ciliated cells that propel cerebrospinal fluid and are classified according to their morphology (cuboidal, tanycytic, or radial; Bruni, 1987; Meletis et al., 2008; Sabelström et al., 2013a). A subpopulation of tanycytic ependymal cells express the neural precursor marker Nestin and extend long processes from the dorsal and ventral poles of the central canal (Hamilton et al., 2009). The functional differences between these different ependymal subpopulations with respect to neural stem cell potential are still largely unknown. Importantly under physiological conditions, ependymal cells do not show multipotency *in vivo*. Rather, their proliferation is limited, occurs in doublets associated with blood vessels, and is restricted to the generation of new ependymal cells (Barnabé-Heider et al., 2010; Hamilton et al., 2009).

SCI triggers significant changes in the proliferative properties and multipotency of ependymal cells. Ependymal cell proliferation increases in a wide range of SCI models, including contusions, compressions, and partial sections that leave the central canal intact (Johansson et al., 1999; Lacroix et al., 2014; Meletis et al., 2008; Mothe and Tator, 2005). This is observed similarly in mice and rats, suggesting it is a fundamentally conserved response to injury (Lytle and Wrathall, 2007). Ependymal proliferation following SCI results in significant expansion of the NSC population, as neurosphere formation is increased and accelerated after partial section of the spinal cord (Barnabé-Heider et al., 2010). Contusion injuries modeling that of humans reveal a prolonged, long-distance ependymal proliferative response, with increased proliferation still observed at the cervical level several weeks after low thoracic SCI (Lacroix et al., 2014). Notably, recent lineage-tracing studies have examined the fate of prelabeled oligodendrocyte progenitors, astrocytes, and ependymal cells and revealed that, at the population level, ependymal cells are the sole population to show multipotency following SCI (Barnabé-Heider et al., 2010; Meletis et al., 2008): FoxJ1+ ependymal cells gave rise both to astrocytes localized at the core of the glial scar and oligodendrocytes in the surrounding spared white matter. It remains to be formally shown whether ependymal-derived astrocytes and oligodendrocytes have the same clonal origin or originate from discrete subpopulations of ependymal cells. Ependymal-derived astrocytes associated with the glial scar are the most abundant cell type generated by the ependymal cell population (Barnabé-Heider et al., 2010), and this is considered in further detail below.

Ependymal and Astrocytic Cell Contribution to the Glial Scar

The glial scar is a prominent and permanent feature of the lesioned mammalian spinal cord, and is most closely associated with reactive astrogliosis. Following SCI, astrocytes proliferate and are recruited to the lesion site, reaching maximal proliferation within 3–7 days and plateauing in number between 2 and 4 weeks after SCI (Norton et al., 1992; Popovich et al., 1997; Sroga et al., 2003; White et al., 2010). These reactive astrocytes express high levels of the intermediate filament proteins GFAP and vimentin, and extend processes that interweave to create a barrier completely surrounding the lesion site (Frisén et al., 1995a; Rowland et al., 2008). Initially regarded as an obstacle to regeneration of severed CNS axons, the potent growth-inhibitory role of the glial scar was elegantly illustrated in experiments by Davies and colleagues, who showed that transplanted adult sensory neurons were capable of robustly extending axons within degenerating white matter tracts, but underwent abrupt growth arrest when they arrived in the vicinity of the glial scar (Davies et al., 1997, 1999). Similarly, mice lacking the intermediate filaments GFAP and vimentin have less reactive astrocytes, and exhibit increased axonal sprouting and growth following SCI (Menet et al., 2003). However, it is now recognized that the glial scar also plays an important beneficial role in containing the secondary damage caused by prolonged lesion site inflammation (Faulkner et al., 2004; Okada et al., 2006). For instance, Faulkner et al. (2004) measured deficits in locomotor recovery when they ablated 95% of proliferating astrocytes with a conditional GFAP-Thymidine kinase transgenic mouse model (where dividing astrocytes are killed with the antiviral agent ganciclovir). At the cellular level, ablation of reactive astrocytes was associated with defective repair of the blood-brain barrier, increase in lesion site CD45+ inflammatory cells, increased loss of local neurons, and increased demyelination (Faulkner et al., 2004). Thus, therapeutic strategies involving the glial scar should be aimed at specifically promoting its beneficial aspects while diminishing its negative consequences.

Recent fate-mapping studies have defined the individual contributions of various cell types to the glial scar following SCI, including ependymal cells (Barnabé-Heider et al., 2010). Interestingly, the progeny of prelabeled oligodendrocyte progenitors, astrocytes, and ependymal cells were found to occupy complementary domains following SCI. Oligodendrocyte progenitors produced oligodendrocyte lineage cells that were scattered around the lesion. Astrocytes generated progeny that were concentrated at the borders of the glial scar. However, ependymal cells generated large numbers of astrocytes that were localized at the core of the glial scar and a small proportion of oligodendrocytes in the surrounding spared white matter (Barnabé-Heider et al., 2010). Impressively, it was estimated that approximately half of scar-associated astrocytes were ependymal-derived (Barnabé-Heider et al., 2010). Importantly, ependymal-derived astrocytes migrated within the core of the scar and produced laminin, a substrate favorable for axonal regrowth (Barnabé-Heider et al., 2010; Frisén et al., 1995b; Meletis et al., 2008). This contrasts the reactive GFAP+ astrocytes derived from pre-existing astrocytes, which are mostly localized around the perimeter of the lesion site and associated with growth inhibitory proteins such as chondroitin sulfate proteoglycans (CSPGs; Silver and Miller, 2004). The distinct characteristics of scar astrocytes that are derived from reactive astrocytes versus ependymal cells suggest that they correspond to growth-inhibitory and growth-promoting astrocyte subtypes, respectively. However, this possibility remains to be demonstrated.

