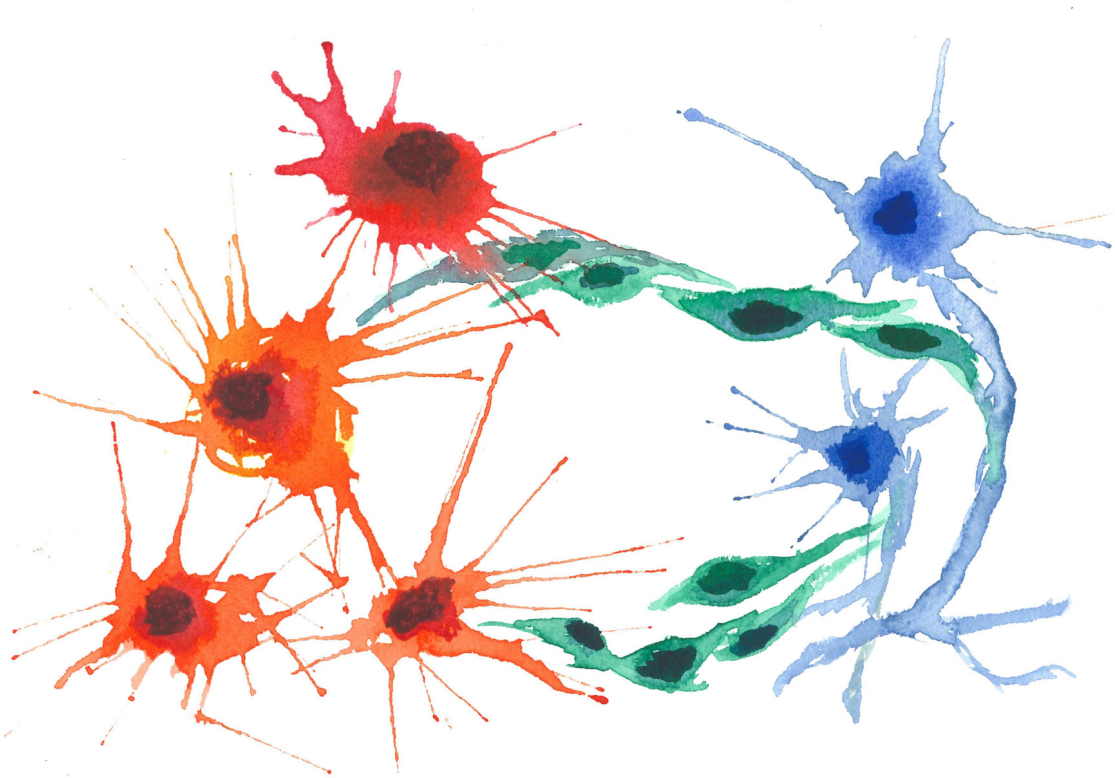
Thesis for doctoral degree (Ph.D.)
2020

The Neurogenic Potential of Astrocytes: A Story of Transformation and Renewal



Giuseppe Santopolo

Karolinska Institutet

From Department of Cell and Molecular Biology
Karolinska Institutet, Stockholm, Sweden

THE NEUROGENIC POTENTIAL OF ASTROCYTES: A STORY OF TRANSFORMATION AND RENEWAL

Giuseppe Santopolo

Karolinska Institutet

Stockholm 2020

Published by Karolinska Institutet.
Printed by US-AB

ISBN 978-91-7831-939-8

On the cover: Representation of the transformation process that leads from astrocytes to neurons. Watercolor by Alexandra Cîrciumaru.

The Neurogenic Potential of Astrocytes: a Story of Transformation and Renewal
THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Giuseppe Santopolo

Principal Supervisor:
Jonas Frisén
Karolinska Institutet
Department of Cell and Molecular Biology

Co-supervisor(s):
Konstantinos Meletis
Karolinska Institutet
Department of Neuroscience

Jens Magnusson
Stanford University
Department of Bioengineering

Opponent:
Malin Parmar
Lund University
Department of Developmental and Regenerative Neurobiology

Examination Board:
Milos Pekny
University of Gothenburg
Department of Clinical Neuroscience

Klas Blomgren
Karolinska Institutet
Department of Women's and Children's Health

Urban Lendahl
Karolinska Institutet
Department of Cell and Molecular Biology

To my family.

“Above all, don’t fear difficult moments. The best comes from them.”

- Rita Levi-Montalcini

ABSTRACT

To not simply repair an organ, but to regenerate and fully recover its function, like there was no injury at all, that is the goal of regenerative medicine. Of all the organs in the human body, the most delicate and difficult to regenerate is the human brain. Our understanding of its physiology changed dramatically in the last decades and the concept of the human brain as a never-changing tissue, where neurons are lost, but not produced after development is no longer valid. Today we know that new neurons constantly form and re-shape the connections between each other and that even in the adult brain, there are populations of stem cells that generate new neurons. Nevertheless, after an injury, a recovery *ad initio* is not possible in the adult human brain.

Different approaches have been tried to change this inability of the brain to regenerate. Stem cells have been the main target for the majority of these strategies, either by stimulating stem cells, which reside in the brain, or by retrieving them from outside sources. The problem with the endogenous stem cells is that, in the adult brain, they are not very active and can generate a very limited number of new neurons, which are insufficient for the recovery of the damaged tissue. As for the external sources of stem cells, these are not always available and they may involve problems of graft rejection. Therefore this is the reason for looking at alternative options within the brain itself.

Astrocytes are one of the most represented cell types in the adult mammalian brain, they can be easily generated and are strikingly similar to the stem cells. Astrocytes have been observed to generate neurons in animal models of Huntington's disease and stroke. Notch signaling seems to be crucial in regulating the neurogenic ability of astrocytes. In animal models where Notch signaling is impaired by deletion of *Rbpj*, astrocyte-derived neurons and neurogenic cells can be identified within the striatum. But many questions are still open: what processes are necessary for the astrocytes to generate neurons? Why do astrocytes from the striatum generate neurons, while astrocytes in other brain regions do not? Can we harness this neurogenic potential and use it in regenerative medicine? If so, how? These are some of the questions I tried to answer with my work.

In **Paper I**, we combined the two models we have previously described to induce astrocyte-derived neurogenesis. We blocked Notch signaling specifically in astrocytes by using an animal model in which *Rbpj* is knocked-out in Cx30-expressing cells. Subsequently, we induced a stroke in the same animals. After seven weeks, we observed an increase in the number of neuroblasts derived from astrocytes, compared to the deletion of *Rbpj* or stroke alone. This study shows that astrocyte-derived neurogenesis is a complex process, which can be induced by different kinds of stimuli, and further enhanced by their interplay.

In **Paper II**, we decided to further investigate what happens in astrocytes, on a transcriptomic level, when they become neurogenic. We used single-cell RNA sequencing to identify the differences between neurogenic and non-neurognic astrocytes at different time-points. We observed that, during differentiation into neurons, striatal astrocytes follow a trajectory that is reminiscent of the one followed by stem cells of the subventricular zone. Moreover, they start to sense the environment for pro-neurogenic factors. One of this is Egf, which administration in the brain of *Rbpj*-deleted mice enhances astrocytes-derived neurogenesis. Remarkably, the effect of Egf is visible even in areas of the striatum where astrocytes do not respond in the absence of injury.

LIST OF SCIENTIFIC PAPERS

I. **Giuseppe Santopolo**, Jens P. Magnusson, Olle Lindvall, Zaal Kokaia, Jonas Frisén (2020). Blocking Notch-Signaling Increases Neurogenesis in the Striatum after Stroke. *Cells* 2020, *9*, 1732.