Sabelström and colleagues recently used a FoxJ1-rasless mouse model to block the generation of ependymal cell-derived progeny and found major defects in scar formation. 14 weeks post-SCI, 79% of the animals did not form

compact scar tissue, and showed deeper and enlarged lesions in contrast to control (Sabelström et al., 2013b). This correlated with the 2-week post-SCI observation of approximately 50% increased neuronal cell loss, and an increase in caspase-3+ cells when compared with control littermates. Interestingly, even though these animals showed a lack of proper glial scar formation, fewer inflammatory cells were observed. This suggests that the astrocytes derived from ependymal cells are not involved in secondary damage restriction. Furthermore, this study suggested that these ependymal cell-derived astrocytes represent a source of neurotrophic factors, such as ciliary neurotrophic factor (CNTF), hepatocyte growth factor (HGF), and insulin-like growth factor (IGF) 1 as these were downregulated in the FoxJ1-rasless mice (Sabelström et al., 2013b). Overall, these data reveal that ependymal cells are a key contributor to the protective properties of the glial scar.

Additional resident and infiltrating cell types also contribute to formation of the glial scar, including pericytes, microglia, and blood-derived neutrophils, macrophages, and lymphocytes (Barnabé-Heider et al., 2010; Grossman et al., 2001; Lytle and Wrathall, 2007; Meletis et al., 2008; Popovich et al., 1997; Sroga et al., 2003; Zai and Wrathall, 2005; for review, see Cregg et al., 2014). Each cell type responds at a different time-point postinjury, and influences the behavior of other cell types within the developing scar. For example, eliminating Ras-mediated proliferation in a subpopulation of pericytes using a glutamate-aspartate transporter (GLAST)-driven Cre recombination approach resulted in improper sealing of the lesion in 33% of the tested animals (Göritz et al., 2011). Thus, understanding the complex cellular interactions between scar-forming cells is an important step toward rational and targeted modulation of scar formation.

Potential Regulators of Ependymal Cell Responses to SCI

The molecular mechanisms regulating the proliferation, migration and differentiation of ependymal cells following SCI remain largely unknown. Activating ependymal cell proliferation requires more than nonspecific tissue degeneration, as proliferation increases have been documented in models of traumatic SCI and amyotrophic lateral sclerosis (ALS; Barnabé-Heider et al., 2010; Chi et al., 2006; Meletis et al., 2008) but not in chemically or autoimmune-mediated models of multiple sclerosis-associated demyelination (Lacroix et al., 2014). However, environmental influences within the injured spinal cord are clearly a dominant factor: ependymal cells show no neuronal differentiation *in vivo* and relatively little oligodendrocyte differentiation following SCI, yet ependymal-derived neurospheres spontaneously produce these cell types *in vitro*. In the following section, we discuss some of the massive pathophysiological changes occurring within the immediate microenvironment of ependymal cells that may play significant roles in determining their properties and behavior after SCI.

Immediate Events. SCI rapidly sets into motion dramatic changes to the cellular and molecular microenvironment of the spinal cord. The blood-spinal cord barrier is normally composed of endothelial cells linked with tight junctions, pericytes, and astrocyte feet processes (Bartanusz et al., 2011; Preston et al., 2001). Disruption and enhanced permeability of this barrier occurs within 5 min after injury (Bartanusz et al., 2011), with several important consequences. First, there is a rapid onset of cell death, as neural cells die within a few minutes of oxygen and glucose deprivation (Jullienne and Badaut, 2013). Necrosis is detectable within the first 2 h following SCI (Eftekharpour et al., 2008; Rowland et al., 2008). By 24-h postcontusion in rats, astrocyte and oligodendrocyte populations are reduced by half in the spared white matter surrounding the lesion site, and there is a growing length of spinal cord devoid of ventral motor neurons (Grossman et al., 2001). Second, death of neurons and oligodendrocytes triggers the release of myelin debris rich in axonal growth-inhibiting molecules such as Nogo, myelin-associated glycoprotein, oligodendrocyte-myelin glycoprotein, semaphorins, and others (Bartsch et al., 1995; Bregman et al., 1995; Cafferty et al., 2010; Chen et al., 2000; Ji et al., 2008; Kottis et al., 2002; Lee et al., 2010; McKerracher et al., 1994; Savio and Schwab, 1990). Little is known concerning the influence of such molecules on neural precursor cells. Third, pericytes are liberated from the damaged blood vessels. Pericytes are recruited to the lesion site where they release fibronectin, collagen I, collagen III, and activate inflammatory cells (i.e., T-cells; Göritz et al., 2011; Sroga et al., 2003), and they play a key role in the formation of the glial scar (Göritz et al., 2011).

Reactive Astrogliosis. Astrogliosis, which begins minutes following injury, has a variety of important consequences beyond simple formation of the glial scar-associated physical barrier. Indeed, reactive astrocytes also scavenge debris, neurotoxic molecules (glutamate) and ions (potassium), produce trophic factors [brain-derived neurotrophic factor (BDNF), neurotrophin-3 [NT-3], IGF, glial cell line-derived neurotrophic factor, nerve growth factor, basic FGF], enhance revascularization, repair the blood-brain barrier, reduce edema, and limit the spread of inflammatory cells (Faulkner et al., 2004; Hamby and Sofroniew, 2010; Okada et al., 2006; Rolls et al., 2009b). Reactive astrocytes also secrete bone morphogenetic proteins (BMPs) that promote astrocytic differentiation (Wang et al., 2011). In addition, reactive astrocytes actively produce a large variety of molecules, such as CSPGs, tenascin, and collagen, which create a biochemical milieu surrounding

the scar that induces neurite retraction and growth cone collapse (Ferreira et al., 2012; Liu et al., 2014; Rolls et al., 2009; Silver and Miller, 2004). CSPGs (neurocan, brevican, and versican) are secreted 24 h following SCI and are expressed for several weeks (Davies et al., 1999). The impact of astrocyte-derived molecules on the recruitment and activation of ependymal cells remains unknown.