II. Jens P. Magnusson, Margherita Zamboni, **Giuseppe Santopolo**, Jeff E. Mold, Mauricio Barrientos-Somarribas, Carlos Talavera-López, Björn Andersson, and Jonas Frisén (2020). Activation of a neural stem cell transcriptional program in parenchymal astrocytes. *eLife*, 2020;9:e59733

CONTENTS

LIST OF ABBREVIATIONS

NSCs	Neural Stem Cells
LV	Lateral Ventricle
SVZ	Subventricular Zone
DG	Dentate Gyrus
GFAP/Gfap	Glial Fibrillary Acid Protein
DCX/Dcx	Doublecortin
PSA-NCAM/Psa-Ncam	Polysialylated-Neural Cell Adhesion Molecule
RMS	Rostral Migratory Stream
OB	Olfactory Bulb
Igf2	Insulin-like Growth Factor 2
BMP/Bmp	Bone Morphogenic Protein
WNT/Wnt	Secreted Wingless
SHH/Shh	Sonic Hedgehog
ASCL1/Ascl1	Achetate-Scute Homolog 1
BBB	Blood Brain Barrier
GF	Growth Factors
VEGF/Vegf	Vascular Endothelial Growth Factor
EGF/Egf	Epidermal Growth Factor
FGF2/Fgf2	Fibroblast Growth Factor
MBD1/Mbd1	Methyl-CpG Binding Protein 1
BCBP/Bcbp	Brain Lipid-Binding Protein
IdU	Iododeoxyuridine
AD	Alzheimer disease
HD	Huntington’s disease
PD	Parkinson’s disease
ES	Embryonic stem (cells)
iPS	Induced pluripotent stem (cells)
Aldh1l1	Aldehyde dehydrogenase 1 family member L1

Cx30	Connexin 30
AAV	Adeno Associated Virus

1 INTRODUCTION

The greatest challenge of regenerative medicine is to understand which mechanisms are crucial not only to control the damage after an injury occurs, but also to reconstruct a tissue with its full functionality. Physiologically, adult mammals lack the ability to fully regenerate tissues after injury (with few exceptions such as the liver from a functional point of view) and this is particularly accentuated in humans. Our understanding of the regenerative process changed dramatically in the last decades. Today we know that the regenerative ability of specific tissues directly correlates with the presence of specialized cells able to proliferate and differentiate into tissue specific cell types. This role is usually undertaken by stem and progenitor cells. Tissues where stem cells are limited in number or in their ability to proliferate and differentiate regenerate poorly. The brain is one of the most striking examples. Even if stem cells within the brain keep generating new neurons throughout adulthood as reported in early works in the field (1–3), their ability to respond to an injury is limited. In humans neuronal death with loss of functionality following injury is often permanent.

Efforts have been made to enhance the regenerative ability of the stem cells in the adult brain, albeit with little success. New approaches aimed at finding different sources of new neurons, within the brain itself or from exogenous sources showed more promising results. Our work focused on a cell type that was initially overlooked in its ability to generate neurons, but holds a great potential: astrocytes. Although counterintuitive at first glance since astrocytes are involved in the formation of scar tissue after acute lesions such as stroke or traumatic injuries, their high numbers and spread distribution within the brain along with their resemblance to stem cells makes them an interesting resource of neuron generation. A neurogenic potential of astrocytes has already been described in the past years. Astrocytes can generate immature and mature neurons in artificial settings, such as when isolated from the rodent brain (4) or through *in vivo* reprogramming techniques (5–7) and also as a physiological response to injury in the mouse striatum (8,9).

The aim of this thesis is to characterize the mechanisms that regulate the neurogenic program in astrocytes. We developed tools that could be used in the identification of important factors to improve astrocyte-derived neurogenesis. We also proved that, by providing appropriate stimuli, the neurogenic response from astrocytes could be enhanced.

2 NEURAL STEM CELLS AND NEUROGENESIS

To fully understand the concept of regenerative medicine, we need to understand how different cell types can be generated in the adult brain. Tissue regeneration is virtually a ubiquitous process, occurring even in the brain and often carried out by stem cells, but very limited in mammals when compared to other animals like fishes and amphibians. Until the 1960s it was believed that neurogenesis, the phenomenon by which new neurons are formed, occurred only during embryonic development. Once the developmental phase was over neurons could die and never be generated anew. Our understanding of the brain changed with the discovery that new neurons are actually formed in the brain of adult mammals (1) by cells with stem cell properties named Neural Stem Cells (NSCs) (2,3,10).

Time, place and numbers of newly generated neurons greatly differ between species (11). In the case of rodents, by far the most common animal model for the study of neurogenesis, NSCs are found in two canonical niches: a region lining the Lateral Ventricle (LV) called Subventricular Zone (SVZ) and the Dentate Gyrus (DG) of the hippocampus. Under physiological conditions, NSCs from these two niches generate new neurons following a well-characterized pathway that will be further discussed in this chapter.

2.1 NEUROGENESIS AND NEUROGENIC NICHES IN RODENTS

2.1.1 The subventricular zone

One of the main neurogenic niches in the mammalian brain is the SVZ, which is an area adjacent to the LV. Within the SVZ reside different cell types, including ependymal cells, astrocytes and the NSCs (Figure 1). The precursor cells from the SVZ have been classified into three categories: type B, C and A. The type B cells, the proper NSC, are identified by the expression of markers such as Glial fibrillary acid protein (Gfap), Nestin and Sox2. Just as their embryonic equivalents, they retain the basic apical-basal polarity and the ability of self-renewal and multipotency (12). Type C cells can be recognized by the expression of the markers Sox2, Dlx and Olig2. They are also called transit-amplifying progenitor cells. They represent an intermediate stage where NSCs activate and undergo several cycles of proliferation into a short period and then terminally differentiate. Type A cells are the migrating neuroblasts expressing Doublecortin (Dcx) and polysialylated-neural cell adhesion molecule (Psa-Ncam). Neuroblasts are immature neurons that towards the end of their differentiation process start to express Tuj1, a typical marker for mature neurons. Chains of migrating neuroblasts trek through the Rostral Migratory Stream (RMS) from the SVZ to reach the Olfactory Bulbs (OB). Here the neuroblasts detach from the chains of migrating cells, disperse within the OB and differentiate into granule and periglomerular neurons (13,14). It is important to note that the nature of the newly generated neuron depends on the origin of the NSCs: deep granule cells and calbindin-expressing periglomerular cells are

generated by NSCs located in the ventral part of the SVZ, whereas superficial granule cells and tyrosine-hydroxylase-expressing periglomerular cells are produced by NSCs from the dorsal SVZ (15). It is still under debate whether this difference in neurogenic potential is intrinsic to the original NSCs or rather dictated by environmental factors.

Like all stem cell populations, NSCs have the ability to self-renew and give rise to differentiated cell types. Initially NSCs were thought to be tri-potent, meaning they could generate neurons, astrocytes and oligodendrocytes. This hypothesis came from *in vitro* experiments which demonstrated the development of these cell types in neurosphere assays of monolayer cell cultures (10,16). Further studies based on population fate-mapping of NSCs from the SVZ revealed however that only neurons and oligodendrocytes can be generated (17) and even more recent *in vivo* clonal analysis has proven that NSCs from the SVZ generate solely neurons (18). Nevertheless, *in vitro* individual NSCs have the ability to generate either neurons or oligodendroglial cells but never both cell types (19). Whether NSCs from the adult mammalian brain have a tri-lineage potential remains a topic that requires further investigation. Some groups proposed that NSCs become specific gradually during development and lose their ability to generate multiple cell types in the adult brain (20). Another model suggests that adult NSCs could be intrinsically tri-potent, but the niche is suppressing their ability to generate specific cell types. The ability of endogenous adult NSCs to self-renew in the long-term is also under debate. Studies involving clonal analysis revealed that, following a phase of expansion, there is a depletion of NSCs (18). Genetic labeling approaches do not come to help in this context, since each model may target different sub-populations of NSCs or the same sub-population at different states, producing inconsistent/discordant/inconclusive results. Understanding the extent to which NSCs have self-renewal capacity will be a critical challenge for the future, with the promise of developing better targeting approaches.

The NSCs have a specialized apical process with a primary cilium, reminiscent of radial glial cells, that allows the NSCs to be in direct contact with the LV. The apical endings of the NSCs are surrounded by ependymal cells, which are necessary for the formation of intercellular junctions between NSCs and ependymal cells within the niche (21). Another role of the ependymal cells is to maintain the molecular composition of the stem cell niche by allowing the cerebrospinal fluid to (re)circulate (22). The cerebrospinal fluid is able to modulate the NSCs behavior because it is rich in soluble factors such as Insulin-like growth factor 2 (Igf2) (23), Bone morphogenic proteins (Bmps) (24,25), secreted Wingless (Wnt), Sonic hedgehog (Shh) and retinoic acid (26,27).

The Notch/Delta signaling maintains NSCs in an undifferentiated status by inhibiting the production of transit-amplifying progenitor cells (28). Type C cells express high levels of Achaete-scute homolog 1 (Ascl1), which is repressed by Hairy and enhancer of split-1 (Hes1), one of Notch's target genes. Ascl1 promotes Notch ligands expression, suggesting that C cells may inhibit the activation and differentiation of NSCs by lateral inhibition (29,30).

The neurogenic environment in the SVZ is further improved by the local characteristics of the Blood Brain Barrier (BBB) that is more permeable around the SVZ, allowing some Growth Factors (GF) to diffuse (31). Furthermore, on the basal end of the NSCs, there are processes that come in direct contact with the blood vessels. This facilitates the flow of vascular factors which are potentially stimulating neurogenesis in the SVZ. Indeed, infusion of Vascular endothelial growth factor (Vegf) promote cell proliferation in the SVZ (32). Epidermal growth factor (Egf) and Fibroblast growth factor 2 (Fgf2) maintain pools of NSCs both *in vitro* and *in vivo*. However, only Fgf2 increases the number of newly generated neurons (33). Egf has been reported to inhibit the differentiation of transit-amplifying progenitor cells into neuroblasts (34). Interestingly, by inducing the expression of the receptor tyrosine-protein kinase ErbB2, Gfap expressing cells in the adult SVZ re-acquire radial glia morphology. This would suggest that Egf has a role in the inhibition of NSCs differentiation (35).

Until here I discussed the role of the major canonical signaling pathways in regulating adult neurogenesis along with other intracellular mechanisms known to affect this process. Several transcription factors play a crucial role in adult neurogenesis. The orphan nuclear receptor Tlx and Bmi-1 are required for the maintenance of adult forebrain NSCs (36). Pax6 induces differentiation of progenitor cells from the SVZ into neurons (37), while Olig2 has an opposite effect (38,39).

Epigenetic modifications are also involved in regulating adult neurogenesis: increased genomic instability is observed in NSCs that lack the Methyl-CpG binding protein 1 (Mbd1), resulting in decreased neuronal differentiation.

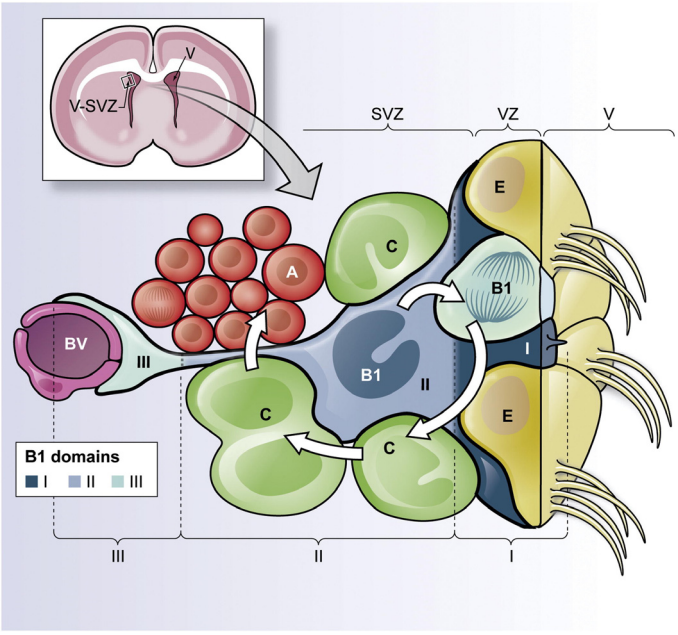


Figure 1: Location of the SVZ in the LV and magnification to illustrate the structure of the neurogenic niche. Type B cells are surrounded by the ependymal cells (E), forming pinwheel-like structures on the ventricular

surface. Type B (NSCs) cells differentiate into type C cells (the transit-amplifying progenitor cells), which divide to generate type A cells, also known as neuroblasts. The NSCs cells retain a process on the apical side containing a cilium responsible to contact the lateral ventricle. On the basal side, instead, there is a long process ending on blood vessels (BV in the figure). (Image from Fuentealba LC, Obernier K, Alvarez-Buylla A. Adult neural stem cells bridge their niche. *Cell Stem Cell*. 2012;10(6):698-708. doi:10.1016/j.stem.2012.05.012. Used with permission from Cell Press).

2.1.2 The dentate gyrus of the hippocampus

The second main neurogenic niche in the mammalian brain is the DG (Figure 2). Here, the NSCs are also called type-1 progenitors and they present radial glia-like features. In the DG, NSCs extend an apical process through the granular cell layer. As in the SVZ, NSCs express Gfap, but also vimentin, and Brain lipid-binding protein (Blbp), common to immature astrocytes and radial glia. Until recently the common understanding was that in normal conditions only 1-2% of the NSCs in the hippocampus undergo active division (40–42), thus the NSCs from the DG were considered quiescent cells. New studies involving single-cell transcriptome analysis of quiescent adult NSCs from the DG showed expression of receptors involved in niche signals and downstream signaling components. Once NSCs become active, many of these signals are down-regulated (43). In particular, decreased levels of GABA (44) or Wnt inhibitor (45) leads to activation of the NSCs. Therefore, quiescence needs to be actively maintained. The idea that stem cells are active as long as the environment does not suppress their replication is becoming more and more established within the scientific community even for other stem cell types and the topic has been reviewed elsewhere (46). Other important signaling pathways involved in the regulation of equilibrium between quiescence and the entrance in cell cycle are the Bmp. The NSCs in the DG express the Bmp-receptor 1A. When activated by its ligands Bmp-2 and Bmp-4, Bmp-receptor 1A start a cascade that enables the maintenance of the quiescence of the NSCs (47). On the other hand, Noggin can bind the Bmp, inducing the NSCs activation and entry into the cell cycle. In addition, Shh and canonical Notch signaling are also responsible for the quiescence and activation of the NSCs in the DG (48,49). Sox1 plays an important role in NSC activation: Sox1 positive NSCs in the DG produce precursors that can further differentiate into neurons and astrocytes (50).

NSCs in the DG divide by asymmetric divisions to generate transit amplifying progenitor cells that in the DG are also called type-2 cells. As in the SVZ, these cells proliferate more intensively than the type-1 and are able to differentiate into the type-3, the neuroblasts. The majority of the neuroblasts is not needed and undergoes apoptosis (51,52). The surviving cells differentiate into mature neurons. Interestingly, the existence of a second population of hippocampal NSCs had been proposed (49). These cells do not present the standard radial structure and are called horizontal hippocampal stem cells. Nevertheless, further characterization of this type of cells is still to be done.

The process described above requires several weeks and once completed newly generated neurons are integrated in the DG. These neurons are hyper-excitable and exhibit a lower threshold for induction of long-term potentiation compared to mature neurons, resembling their developmental counterparts (53). Adult-born neurons also induce plasticity, by creating new synaptic connections with the pre-existing neurons. These features contribute to create variability in the information coding within the brain circuits.

By this point one question remains unanswered: what could be the function of these newly generated neurons? New neurons, both in the hippocampus and in the OB seem to be critical for the process of new memory formation (54–56). In particular, neurogenesis in the DG contributes to long-term spatial memory and pattern separation (57–59). In the OB, interneurons are involved in short-term olfactory memory and olfactory associative learning (60).