Oligodendrocyte Progenitors. Oligodendrocyte progenitors (OPCs) represent the major population of proliferating cells in the noninjured spinal cord (Barnabé-Heider et al., 2010; Horner et al., 2000), and following SCI, these cells are likely to be in close proximity to proliferating, migrating, and differentiating ependymal cells. Recent studies using platelet-derived growth factor receptor alpha (PDGFRα)-driven reporter lines have revealed that OPCs undergo substantial proliferation surrounding spinal cord lesion sites, continuing to generate myelinating oligodendrocytes for several months following spinal cord contusions in rats (Hesp et al., 2015). Indeed, spared rubrospinal and corticospinal axons have been shown to be extensively remyelinated in the chronically injured rat spinal cord (Powers et al., 2012), and NG2+ OPCs (pre-existing or newly generated) are the source of this remyelination (Powers et al., 2013). How OPC proliferation and differentiation into remyelinating oligodendrocytes affects the fate of ependymal-derived progeny has yet to be investigated.

Inflammation. The inflammatory microenvironment is likely to be a significant determinant of ependymal cell fate, as pro- and anti-inflammatory cytokines exhibit opposing effects on neural precursors (Kokaia et al., 2012). Chronic inflammation at the lesion site is responsible for the majority of the secondary damage occurring after injury (Jones et al., 2005). SCI activates immune cells and triggers the release of complex patterns of pro- and anti-inflammatory cytokine (Pineau and Lacroix, 2007). Initial proinflammatory signals are released from resident neural cells and are subsequently sustained by immune cells. Microglia, resident phagocytes, are highly responsive to tissue perturbations, and react immediately upon injury. Microglial activation is associated with morphological changes (from branch-stellate to round shape) and an altered panel of cytokine expression (Jones et al., 2005). Circulating neutrophils and monocyte-derived macrophages are also rapidly activated and invade the spinal cord parenchyma at the lesion site, contributing to the later recruitment of lymphocytes (Popovich et al., 1997; Zhang and Gensel, 2014). While the neutrophil response normalizes by 2 weeks following injury in rats, macrophages and lymphocytes remain present at the lesion for months (Prüss et al., 2011). Similar to astrocytes, the role of resident microglia and blood-derived macrophages is highly complex and presents beneficial (protects and activates oligodendrocyte precursor cell proliferation) and detrimental (causes cytotoxicity and demyelination) effects on recovery from SCI (David and Kroner, 2011). Proinflammatory cytokines, such as interleukin (IL) 1β, IL-6, and tumor necrosis factor-α (TNFα), are released early postinjury by microglia and macrophages. These factors mediate secondary tissue damage, modulate axonal remyelination, stimulate astrogliosis, and induce expression of adhesion molecules and the production of chemotactic factors, such as IL-8, monocyte chemotactic protein (MCP)-1, and macrophage inhibitory protein (MIP)-1α (Bareyre and Schwab, 2003; Jones et al., 2005). Selective depletion of monocyte-derived macrophages improves recovery of function, preserves myelination, and stimulates sprouting and regeneration of axons following SCI (Jones et al., 2005; Popovich et al., 1999). In contrast, microglia/macrophages also produce neuroprotective molecules, such as transforming growth factor (TGF)-β, and NT-3. BDNF and NT-3 promote neuronal survival, and together with TGF-β, inhibit further microglia activation, which includes proinflammatory cytokine and reactive oxygen intermediate production (Jones et al., 2005). The dual role of the microglial/macrophage population can be explained by the presence of at least two subpopulations, M1 and M2, showing neurotoxic and proregenerative properties, respectively (Kigerl et al., 2009). Shifting the polarization of these cells to the M2 phenotype is an active area of investigation (David and Kroner, 2011). It is unknown whether modulating the levels of inflammation-associated cells or their secreted factors can enhance the responses of endogenous neural stem cells to CNS injury.

Therapeutic Perspectives

Stimulation of endogenous neural stem cells is a logical approach for attempting to replace neuronal and glial cells lost after stroke and SCI. However, as discussed in the preceding sections, therapeutic strategies aimed at achieving this goal are faced with overcoming the extensive cellular and molecular changes within the neural stem cell microenvironment. These include cell death, inflammation, reactive changes in diverse cell populations, and alterations in the molecular composition of the extracellular milieu. Nevertheless, recent studies suggest that the behavior of endogenous NSCs during pathological conditions can indeed be modulated to promote functional recovery after CNS injury (Erlandsson et al., 2011; Kolb et al., 2007). For example, using immune-deficient NOD/SCID mice or treatment with Cyclosporine A in a PVD model, Erlandsson and colleagues found that suppressing the immune response: (1) enhanced migration of SVZ-derived cells to the ischemic injury site, (2) shifted cellular differentiation to a gliogenic profile, and (3) stimulated motor improvement.

Several therapeutically relevant strategic goals can be identified. First, modulating astrocytic contribution to the glial scar. As discussed earlier, neural stem cell-derived astrocytes play a protective role following stroke and SCI (Benner et al., 2013; Sabelström et al., 2013b). Moreover, it appears that the astrocyte population contains both growth-promoting and growth-inhibiting subtypes (Bayraktar et al., 2014; Davies et al., 1997, 1999; Fitch and Silver, 2008). Thus, it is relevant to test whether enhancing the production of neural stem cell-derived astrocytes can further reduce secondary damage and/or create a more growth-promoting environment at the injury site.

Second, enhancing oligodendrocyte differentiation. Unrepaired zones of demyelination are a significant feature following SCI, and lineage-tracing studies showed that oligodendrocytes represented approximately 3% of the progeny of reactive ependymal cell (Barnabé-Heider et al., 2010). This low percentage is likely due to the high expression of astrocyte-promoting factors such as IL-6-related cytokines and BMPs in the injured spinal cord. Supporting the rationale for stimulating oligodendrocyte fate of spinal cord neural stem cells, neurogenin-2 overexpressing neurospheres showed greater oligodendrocyte differentiation, enhanced myelination and resulted in beneficial changes in motor and sensory function when transplanted into the injured spinal cord (Hofstetter et al., 2005).

Third, enhancing neuronal differentiation. Neuronal loss at the site of injury is a major reason for functional deficits following stroke in particular. Stimulating neurogenesis and integration of new neurons could promote recovery either by directly replacing the lost neurons, or by providing a neuronal substrate for electrical signals to bridge or circumvent the lesion area. As discussed earlier, there is little information on whether SVZ-derived neurons functionally integrate following stroke, and neurogenesis has yet to be documented in the injured adult spinal cord.