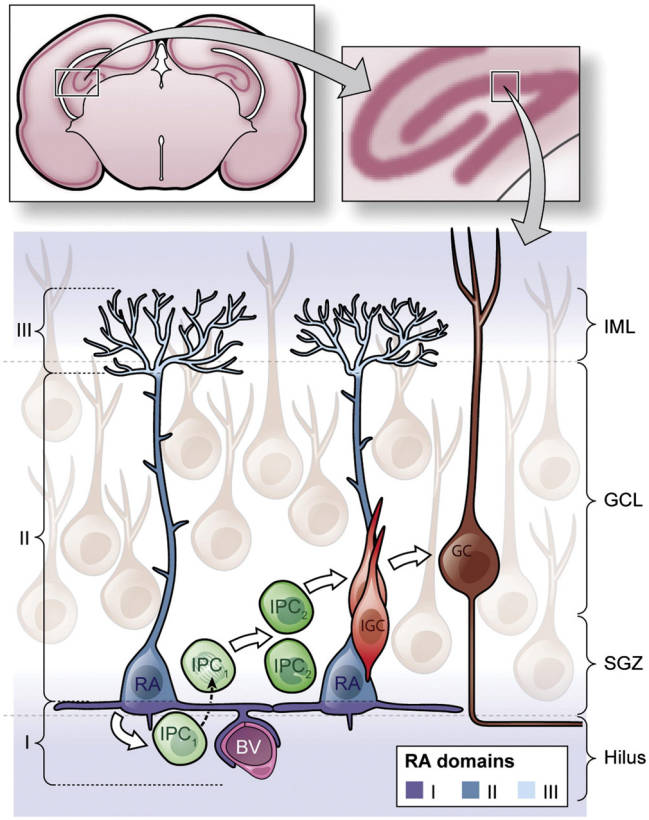


Figure 2: Location of the DG in the hippocampus and magnification of the stem cell niche. Type-1 cells are the radial glia like cells (RA). Tyoe-2 are the intermediate progenitors (IPC 1 and 2 in the figure) that can differentiate into type-3 cells, the neuroblasts (IGC). Finally, the type-3 cells will migrate and differentiate mature granule cells (GC in the figure). (Image from Fuentealba LC, Obernier K, Alvarez-Buylla A. Adult

neural stem cells bridge their niche. *Cell Stem Cell*. 2012;10(6):698-708. doi:10.1016/j.stem.2012.05.012. Used with permission from Cell Press).

2.1.3 Neurogenesis outside the canonical niches

Throughout the years neurogenesis has been described in other brain regions than the SVZ and the DG, but, since these areas are not conserved between species or experience neurogenesis only as a response to injury, they are not considered canonical niches.

In different rodent species, pigs and primates the following areas have been observed to harbor neuroblasts: the striatum, neocortex, amygdala, piriform cortex and adjoining perirhinal cortex (61,62). In rabbits, which on the evolutionary scale are as distant from humans as mice, striatal neurogenesis has been observed in physiological conditions (63). Similar observations have been done in rats (64,65) and non-human primates (66), although these results have not been replicated in other studies and require caution (67,68). Under physiological conditions, neurogenic cells are never observed very far from the SVZ. This may be because the niche plays a critical role in the creation of a pro-neurogenic microenvironment.

Putting together all the information listed until now, it is clear that our understanding of adult neurogenesis in mammals walked a long way since the 1960s. But one question arises when reading what is known about rodents: what do we know about the adult human brain?

2.2 ADULT NEUROGENESIS IN THE HUMAN BRAIN

The first part of this thesis discussed the topic of adult neurogenesis in the mammalian brain and the animal models; rodents in particular, have been considered the paradigm for it. Nevertheless, the interest of the scientific community and of society is aimed at understanding how much of this knowledge applies to humans. In many ways, humans are very different from the rodents and the brain in particular has many layers of complexity that the rodent brain cannot represent. That is why much effort is aimed at understanding if and how neurogenesis occurs in the adult human brain.

2.2.1 Neurogenesis in the human subventricular zone and striatum

To assess the neurogenic potential of the human brain the first step is the identification of the NSCs and the stem cells niches (or their equivalents). The natural places to start are the LV and the hippocampus (Figure 3). A meticulous investigation of the human LV and SVZ identified the presence of a ribbon of GFAP-expressing astrocytes, but lacked the chain migration of neuroblasts typical of mouse SVZ (69). A later study also revealed, within the human SVZ the presence of PSA-NCAM positive cells, a typical marker for neuroblasts, but

failed to identify DCX expressing cell (70). It is important to mention at this point that the presence of specific cellular markers widely used in rodents are not as reliable when characterizing the human brain. Due to some differences in expression patterns and technical difficulties in the tissue processing, the presence or absence of markers like DCX or PSA-NCAM should not be used as a definitive proof. An example is given by the marker DCX which is not restricted to neuroblasts in the human brain (71). More about this topic will be discussed later.

To understand if some of these differences are due to incomparable neurogenic mechanisms between rodents and humans, studies of the infant and fetal human brain have been conducted. Both the fetal and post-natal human forebrains have been found to be populated by high numbers of cells that are actively proliferating and expressing neurogenic markers such as DCX and PSA-NCAM (72), and vimentin (73). Moreover, although some neuroblasts in the fetal and post-natal human brains do form chains of migrating cells seen instead in the rodent and the primate brains, most neuroblasts migrate tangentially to the LV. Some of the neuroblasts that migrate in chains have also been observed to detach from the RMS and trek towards the prefrontal cortex, leading to speculation regarding the role of these neuroblasts in the human brain compared to the rodents and the target regions of adult human neurogenesis. Neurogenesis from the SVZ in humans seems to decline very rapidly and become almost undetectable 9 months after birth (73). After this initial finding, later studies questioned the existence of the RMS in humans with contrasting results. Proliferative cells have been reported surrounding an extension of the SVZ towards the OB (74), but later studies, that confirmed the existence of the described structure, could not identify the proliferating and migrating neuroblasts (72,73). Nevertheless, such differences in results may be due to the panel of markers chosen to assess proliferation.

As previously discussed, SVZ derived neurogenesis in rodents is a mechanism, which ensures the formation of new neurons that will integrate into the pre-existing circuits of the OB. This is not the case for humans and this may be easily explained by the fact that olfactory perception in our species does not play such an important role as for rodents.

Quantification of neurogenesis in humans has also been a challenge in the field. Considering the rarity and ambiguity of NSCs in the human SVZ and the difficulty in tracing neuroblasts and newly generated neurons, studies based on staining were not capable of estimating the number of neurons that are generated in the adult human brain. The application of techniques based on the analysis of the ^{14}C levels in the neuronal population provided an answer to this question. The rationale behind this technique is that ^{14}C concentration in the human body closely mirrors the atmospheric levels (75–77). Due to the aboveground tests for nuclear weapons during the 1950s and ‘60s, the levels of ^{14}C in the atmosphere almost doubled, to then slowly go back towards pre-tests levels after the Test Ban Treaty of 1963. These changes in the levels of atmospheric ^{14}C concentration have been used to date the age of cells. When a cell will undergo mitosis, ^{14}C will be integrated into the newly synthetized DNA. The concentration of ^{14}C in the DNA, will therefore mirror the concentration in the atmosphere at

that precise moment (78–81). When such technique was used to estimate the neuronal turnover in the human OB the ^{14}C concentration in the DNA was not different from the concentration at the time of birth and mathematical modeling of the results estimated that no more than 1% of the neurons from the OB could have been replaced during adulthood 82. Taken together, this points out that adult neurogenesis in the human SVZ is not aimed at replacing neurons in the OB.

Given that NSCs in the human SVZ differentiate into neuroblasts and that these neuroblasts do not seem to generate neurons destined for the OB or undergo apoptosis (83), one could wonder as to where are the new neurons generated in the human brain? The observations in the OB where not surprising after all since, as mentioned, the OB in humans are less complex/sensitive than the rodents'. However, there is another area in the human brain that would instead experience an increase in complexity compared to rodents and that is localized close to the SVZ: the striatum. The analysis of iododeoxyuridine (IdU) incorporation in the brain of patients treated for cancer revealed IdU positive interneurons in the striatum (83). This meant that these interneurons must have been generated during adulthood, when the patients were treated with IdU. In the same study, ^{14}C concentration in the neuronal population from the striatum revealed that only DARP23 negative interneurons are generated, with a turnover of approximately 2.7%/year according to the most likely mathematical model (83). Although no formal demonstration is given of the SVZ origin of these adult-generated interneurons, considering the distance from the SVZ and the presence of NSCs and neuroblasts that migrate tangentially from the LV, it is interesting to speculate if, during the evolution of the human brain, the increasing complexity of the striatum compared to the OB and the need for new neurons to be generated induced a change in the final destination of the SVZ-derived neuroblasts. Later studies failed to replicate such results and instead suggest that there is no neurogenesis in the human striatum (68) and more research is needed to get a definitive answer.

2.2.2 Neurogenesis in the human hippocampus

The second neurogenic niche common in mammals is the hippocampus, which in recent years attracted interest, due to contrasting results and the re-evaluation of the topic using new techniques. As already shown for the SVZ, the first approach to verify the existence of NSCs and neurogenesis in the human hippocampus was based on quantification of bromodeoxyuridine (BrdU) positive neurons in the brains from patients treated for cancer. As for IdU quantification in the striatum, BrdU is incorporated in the DNA during cell division. The presence of BrdU in the neurons of the patients could mean that these neurons derive from NSCs that underwent division while the patient was under treatment (84).

Later studies aimed at validating the information accumulated about murine hippocampal neurogenesis in humans. Cells positive for DCX were identified in the DG dissected from individuals of age ranging from 1 to 100 years (85). These cells not only expressed DCX, but

also a panel of markers associated with hippocampal neurogenesis in mice, such as the proliferation markers Ki67 and Mem2, the transcription factor Prox1 (associated with granule cell development) and the calcium binding protein calretinin. For older individuals, DCX expressing cells were often found to be positive for the neuronal marker NeuN, information that today has to be considered more carefully, considering the concerns regarding DCX expression in humans (discussed in (86)). As observed for the SVZ, it was clear that the DG suffers a decline in neurogenesis within the first years after birth (85).

Analysis of the ^{14}C concentration in the human hippocampus confirmed that both the non-neuronal and neuronal populations experience turnover throughout life. In particular, it seems that two populations of neurons constitute the human hippocampus: one with a turnover rate of approximately 1.75% per year, while the second is not renewed at all (87). By using this method, it was also possible to model the decline in hippocampal neurons and verify that it closely resembles the decline described in neuroblasts.

Recently, the debate on human neurogenesis in the adult DG was re-ignited by contrasting results regarding the presence of DCX positive cells in the adult human hippocampus (88,89). The scientific community now questions the use of DCX and PSA-NCAM as markers for neurogenesis in humans, as it seems that these markers can easily degrade and become difficult to stain if the post-mortem interval is prolonged (86,90). Moreover, independently of the presence of DCX and PSA-NCAM positive cells, the results obtained with BrdU and ^{14}C quantification are not disproven.

Many questions remain unanswered on what is the role of neurogenesis in the adult human brain, its potential in regenerative medicine and to which extent we can control it in order to develop new therapies. More about this will be discussed in the next chapter.

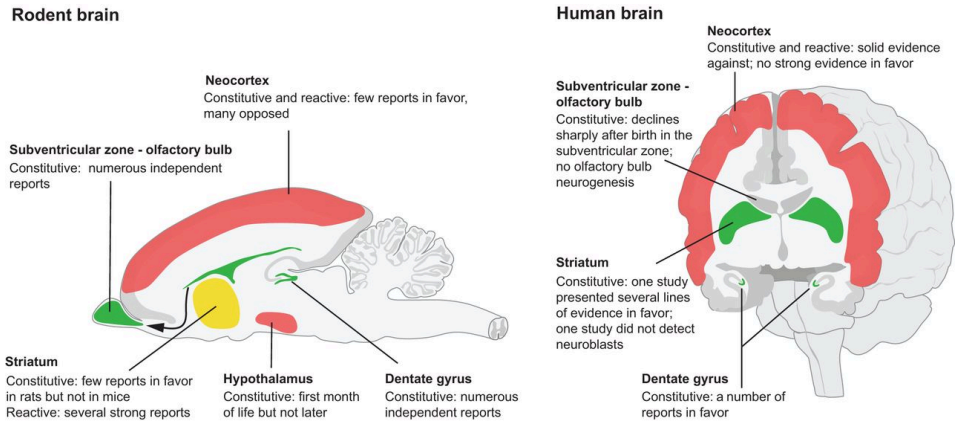


Figure 3: Comparison of the neurogenic niches between mouse and human. A) Location of the LV and DG in the mouse brain. B) Location of LV, striatum and DG in the human brain (Image from Magnusson JP, Frisén J. Stars from the darkest night: unlocking the neurogenic potential of astrocytes in different brain

regions. *Development*. 2016;143(7):1075-1086. doi:10.1242/dev.133975. Used with permission from The Company of Biologists).

3 REGENERATIVE MEDICINE OF THE BRAIN

The discovery of adult NSCs and their ability to generate new neurons raised hopes that they could be involved in tissue repair following injury. However, apart from conferring an additional layer of plasticity to the brain, it seems that NSCs in the adult mammalian brain are not adapted to efficiently regenerate large portions of tissue. Nevertheless, as discussed in this chapter, NSCs respond to different kind of injuries and neurodegenerative disease. Understanding which mechanisms are involved in the neurogenic response and what are the factors playing against it brings the possibility of manipulation in the view of improving the regenerative capacity of the human brain. In this chapter I will discuss the neurogenic response to some types of injuries and diseases, with a particular focus on stroke. In the second part, I will summarize the approaches that have been taken until now to enhance the neurogenic response to damage.

3.1 REGENERATIVE RESPONSE TO INJURY

An endogenous neurogenic response has been observed following injury during neurodegenerative diseases. Quantifications of Ki67 expression or BrdU incorporation have proven that NSCs undergo proliferation following seizure activity in the rodent DG and SVZ (91–94). This seems to be triggered by the ability of NSCs to sense the electrical activity around them (59), by activation of their GABA receptors (44,95,96) or epigenetic changes induced by seizure (97,98). The increase in number of proliferating cells after seizures is mirrored by an increased number of new neurons, as assessed by the expression of neuronal markers in BrdU positive cells 4 weeks after seizure (91,93). Many of these new neurons show signs of abnormalities, that can potentially alter the connectivity within the hippocampus (99–101). In the SVZ, the seizure-induced neurons migrate more rapidly than in normal conditions, often leaving the RMS prematurely (94).

Neurogenesis and the neurogenic niches are also affected in different neurodegenerative diseases, but in different ways depending on the disease. From post-mortem brain analysis, researchers described increased cell proliferation in the DG of patients affected by Alzheimer disease (AD) and in the SVZ of patients with Huntington's disease (HD), but neurogenesis was decreased in the DG and SVZ of patients with Parkinson's disease (PD), as discussed in the rest of this chapter.

Neurogenesis in AD is a controversial topic, while some early studies found that markers for neurogenesis and for neuronal cell types are overexpressed in the human DG (102), suggesting that new neurons are formed, more recent studies observed a steep decrease in neurogenesis in AD brain (103,104). Many animal models have been generated to study how neurogenesis is affected in the AD brain, but the results are conflicting. Some studies reported that mice carrying mutations in the *presenilins-1* gene had reduced proliferation in

the hippocampus and only the wild-type human presenilin-1 gene overexpression promoted survival and differentiation of NSCs into immature neurons (105,106). Nevertheless, different mutations of presenilin-1 have instead been associated with enhanced proliferation and neurogenesis in the mouse brain (107). Regardless of whether these mutations induce an increase or a decrease in hippocampal neurogenesis, it seems clear that presenilin-1 mutations affect the neurogenic process in the adult hippocampus, and this may have a significant contribution in the pathogenesis of AD.

Similar inconsistencies in results have been observed in other mouse models for AD: mutations of the gene encoding amyloid precursor protein have been associated with both decreased (108–110) and increased (111) proliferation and neuronal survival.

Other neurodegenerative diseases have been modeled in animals by overexpressing the wild type or mutated form of α-synuclein that accumulates in PD, dementia with Lewy bodies and multiple system atrophy. Overexpression of the wild-type protein is associated with decreased neuronal survival in both the DG and SVZ. While expression of wild-type α-synuclein does not affect the NSCs proliferation rate, mutations of this protein are associated with decrease proliferation in the SVZ (112).