Fourth, activating NSCs that are unresponsive in other diseases. Uncovering the mechanisms triggering NSC proliferation, migration, and differentiation following SCI and stroke may enable us to recruit NSCs in other conditions. For example, myelin repair is a key objective in multiple sclerosis, but neither chemically mediated focal demyelination nor autoimmune-mediated multifocal demyelination is sufficient to activate the proliferation of central canal ependymal cells (Lacroix et al., 2014).

While great strides have been taken over the past 15–20 years, many key challenges remain to be overcome before endogenous neural stem cells can be used for therapeutic benefit in stroke and SCI. In particular, it will be important to understand the complex influences of the glial scar on recruitment, migration, differentiation, and integration of NSC-derived progeny. Interestingly, recent discoveries suggest that NSCs can also be induced and/or recruited to sites of CNS injury from regions other than the traditionally defined stem cell niches. For example, astrocytes in non-neurogenic regions (i.e., the cortex and striatum) can acquire neurosphere-forming ability and can generate neurons in response to tissue pathology or to modulation of key signaling pathways (Buffo et al., 2008; Sirko et al., 2013). Similarly, fate-mapping recently revealed that striatal astrocytes undergo an *in vivo* neurogenic response to MCAO, which can be recreated in the absence of stroke by blocking Notch signaling (Magnusson et al., 2014). Such discoveries offer hope that endogenous sources of neural regenerative capacity are waiting to be harnessed.

References

Arvidsson A, Collin T, Kirik D, Kokaia Z, Lindvall O. 2002. Neuronal replacement from endogenous precursors in the adult brain after stroke. Nat Med 8: 963–970.

Barbaro NM, Gupta N, Kunwar S. 2004. Unique astrocyte ribbon in adult human brain contains neural stem cells but lacks chain migration. Nature 427: 740–744.

Bareyre FM, Schwab ME. 2003. Inflammation, degeneration and regeneration in the injured spinal cord: Insights from DNA microarrays. Trends Neurosci 26:555–563.

Barnabé-Heider F, Göritz C, Sabelström H, Takebayashi H, Pfrieger FW, Meletis K, Frisén J. 2010. Origin of new glial cells in intact and injured adult spinal cord. Cell Stem Cell 7:470–482.

Bartanusz V, Jezova D, Alajajian B, Digicaylioglu M. 2011. The blood–spinal cord barrier: Morphology and clinical implications. Ann Neurol 70:194–206.

Bartsch U, Bandtlow CE, Schnell L, Bartsch S, Spillmann AA, Rubin BP, Hillenbrand R, Montag D, Schwab ME, Schachner M. 1995. Lack of evidence that myelin-associated glycoprotein is a major inhibitor of axonal regeneration in the CNS. Neuron 15:1375–1381.

Bastien D, Lacroix S. 2014. Cytokine pathways regulating glial and leukocyte function after spinal cord and peripheral nerve injury. Exp Neurol 258C:62–77.

Bayraktar OA, Fuentealba LC, Alvarez-Buylla A, Rowitch DH. 2014. Astrocyte development and heterogeneity. Cold Spring Harb Perspect Biol. 7, a020362.

Benner EJ, Luciano D, Jo R, Abdi K, Paez-Gonzalez P, Sheng H, Warner DS, Liu C, Eroglu C, Kuo CT. 2013. Protective astrogenesis from the SVZ niche after injury is controlled by Notch modulator Thbs4. Nature 497:369–373.

Bonfanti L, Peretto P. 2011. Adult neurogenesis in mammals—A theme with many variations. Eur J Neurosci 34:930–950.

Bregman BS, Kunkel-Bagden E, Schnell L, Dai HN, Gao D, Schwab ME. 1995. Recovery from spinal cord injury mediated by antibodies to neurite growth inhibitors. Nature 378:498–501.

Bruni JE. 1987. Ependyma of the central canal of the rat spinal cord: A light and transmission electron microscopic study. J Anat 152:55–70.

Buffo A, Rite I, Tripathi P, Lepier A, Colak D, Horn A, Mori T, Götz M. 2008. Origin and progeny of reactive gliosis: A source of multipotent cells in the injured brain. Proc Natl Acad Sci USA 105:3581–3586.

Cafferty WBJ, Duffy P, Huebner E, Strittmatter SM. 2010. MAG and OMgp synergize with Nogo-A to restrict axonal growth and neurological recovery after spinal cord trauma. J Neurosci 30:6825–6837.

Carlén M, Meletis K, Göritz C, Darsalia V, Evergren E, Tanigaki K, Amendola M, Barnabé-Heider F, Yeung MSY, Naldini L, Honjo T, Kokaia Z, Shupliakov O, Cassidy RM, Lindvall O, Frisén J. 2009. Forebrain ependymal cells are Notch-dependent and generate neuroblasts and astrocytes after stroke. Nat Neurosci 12:259–267.

Chen MS, Huber AB, van der Haar ME, Frank M, Schnell L, Spillmann AA, Christ F, Schwab ME. 2000. Nogo-A is a myelin-associated neurite outgrowth inhibitor and an antigen for monoclonal antibody IN-1. Nature 403:434–439.

Cheriyan T, Ryan DJ, Weinreb JH, Cheriyan J, Paul JC, Lafage V, Kirsch T, Errico TJ. 2014. Spinal cord injury models: A review. Spinal Cord 52:588–595.

Chi L, Ke Y, Luo C, Li B, Gozal D, Kalyanaraman B, Liu R. 2006. Motor neuron degeneration promotes neural progenitor cell proliferation, migration, and neurogenesis in the spinal cords of amyotrophic lateral sclerosis mice. Stem Cells 24:34–43.

Cregg JM, DePaul M a., Filous AR, Lang BT, Tran A, Silver J. 2014. Functional regeneration beyond the glial scar. Exp Neurol 253:197–207.

David S, Kroner A. 2011. Repertoire of microglial and macrophage responses after spinal cord injury. Nat Rev Neurosci 12:388–399.

Davies SJA, Fitch MT, Silver J. 1997. Regeneration of adult axons in white matter tracts of the central nervous system. Nature 390:372–375.