All the injury models presented so far affect the neurogenic process and cause a loss of neurons. Many more could be included in this list, i.e. inflammation and cancer, however our focus resides in one particular model: stroke.

3.2 STROKE: DISEASE AND NEUROGENIC RESPONSE TO INJURY

3.2.1 Ischemic stroke

Stroke is one of the most common neurological diseases in the western world and 13.7 million people experience a stroke every year. Stroke is fatal in almost half of the cases and it has debilitating outcomes for 5.5 millions people every year (113). There are three types of stroke: hemorrhagic stroke, in which a blood vessel rupture causes bleeding in the brain; transient ischemic stroke, a temporary blockage or reduce in the blood flow, that usually does not have a longstanding effect; and ischemic stroke, when a blood vessel is occluded. The latter is the most common form of stroke, accounting for approximately 87% of all cases. The ischemic stroke is characterized by three distinct phases: the acute phase, when the ischemic attack is followed by reperfusion; the sub-acute phase, with the formation of the glial scar (a process that requires few weeks); and lastly the chronic phase, which is characterized by the continuation of the inflammatory process and neurogenic response. The outcomes of the ischemic stroke are visible for the rest of the affected person's life and often include conditions such as diminished motor function of the paretic side, impairment of speech and loss of vision, which gravely reduce the quality of life and increase the overall mortality. More than 20% of the people that will experience a stroke will be dependent on others for assistance; as a consequence health care costs are increased in this category of population.

Risk factors for stroke are both modifiable (smoking, high alcohol consumption, physical inactivity, unhealthy diet) and non-modifiable (sex and genetic predisposition). Both sex and age are important factors, with women below 85 years of age having a lower risk compared to men. However after this age limit this trend reverses. Stroke patients often have one or more concomitant comorbidities such as diabetes, cardiovascular diseases or kidney disease, which further contribute to the poor prognosis of recovery and predispose to higher mortality rates than the healthy population. In 2010, the direct and indirect costs of stroke amounted to 36.5 billion US dollars just in the United States (114).

After the ischemic event, it is possible to identify two distinct areas: the ischemic core and the penumbra. The core is characterized by blood flow below 10-25% (10-12ml/100g/min or less) of the normal levels and low ATP levels. This is the area with the highest degree of cell death; neurons are the cell type that is the most susceptible, but many of the glial and supporting cells are lost as well. The damage within the core is such that even if treatment is provided within four hours from the ischemic event and normal blood flow and ATP levels are restored, cell death is inevitable. The penumbra, instead, is the area between the necrotic core and the unaffected tissue where the lack of oxygen and nutrient is not such to cause irreversible damage (60ml/100g/min). Many of the cells in the penumbra may even survive and recover after the ischemic event, as long as treatment is provided within few hours. The penumbra is also where the endogenous regenerative process takes place in the weeks and months following a stroke.

The main events that lead to cell death during and after an ischemic event are depletion of oxygen and glucose. These will cause a drop in the levels of ATP that will result in the impairment of many processes necessary for cell survival. Duration, intensity and location of the ischemic event are directly correlated with the extent of damage. Within the first minutes after the ischemic event, the pre-synaptic voltage dependent Ca^{2+} channels are activated, leading to uncontrolled release of excitatory amino acids that are left in the extracellular space. The accumulation of such amino acids creates a toxic environment with uncontrolled activation of N-methyl-D-aspartate receptor (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) receptors, resulting in the activation of proteases, lipases and nucleases that will lead to cell membrane damage and cell death (115).

All the processes described above are responsible for the immediate damage and cell death by necrosis that takes place during the acute phase within the ischemic core. During the later phases of stroke, peripheral neurons are exposed to adverse conditions that may lead to apoptotic cell death. Genes like *Bcl2* and *p53* are up-regulated and induce the release of pro-apoptotic signals like cytochrome c, which speeds up the activation of the Caspase cascade (116). Other cell death pathways induced by the ischemic event include p53 (117), Jnk (118) , c-Jun, p38 and Cdk-5 (119).

The ischemic event and the subsequent hypoxia induce the release of proteases in the surrounding tissues, which cause disruption of the extracellular matrix. The BBB is also impaired, allowing uncontrolled diffusion and secondary damage (reviewed by (120)).

Inflammatory processes are also associated with further propagation of the injury and they are typical of the chronic phase of the disease.

As mentioned in this chapter, stroke is a severe disease, but many of the patients that are affected will recover to some degree. This is possible because the brain responds to the injury by activating a neurogenic response that may be responsible for functional recovery (121), but more evidence are needed. In the next part of this chapter I will discuss the characteristics of the endogenous regenerative process following stroke and how the scientific community has tried to modulate it and enhance it.

3.2.2 Neurogenic response to stroke

Following the ischemic event, NSCs in the rodent SVZ become activated and increase their neurogenic ability (122–124) (process summarized in Figure 4). Injection of BrdU in animals following the induction of an ischemic event allowed the identification of cells either BrdU and DCX/PSA-NCAM positive or BrdU and NeuN positive (64,123).

Many of the neuroblasts that are generated as a response to stroke do not migrate through the RMS, but detach from it and migrate towards the injured area, both in the cortex and in the striatum. In the striatum, neuroblasts start to express markers typical of the striatal medium spiny neurons (like Pbx and Meis2 and DARP-32 after complete differentiation) that are the most affected cells during stroke (64). NSCs in the SVZ of rodents increase their neurogenic potential immediately after stroke, but they stay activated for several months during the chronic phase of the disease (125) and neuroblasts are observed to migrate into the lesion site for up to one year (126).

To be able to detach from the RMS and migrate to the penumbra of the ischemic area neuroblasts use the vasculature as a scaffold (127), taking advantage of the neo-angiogenic process taking place within the injured brain. Within the striatum of rodents following stroke, neuroblasts are often observed adjacent to blood vessels and their migration can be modulated by infusion of factors able to influence the angiogenic activity (124,125,128). Moreover, The Slit-Robo signaling is used by neuroblasts to migrate through the glial scar and by manipulating it is possible to allow further migration into the injured area (129).

Detachment of neuroblasts from the RMS and migration into the ischemic area is also regulated by the release of metalloproteases, chemokines and chemokine receptors (like CXCR4) (125,128,130–133), which I previously mentioned as factors released in the area of the brain that experiences an ischemic event. The detachment of the neuroblasts from the SVZ and their migration towards the striatum comes at the expenses of neurogenesis in the OB. After stroke, less neurons will be formed in the OB located on the same hemisphere as the stroke.

However, only a small portion of the neurons is replaced in the long term following stroke and more than 80% of them die between 2 and 6 weeks after stroke (64). Nevertheless, the presence of these short-lived cells is correlated with a better outcome and recovery, at least in rodents (134), suggesting that they may play a role in the creation of a stable pro-regenerative environment. The inflammatory environment still present in the brain may affect neuronal long-term survival long after the stroke. Administration of anti-inflammatory indomethacine, a molecule that suppresses inflammation and microglia activation, stimulates the formation of newborn cells in the striatum following stroke (135). Tnf-α signaling is also involved in the neurogenic process after the ischemic event and it has a detrimental role (136). Microglia has been shown to have a positive effect on SVZ neurogenesis (137) and in different models of wound injury in mammals the modulatory role of the immune system is beneficial for the regenerative process.

Nevertheless, the regenerative response following stroke in mammals has its limitations. First, stroke is a disease that is usually experienced later in life, when the NSCs are fewer and not as active compared to younger ages. Another factor that has been previously mentioned is the formation of the glial scar, which blocks neuroblasts migration. These are the reasons behind the importance of developing new strategies in order to increase the regenerative response following stroke.