Davies SJA, Goucher DR, Doller C, Silver J. 1999. Robust regeneration of adult sensory axons in degenerating white matter of the adult rat spinal cord. J Neurosci 19:5810–5822.

Diaz Quiroz JF, Echeverri K. 2013. Spinal cord regeneration: Where fish, frogs and salamanders lead the way, can we follow? Biochem J 451:353–364.

Doetsch F, Caillé I, Lim DA, García-Verdugo JM, Alvarez-Buylla A. 1999. Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. Cell 97:703–716.

Dromard C, Guillon H, Rigau V, Ripoll C, Sabourin JC, Perrin FE, Scamps F, Bozza S, Sabatier P, Lonjon N, Duffau H, Vachiery-Lahaye F, Prieto M, Tran Van Ba C, Deleyrolle L, Boularan A, Langley K, Gaviria M, Privat A, Hugnot JP, Bauchet L. 2008. Adult human spinal cord harbors neural precursor cells that generate neurons and glial cells in vitro. J Neurosci Res 86:1916–1926.

Eftekharpour E, Karimi-Abdolrezaee S, Fehlings MG. 2008. Current status of experimental cell replacement approaches to spinal cord injury. Neurosurg Focus 24:E19.

Erlandsson A, Lin C-HA, Yu F, Morshead CM. 2011. Immunosuppression promotes endogenous neural stem and progenitor cell migration and tissue regeneration after ischemic injury. Exp Neurol 230:48–57.

Faulkner JR, Herrmann JE, Woo MJ, Tansey KE, Doan NB, Sofroniew MV. 2004. Reactive astrocytes protect tissue and preserve function after spinal cord injury. J Neurosci 24:2143–2155.

Fei J-F, Schuez M, Tazaki A, Taniguchi Y, Roensch K, Tanaka EM. 2014. CRISPR-Mediated genomic deletion of sox2 in the axolotl shows a requirement in spinal cord neural stem cell amplification during tail regeneration. Stem Cell Reports 3:444–459.

Ferreira LMR, Floriddia EM, Quadrato G, Di Giovanni S. 2012. Neural regeneration: Lessons from regenerating and non-regenerating systems. Mol Neurobiol 46:227–241.

Fitch MT, Silver J. 2008. CNS injury, glial scars, and inflammation: Inhibitory extracellular matrices and regeneration failure. Exp Neurol 209:294–301.

Frisén J, Johansson CB, Török C, Risling M, Lendahl U. 1995. Rapid, widespread, and longlasting induction of nestin contributes to the generation of glial scar tissue after CNS injury. J Cell Biol 131:453–464.

Gaete M, Muñoz R, Sánchez N, Tampe R, Moreno M, Contreras EG, Lee-Liu D, Larraín J. 2012. Spinal cord regeneration in Xenopus tadpoles proceeds through activation of Sox2-positive cells. Neural Dev 7:13.

Gheusi G, Lepousez G, Lledo P. 2013. Adult-born neurons in the olfactory bulb: Integration and functional consequences. Curr Top Behav Neurosci 15: 49–72.

Goldshmit Y, Sztal TE, Jusuf PR, Hall TE, Nguyen-Chi M, Currie PD. 2012. Fgf-dependent glial cell bridges facilitate spinal cord regeneration in zebrafish. J Neurosci 32:7477–7492.

Gonzalez-Perez O, Romero-Rodriguez R, Soriano-Navarro M, Garcia-Verdugo JM, Alvarez-Buylla A. 2009. Epidermal growth factor induces the progeny of subventricular zone type B cells to migrate and differentiate into oligodendrocytes. Stem Cells 27:2032–2043.

Göritz C, Dias DO, Tomilin N, Barbacid M, Shupliakov O, Frisén J. 2011. A pericyte origin of spinal cord scar tissue. Science 333:238–242.

Grossman SD, Rosenberg LJ, Wrathall JR. 2001. Temporal-spatial pattern of acute neuronal and glial loss after spinal cord contusion. Exp Neurol 168: 273–282.

Hamby ME, Sofroniew MV. 2010. Reactive astrocytes as therapeutic targets for CNS disorders. Neurotherapeutics 7:494–506.

Hamilton LK, Truong MK V, Bednarczyk MR, Aumont A, Fernandes KJL. 2009. Cellular organization of the central canal ependymal zone, a niche of latent neural stem cells in the adult mammalian spinal cord. Neuroscience 164:1044–1056.

Hesp ZC, Goldstein E a., Miranda CJ, Kaspar BK, McTigue DM. 2015. Chronic oligodendrogenesis and remyelination after spinal cord injury in mice and rats. J Neurosci 35:1274–1290.

Hofstetter CP, Holmström N AV, Lilja JA, Schweinhardt P, Hao J, Spenger C, Wiesenfeld-Hallin Z, Kurpad SN, Frisén J, Olson L. 2005. Allodynia limits the usefulness of intraspinal neural stem cell grafts; directed differentiation improves outcome. Nat Neurosci 8:346–353.

Horky LL, Galimi F, Gage FH, Horner PJ. 2006. Fate of endogenous stem/progenitor cells following spinal cord injury. J Comp Neurol 498:525–538.

Horner, PJ, Power, AE, Kempermann, G, Kuhn, HG, Palmer, TD, Winkler, J, Thal, LJ, Gage, FH. 2000. Proliferation and differentiation of progenitor cells throughout the intact adult rat spinal cord. J Neurosci 20:2218–2228.

Hugnot JP, Franzen R. 2011. The spinal cord ependymal region: A stem cell niche in the caudal central nervous system. Front Biosci (Landmark Ed) 16: 1044–1059.

Ji B, Case LC, Liu K, Shao Z, Lee X, Yang Z, Wang J, Tian T, Shulga-Morskaya S, Scott M, He Z, Relton JK, Mi S. 2008. Assessment of functional recovery and axonal sprouting in oligodendrocyte-myelin glycoprotein (OMgp) null mice after spinal cord injury. Mol Cell Neurosci 39:258–267.

Jin K, Minami M, Lan JQ, Mao XO, Batteur S, Simon RP, Greenberg DA. 2001. Neurogenesis in dentate subgranular zone and rostral subventricular zone after focal cerebral ischemia in the rat. Proc Natl Acad Sci USA 98: 4710–4715.