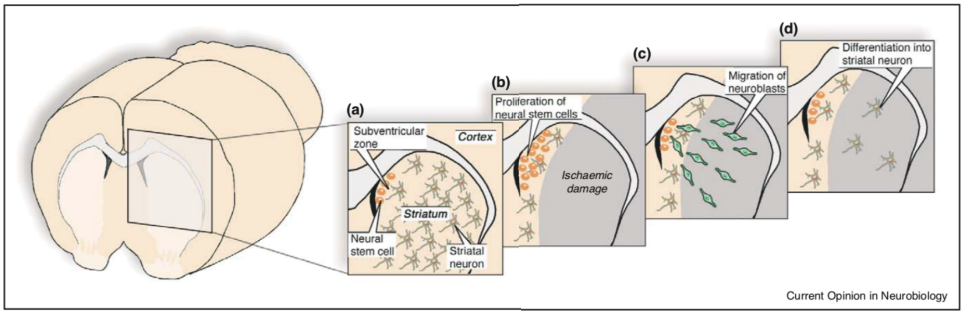


Figure 4: Neurogenesis in the mouse brain following stroke. A) Cellular composition of the healthy striatum. B) Neuronal loss and formation of core and penumbra following the ischemic event. C) Neurogenic process to repair the injured striatum. D) New neurons formed in the mouse brain. (Image from Kokaia Z, Lindvall O. Neurogenesis after ischaemic brain insults. *Curr Opin Neurobiol.* 2003;13(1):127-132. doi:10.1016/s0959-4388(03)00017-5. Used with permission from Elsevier).

3.3 REGENERATIVE MEDICINE OF STROKE

As discussed in the previous chapter, the neurogenic response starts during the acute and sub-acute phase of the disease, but continues several months into the chronic phase. This last phase of the disease is the one that represents the main target for the therapeutic approaches aimed at increasing the regenerative capacity of the brain.

The approaches of foremost interest are the ones based on the use of stem or progenitor cells and they can be divided in two main categories: transplantation from exogenous sources or stimulation of the endogenous population.

3.3.1 Transplantation from exogenous sources

3.3.1.1 Human fetal NSCs

The stimulation of the endogenous stem cell niches presents some intrinsic limitation. As mentioned previously, the NSCs in the adult mammalian brain are less active and more difficult to stimulate. That is why many groups are addressing the concept of using cells from exogenous sources. This way it is possible to have active stem or progenitor cells and to transplant them in high numbers. The role of the transplanted cells would be both to replace the dead cells and to provide support and thus create a pro-regenerative environment, for example by secreting pro-neurogenic factors.

Transplantation protocols have been attempted with fetal stem cells, embryonic stem (ES) cells and induced pluripotent stem (iPS) cells (Figure 5). Similar approaches have already been tested in humans with positive results for treatment of PD (for extensive reviews see (138,139)), giving hope for similar outcomes in patients with stroke. The most promising studies are the ones where human cells have been tested for their capacity to generate neurons after transplantation into the brain of rodents following stroke. Many of these attempts have been based on human fetal stem cells (140–143) but also on iPS cells (144).

Human fetal stem cells transplanted into the brain of rodents following induction of stroke were able to migrate into the lesion site and differentiate into neurons both in the striatum (141) and in the cortex (143). The studies in the cortex also revealed that transplantation outside the ischemic area greatly improves survival of the transplanted cells. Moreover, brains in which the immune response was stronger were less favorable for the survival of the transplanted cells (143), but fetal NSCs grafted into the stroked rodent brain have been reported to decrease the levels of inflammatory molecules (145). The transplanted cells were able to mimic the traditional differentiation process: after transplantation, they would start to express neuroblast markers like Dcx and migrate towards the lesion site, where they would lose the migratory phenotype and differentiate into immature neurons (recognized by their expression of βIII-tubulin). These cells are not only able to generate new neurons, but they also secrete factors such as VEGF that can improve the regenerative environment in the brain (142). The transplantation of fetal human stem cells also improved the connectivity and dendritic plasticity in the uninjured hemisphere (140). Moreover, pre-differentiation into GABAergic neurons before transplantation was reported to improve the recovery after stroke (146).

One of the goals of the studies mentioned above was also to test whether human fetal stem cells isolated from different regions harbor a different regenerative potential once

transplanted in different brain regions. NSCs isolated from the fetal cortex or striatum are both able to survive and migrate through the injured striatum of rodents after transplantation. This further supports the idea that the striatum is an area permissive for neurogenesis and the environment plays an important role in the regenerative capacity of stem cells. Nevertheless, NSCs isolated from the striatum were able to migrate further than their cortical counterpart, allowing for the regeneration of bigger portions of tissue (141). It is interesting to speculate whether this ability is intrinsic to striatal NSCs or if it is simply because they are in an environment that was more similar to the one they have been isolated from.

The ideal timing and number of cells for transplantation have also been investigated (147). Cells transplanted 48 hours after the induction of the ischemic event survive better than cells transplanted 6 weeks after the stroke. This observation may be explained by the fact that at later time points the glial scar is completely formed and the inflammatory cells migrated in the area create a stabilized environment rather than one prone to regeneration where most of the damaged tissue is irremediably impaired. What's more, the same study also showed that increasing the number of transplanted cells does not improve the regenerative process.

Similar studies have been repeated in monkeys, an animal model closer to humans (148). Human fetal NSCs survived in the monkey brain for the entire length of the study (105 days) when transplanted 1 week after the ischemic event. The transplanted cells also started to express βIII-tubulin, a marker for immature neurons.

All the studies presented until here are based on intracranial transplantation of the stem cells, but systemic delivery approaches have also been tested in the past. Transplantation of human fetal NSCs in the brains of rats 24h after stroke induced an improvement in the behavioral recovery of the animals (149). Survival and differentiation of the transplanted cells was assessed up to 540 days after transplantation, but they were also observed in other tissues (kidneys, lungs and spleen). The improvement in these animals does not seem to be induced by the formation of new neurons, but rather by white matter recovery and release of neurotrophic factors, as speculated in similar studies (150–152). Another mechanism that improves recovery following transplantation is the up-regulation of the glutamate transporter Glt-1 in astrocytes (153).

Transplanted human fetal NSCs have also been observed to improve endogenous neurogenesis (154). In this study, fetal cells were transplanted 48h following stroke and 1 to 2 weeks after transplantation it was possible to observe graft-derived neuroblasts. SVZ-derived neurogenesis was also improved in these animals and they all performed better at several behavioral tests.

Optimizations of the protocols have been applied to increase cell survival, migration, differentiation and overall recovery. It is possible to improve the survival of fetal-derived NSCs by pretreatment with Tnf-α (155), overexpression of Cu/Zn-Superoxide dismutase (156) and Il-6 (157).

Human fetal NSCs have been used in a phase 1 clinical trial in patients affected by stroke (158). In this study it was observed that transplanted cells were able to produce factors responsible for inducing angiogenesis, neurogenesis and suppressing inflammation. No adverse effects were observed in the eleven patients involved in the study. Moreover, neurological improvements were visible already one month after transplantation and were stable for up to two years later. A second clinical trial followed in patients with motor dysfunction of the upper limb consecutive to a stroke (159). Patients were transplanted with 20 million human fetal NSCs and, at one year from transplantation, no adverse effect was observed. Improvements in different clinical parameters were observed in fifteen patients out of twenty-three.

3.3.1.2 Embryonic stem cells

ES cells have also been transplanted in the rodent brain following stroke. These cells were able to migrate towards the injured area and induce behavioral improvement (160). The transplanted ES cells are also very efficient at differentiating into mature functional neurons and it has been estimated that around 30% of the transplanted cells become positive for neuronal markers (161,162). The neurons generated by human ES cells in the brain of rodents following stroke are also able to develop axonal projections that extend for long distances, similar to the ones observed in the uninjured rodent brain (163). Cells implanted in the motor cortex developed axons into the cervical spinal cord and into the cortex of the contralateral hemisphere. Pre-treatment of ES cells with BDNF was reported to improve cell survival and functional recovery in ischemic mice (164).

The advantage of human ES cells is that they can generate a virtually unlimited number of NSCs and can be directed into any neuronal subtype. However, this also represents the biggest drawback of this system. Unlike human fetal stem cells, tumor derived from the transplanted ES cells have been observed (165). The most recent studies on human ES cells are introducing steps of pre-differentiation to limit the tumorigenic potential of the transplanted cells, which seems to be a successful strategy. Nevertheless, more studies are needed to better characterize this phenomenon.