Jin K, Sun Y, Xie L, Peel A, Mao XO, Batteur S, Greenberg DA. 2003. Directed migration of neuronal precursors into the ischemic cerebral cortex and striatum. Mol Cell Neurosci 24:171–189.

Johansson CB, Momma S, Clarke DL, Risling M, Lendahl U, Frisén J. 1999. Identification of a neural stem cell in the adult mammalian central nervous system. Cell 96:25–34.

Jones LL, Sajed D, Tuszynski MH. 2003. Axonal regeneration through regions of chondroitin sulfate proteoglycan deposition after spinal cord injury: A balance of permissiveness and inhibition. J Neurosci 23:9276–9288.

Jones T, McDaniel E, Popovich P. 2005. Inflammatory-mediated injury and repair in the traumatically injured spinal cord. Curr Pharm Des 11:1223–1236.

Jullienne A, Badaut J. 2013. Molecular contributions to neurovascular unit dysfunctions after brain injuries: Lessons for target-specific drug development. Future Neurol 8:677–689.

Kernie SG, Parent JM. 2010. Forebrain neurogenesis after focal Ischemic and traumatic brain injury. Neurobiol Dis 37:267–274.

Kigerl KA, Gensel JC, Ankeny DP, Alexander JK, Donnelly DJ, Popovich PG. 2009. Identification of two distinct macrophage subsets with divergent effects causing either neurotoxicity or regeneration in the injured mouse spinal cord. J Neurosci 29:13435–13444.

Kirkham M, Hameed LS, Berg D a, Wang H, Simon A. 2014. Progenitor cell dynamics in the newt telencephalon during homeostasis and neuronal regeneration. Stem Cell Rep 2:507–519.

Kokaia Z, Martino G, Schwartz M, Lindvall O. 2012. Cross-talk between neural stem cells and immune cells: The key to better brain repair? Nat Neurosci 15:1078–1087.

Kolb B, Morshead C, Gonzalez C, Kim M, Gregg C, Shingo T, Weiss S. 2007. Growth factor-stimulated generation of new cortical tissue and functional

recovery after stroke damage to the motor cortex of rats. J Cereb Blood Flow Metab 27:983–997.

Kottis V, Thibault P, Mikol D, Xiao Z-C, Zhang R, Dergham P, Braun PE. 2002. Oligodendrocyte-myelin glycoprotein (OMgp) is an inhibitor of neurite outgrowth. J Neurochem 82:1566–1569.

Kriegstein A, Alvarez-Buylla A. 2009. The glial nature of embryonic and adult neural stem cells. Annu Rev Neurosci 32:149–184.

Lacroix S, Hamilton LK, Vaugeois A, Beaudoin S, Breault-Dugas C, Pineau I, Lévesque SA, Grégoire CA, Fernandes KJL. 2014. Central canal ependymal cells proliferate extensively in response to traumatic spinal cord injury but not demyelinating lesions. PLoS One 9:e85916.

Lee JK, Geoffroy CG, Chan AF, Tolentino KE, Crawford MJ, Leal MA, Kang B, Zheng B. 2010. Assessing spinal axon regeneration and sprouting in Nogo-, MAG-, and OMgp-deficient mice. Neuron 66:663–670.

Lee Y, Lee S, Lee S-R, Park K, Hong Y, Lee M, Park S, Jin Y, Chang K-T, Hong Y. 2014. Beneficial effects of melatonin combined with exercise on endogenous neural stem/progenitor cells proliferation after spinal cord injury. Int J Mol Sci 15:2207–2222.

Lee-Liu D, Edwards-Faret G, Tapia VS, Larraín J. 2013. Spinal cord regeneration: Lessons for mammals from non-mammalian vertebrates. Genesis 51: 529–544.

Li B, Piao C-S, Liu X-Y, Guo W-P, Xue Y-Q, Duan W-M, Gonzalez-Toledo ME, Zhao L-R. 2010. Brain self-protection: The role of endogenous neural progenitor cells in adult brain after cerebral cortical ischemia. Brain Res 1327:91–102.

Li Y, Chen J, Chopp M. 2002. Cell proliferation and differentiation from ependymal, subependymal and choroid plexus cells in response to stroke in rats. J Neurol Sci 193:137–146.

Lin R, Cai J, Nathan C, Wei X, Schleidt S, Rosenwasser R, Iacovitti L. 2014. Neurogenesis is enhanced by stroke in multiple new stem cell niches along the ventricular system at sites of high BBB permeability. Neurobiol Dis 74: 229–239.

Liu Z, Li Y, Cui Y, Roberts C, Lu M, Wilhelmsson U, Pekny M, Chopp M. 2014. Beneficial effects of gfap/vimentin reactive astrocytes for axonal remodeling and motor behavioral recovery in mice after stroke. Glia 62: 2022–2033.

Longa EZ, Weinstein PR, Carlson S, Cummins R. 1989. Reversible middle cerebral artery occlusion without craniectomy in rats. Stroke 20:84–91.

Luo J, Shook BA, Daniels SB, Conover JC. 2008. Subventricular zone-mediated ependyma repair in the adult mammalian brain. J Neurosci 28: 3804–3813.

Lytle JM, Wrathall JR. 2007. Glial cell loss, proliferation and replacement in the contused murine spinal cord. Eur J Neurosci 25:1711–1724.

Magnusson JP, Göritz C, Tatarishvili J, Dias DO, Smith EMK, Lindvall O, Kokaia Z, Frisén J. 2014. A latent neurogenic program in astrocytes regulated by Notch signaling in the mouse. Science 346:237–241.

Martens DJ, Seaberg RM, van der Kooy D. 2002. In vivo infusions of exogenous growth factors into the fourth ventricle of the adult mouse brain increase the proliferation of neural progenitors around the fourth ventricle and the central canal of the spinal cord. Eur J Neurosci 16:1045–1057.

McHedlishvili L, Mazurov V, Grassme KS, Goehler K, Robl B, Tazaki A, Roensch K, Duemmler A, Tanaka EM. 2012. Reconstitution of the central and peripheral nervous system during salamander tail regeneration. Proc Natl Acad Sci USA 109:E2258–E2266.

McKerracher L, David S, Jackson DL, Kottis V, Dunn RJ, Braun PE. 1994. Identification of myelin-associated glycoprotein as a major myelin-derived inhibitor of neurite growth. Neuron 13:805–811.

Meletis K, Barnabé-Heider F, Carlén M, Evergren E, Tomilin N, Shupliakov O, Frisén J. 2008. Spinal cord injury reveals multilineage differentiation of ependymal cells. PLoS Biol 6:e182.

Menet V, Prieto M, Privat A, Gime M. 2003. Axonal plasticity and functional recovery after spinal cord injury in mice deficient in both glial fibrillary acidic protein and vimentin genes. Proc Natl Acad Sci USA 100: 8999–9004.

Menn B, Garcia-Verdugo JM, Yaschine C, Gonzalez-Perez O, Rowitch D, Alvarez-Buylla A. 2006. Origin of oligodendrocytes in the subventricular zone of the adult brain. J Neurosci 26:7907–7918.

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: 265–278.

Mothe AJ, Tator CH. 2005. Proliferation, migration, and differentiation of endogenous ependymal region stem/progenitor cells following minimal spinal cord injury in the adult rat. Neuroscience 131:177–187.

Mothe AJ, Zahir T, Santaguida C, Cook D, Tator CH. 2011. Neural stem/progenitor cells from the adult human spinal cord are multipotent and self-renewing and differentiate after transplantation. PLoS One 6:e27079.

Norton WT, Aquino DA, Hozumi I, Chiu F, Brosnan CF. 1992. Quantitative aspects of reactive gliosis: A review. Neurochem Res 17:877–885.

Okada S, Nakamura M, Katoh H, Miyao T, Shimazaki T, Ishii K, Yamane J, Yoshimura A, Iwamoto Y, Toyama Y, Okano H. 2006. Conditional ablation of Stat3 or Socs3 discloses a dual role for reactive astrocytes after spinal cord injury. Nat Med 12:829–834.

Ortega F, Gascón S, Masserdotti G, Deshpande A, Simon C, Fischer J, Dimou L, Chichung Lie D, Schroeder T, Berninger B. 2013. Oligodendrogliogenic and neurogenic adult subependymal zone neural stem cells constitute distinct lineages and exhibit differential responsiveness to Wnt signalling. Nature Cell Biology 15:602–613.

Ovbiagele B, Goldstein LB, Higashida RT, Howard VJ, Johnston SC, Khavjou OA, Lackland DT, Lichtman JH, Mohl S, Sacco RL, Saver JL, Trogdon JG. 2013. Forecasting the future of stroke in the United States: A policy statement from the American Heart Association and American Stroke Association. Stroke 44:2361–2375.

Parent JM, Vexler ZS, Gong C, Derugin N, Ferriero DM. 2002. Rat forebrain neurogenesis and striatal neuron replacement after focal stroke. Ann Neurol 52:802–813.

Pineau I, Lacroix S. 2007. Proinflammatory cytokine synthesis in the injured mouse spinal cord: Multiphasic expression pattern and identification of the cell types involved. J Comp Neurol 500:267–285.

Popovich PG, Guan Z, Wei P, Huitinga I, van Rooijen N, Stokes BT. 1999. Depletion of hematogenous macrophages promotes partial hindlimb recovery and neuroanatomical repair after experimental spinal cord injury. Exp Neurol 158:351–365.

Popovich PG, Wei P, Stokes BT. 1997. Cellular inflammatory response after spinal cord injury in Sprague-Dawley and Lewis rats. J Comp Neurol 464: 443–464.

Powers BE, Lasiene J, Plemel JR, Shupe L, Perlmutter SI, Tetzlaff W, Horner PJ. 2012. Axonal thinning and extensive remyelination without chronic demyelination in spinal injured rats. J Neurosci 32:5120–5125.

Powers BE, Sellers DL, Lovelett E a, Cheung W, Aalami SP, Zapertov N, Maris DO, Horner PJ. 2013. Remyelination reporter reveals prolonged refinement of spontaneously regenerated myelin. Proc Natl Acad Sci USA 110: 4075–1080.

Preston E, Webster J, Small D. 2001. Characteristics of sustained blood-brain barrier opening and tissue injury in a model for focal trauma in the rat. J Neurotrauma 18:83–92.

Prüss H, Kopp MA, Brommer B, Gatzemeier N, Laginha I, Dirnagl U, Schwab JM. 2011. Non-resolving aspects of acute inflammation after spinal cord injury (SCI): Indices and resolution plateau. Brain Pathol 21:652–660.

Quiñones-Hinojosa A, Sanai N, Soriano-Navarro M, Gonzalez-Perez O, Mirzadeh Z, Gil-Perotin S, Romero-Rodriguez R, Berger MS, Garcia-Verdugo JM, Alvarez-Buylla A. 2006. Cellular composition and cytoarchitecture of the adult human subventricular zone: A niche of neural stem cells. J Comp Neurol 494:415–434.

Reynolds BA, Weiss S. 1992. Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. Science 255: 1707–1710.

Rolls A, Shechter R, Schwartz M. 2009. The bright side of the glial scar in CNS repair. Nat Rev Neurosci 10:235–241.

Rowland JW, Hawryluk GWJ, Kwon B, Fehlings MG. 2008. Current status of acute spinal cord injury pathophysiology and emerging therapies: Promise on the horizon. Neurosurg Focus 25:E2.

Sabelström H, Stenudd M, Frisén J. 2013a. Neural stem cells in the adult spinal cord. Exp Neurol 260:44–49.

Sabelström H, Stenudd M, Réu P, Dias DO, Elfineh M, Zdunek S, Damberg P, Göritz C, Frisén J. 2013b. Resident neural stem cells restrict tissue damage and neuronal loss after spinal cord injury in mice. Science 342:637–640.

Savio T, Schwab ME. 1990. Lesioned corticospinal tract axons regenerate in myelin-free rat spinal cord. Proc Natl Acad Sci USA 87:4130–4133.

Silver J, Miller JH. 2004. Regeneration beyond the glial scar. Nat Rev Neurosci 5:146–156.

Sirko S, Behrendt G, Johansson PA, Tripathi P, Costa M, Bek S, Heinrich C, Tiedt S, Colak D, Dichgans M, Fischer IR, Plesnila N, Staufenbiel M, Haass C, Snapyan M, Saghatelyan A, Tsai L-H, Fischer A, Grobe K, Dimou L, Götz M. 2013. Reactive glia in the injured brain acquire stem cell properties in response to sonic hedgehog. [corrected]. Cell Stem Cell 12:426–439.

Sofroniew MV, Pearson RCA, Eckenstein F, Cuello AC, Powell TPS. 1983. Retrograde changes in cholinergic neurons in the basal forebrain of the rat following cortical damage. Brain Res 289:370–374.

Sroga JM, Jones TB, Kigerl KA, McGaughy VM, Popovich PG. 2003. Rats and mice exhibit distinct inflammatory reactions after spinal cord injury. J Comp Neurol 462:223–240.

Tanaka EM, Ferretti P. 2009. Considering the evolution of regeneration in the central nervous system. Nat Rev Neurosci 10:713–723.

The National Spinal Cord Injury Statistical Center. 2013. Spinal cord injury facts and figures at a glance. Available at: www.uab.edu/nscisc. Accessed October 7th, 2014.

World Health Organization. 2002. World Health Report 2002. Available at: http://www.who.int/whr/2002/en/. Accessed October 5th, 2014.

Tonchev AB, Yamashima T, Sawamoto K, Okano H. 2005. Enhanced proliferation of progenitor cells in the subventricular zone and limited neuronal production in the striatum and neocortex of adult macaque monkeys after global cerebral ischemia. J Neurosci Res 81:776–788.

Tone O, Miller JC, Bell JM, Rapoport SI. 1987. Regional cerebral palmitate incorporation following transient bilateral carotid occlusion in awake gerbils. Stroke 18:1120–1127.

Voigt T. 1989. Development of glial cells in the cerebral wall of ferrets: Direct tracing of their transformation from radial glia into astrocytes. J Comp Neurol 289:74–88.

Walder S, Zhang F, Ferretti P. 2003. Up-regulation of neural stem cell markers suggests the occurrence of dedifferentiation in regenerating spinal cord. Dev Genes Evol 213:625–630.

Wang Y, Cheng X, He Q, Zheng Y, Kim DH, Whittemore SR, Cao QL. 2011. Astrocytes from the contused spinal cord inhibit oligodendrocyte differentiation of adult oligodendrocyte precursor cells by increasing the expression of bone morphogenetic proteins. J Neurosci 31:6053–6058.

Weiss S, Dunne C, Hewson J, Wohl C, Wheatley M, Peterson AC, Reynolds BA. 1996. Multipotent CNS stem cells are present in the adult mammalian spinal cord and ventricular neuroaxis. J Neurosci 16:7599–7609.

Wester P, Watson BD, Prado R, Dietrich WD. 1995. A photothrombotic "ring" model of rat stroke-in-evolution displaying putative penumbral inversion. Stroke 26:444–450.

Westgren N, Levi R. 1998. Quality of life and traumatic spinal cord injury. Arch Phys Med Rehabil 79:19–21.

White RE, McTigue DM, Jakeman LB. 2010. Regional heterogeneity in astrocyte responses following contusive spinal cord injury in mice. J Comp Neurol 518:1370–1390.

Yamashita T, Ninomiya M, Hernández Acosta P, García-Verdugo JM, Sunabori T, Sakaguchi M, Adachi K, Kojima T, Hirota Y, Kawase T, Araki N, Abe K, Okano H, Sawamoto K. 2006. Subventricular zone-derived neuroblasts migrate and differentiate into mature neurons in the post-stroke adult striatum. J Neurosci 26:6627–6636.

Zai LJ, Wrathall JR. 2005. Cell proliferation and replacement following contusive spinal cord injury. Glia 50:247–257.

Zhang B, Gensel JC. 2014. Is neuroinflammation in the injured spinal cord different than in the brain? Examining intrinsic differences between the brain and spinal cord. Exp Neurol 258:112–120.

Zhang R, Zhang Z, Wang L, Wang Y, Gousev A, Zhang L, Ho K-L, Morshead C, Chopp M. 2004a. Activated neural stem cells contribute to stroke-induced neurogenesis and neuroblast migration toward the infarct boundary in adult rats. J Cereb Blood Flow Metab 24:441–448.

Zhang R, Zhang Z, Zhang C, Zhang L, Robin A, Wang Y, Lu M, Chopp M. 2004b. Stroke transiently increases subventricular zone cell division from asymmetric to symmetric and increases neuronal differentiation in the adult rat. J Neurosci 24:5810–5815.

Zhang RL, Chopp M, Roberts C, Liu X, Wei M, Nejad-Davarani SP, Wang X, Zhang ZG. 2014. Stroke increases neural stem cells and angiogenesis in the neurogenic niche of the adult mouse. PLoS One 9:e113972.

Zhang RL, LeTourneau Y, Gregg SR, Wang Y, Toh Y, Robin AM, Zhang ZG, Chopp M. 2007a. Neuroblast division during migration toward the ischemic striatum: A study of dynamic migratory and proliferative characteristics of neuroblasts from the subventricular zone. J Neurosci 27:3157–3162.

Zhang RL, Zhang ZG, Wang Y, LeTourneau Y, Liu XS, Zhang X, Gregg SR, Wang L, Chopp M. 2007b. Stroke induces ependymal cell transformation into radial glia in the subventricular zone of the adult rodent brain. J Cereb Blood Flow Metab 27:1201–1212.

Zhang RL, Zhang ZG, Zhang L, Chopp M. 2001. Proliferation and differentiation of progenitor cells in the cortex and the subventricular zone in the adult rat after focal cerebral ischemia. Neuroscience 105:33–41.

Zukor K, Belin S, Wang C, Keelan N, Wang X, He Z. 2013. Short hairpin RNA against PTEN enhances regenerative growth of corticospinal tract axons after spinal cord injury. J Neurosci 33:15350–15361.