3.3.1.3 Induced pluripotent stem cells

As ES cells, iPS cells are able to differentiate into specific neuronal subtypes. Another advantage of using iPS cells is that they can be generated from cells isolated directly from the patient, avoiding any compatibility and rejection outcomes or ethical issues (166).

Human iPS cells transplanted into the brain of rats following stroke have the ability to migrate into the ischemic area and participate to the recovery processes already 4 to 16 days after transplantation (167). These results need to be considered carefully, as a separate study

failed to assess any improvement, although survival of the transplanted cells and neuronal differentiation was verified (168,169). Observations in these studies were limited, since many important aspects such as formation of tumors and differentiation into neuronal subtypes were not properly addressed. The tumorigenic potential of iPS cells has been proven when undifferentiated cells have been used for transplantation (169,170). Moreover, the ischemic environment in the brain seems to induce a stronger tumorigenic potential compared to the healthy brain (169). Survival and differentiation into mature and functional neurons were later shown in another study (144). In this case, fibroblast-derived iPS cells were transplanted into the striatum of rats following stroke. The iPS-derived neurons were forming axonal projections directed towards the globus pallidus. Motor recovery improvement was also observed following transplantation. As seen in the other studies, the improvement in behavior was rapid (1 week), suggesting mechanisms that are independent of the formation of new neurons.

Subsequent studies developed new systems to obtain iPS cells from blood derived monocytes (171–173), in particular, the use of non-viral systems is able avoid the integration of viral sequences in the genome. iPS cells derived through non-viral methods and differentiated into NSCs have been transplanted in the rodent brain following stroke, with successful recovery (174). Good results have also been obtained by transplanting human iPS-derived NSCs in the brain of mice and rats that experienced an ischemic event (175–178).

The mechanism by which iPS cells induce recovery after stroke is still to be clarified: iPS-derived neurons develop functional synapses and integrate into the pre-existing neuronal circuit (175,176), but other studies suggest that the main support provided by the transplanted cells is via secretion of trophic factors, modulation of inflammation, support of the surviving tissue and reduction of brain atrophy (177,179–182). Improvement of recovery following transplantation of iPS-derived cells in the ischemic rodent brain have been observed even in aged animals (183).

No clinical trial is ongoing at the moment with iPS cells for the treatment of ischemic stroke. Several studies are instead focused on the evaluation of this system on larger animal models (184,185).

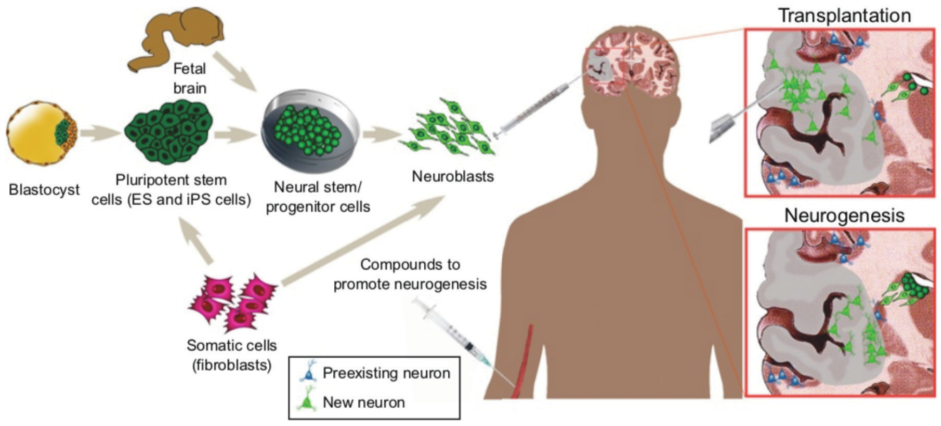


Figure 5: Different approaches for NSCs transplantation in the human brain after stroke. Cells of different origins have been tested for transplantation protocols, including: fetal NSCs, ES cells, and iPS cells obtained from somatic cells. (Image from Kokaia Z, Lindvall O. Stem cell repair of striatal ischemia. *Prog Brain Res*. 2012;201:35-53. doi:10.1016/B978-0-444-59544-7.00003-2. Used with permission from Elsevier).

3.3.1.4 Other strategies

Stem cells derived from other tissues have also been tested. Stem cells from umbilical cord blood, bone marrow and mesenchymal stem cells have all shown to improve the outcome of the disease when transplanted in rodents following stroke.

Mesenchymal cells have been extensively used in recent years for the treatment of stroke (186). Mesenchymal cells are able to differentiate into neurons, reduce inflammation and apoptosis and promote angiogenesis (reviewed by (187)).

In recent years, transplantation of bone marrow mononuclear cells attracted the attention of scientists because of the possibility to collect them autologously prior to administration. These cells could be use to modulate inflammation following stroke and the formation of new blood vessels, which may further support the neurogenic process. Bone marrow mononuclear cells have been reported to differentiate into smooth muscle cells when transplanted in the brain of rats following ischemia (188,189). In the same studies it was reported that the transplanted cells contributed also to angiogenesis and the restoration of physiological blood flow. These effects seems to be mediated by the uptake of Vegf into endothelial cells, which is promoted by the bone marrow mononuclear cells (190). Results of clinical trials where patients have been treated with bone marrow mononuclear cells suggest that an early infusion of cells is beneficial for patients' recovery (191,192), but not long after stroke (193).

3.3.2 Stimulation of endogenous neurogenesis

Endogenous neurogenesis following stroke is often not sufficient to guarantee a full recovery. Many steps of the neurogenic process could be targeted to increase the regenerative ability of the brain including: NSCs proliferation, differentiation in neuroblasts, cell migration and survival, maturation into neurons and integration into the pre-existing neuronal circuits.

Molecules like Vegf have been involved in the process of neurogenesis and it is now known that they are necessary for NSCs activity (194). Injection of the Neuropeptide Y into the LV stimulates NSCs to proliferate and migrate both in the OB and in the striatum (195). Similarly, Egf is a potent mitogen for NSCs and if infused in the LV induces a migratory phenotype (10,34), while erythropoietin has been reported to induce neuronal differentiation (196). Studies based in the injection of Egf and erythropoietin in the rodent cortex following stroke have shown an increase in the regenerative capacity of the tissue and recovery of motor functions (197). Both the stromal cell-derived factor 1α and the monocyte chemoattractant protein 1 have been involved in cell migration after stroke (130,131). Neuronal survival has been successfully improved after stroke by administration of anti-inflammatory and anti-apoptotic molecules (198–200).

Forced expression of *Ascl1* has the ability to induce reactive astrocytes differentiation into neurons in the mouse cortex following stroke (201). This could be used with the double goal to reduce gliogenesis and enhance neurogenesis.

To be able to move these strategies from the bench to the clinic, the connection between increased neurogenesis and functional recovery needs to be proven. Moreover, direction of migration towards the lesion site may be more problematic, considering that the distance that has to be covered in the human brain is greater than what can be modeled in the rodent brain.

All the approaches presented above have some intrinsic limitations. For endogenous neurogenesis the weak response to injury, the limited number of cells that can be generated by the adult NSCs and the distance these cells would have to migrate restrain their usage. As for the transplantation techniques, the potential rejection of the transplanted cells along with the ethical problems and the tumorigenicity has a negative impact on their adoption for further clinical use. This is the reason I started to look at a strategy that could avoid all these problems.

4 ASTROCYTES

Astrocytes are one of the most common cells in the mammalian brain and they are distributed in every part of it (202). They share expression markers (9,203) and electrophysiological profile (204) with stem cells and have been observed to be prone to acquire stem cell properties in different conditions (the topic will be discussed later in this chapter). Because of these peculiar characteristics they attracted our attention as a source of new neurons. If astrocytes could be used to regenerate portions of damaged tissue, many of the limitations listed in chapter 3 would be overcome. Astrocytes could be a source of new neuronal cells that can be stimulated from within the brain and that does not have to migrate, since stimulation could occur locally in the damaged area.

4.1 CHARACTERISTICS AND FUNCTIONS OF ASTROCYTES

The name astrocyte comes from the description of their star-shaped morphology as described using the Golgi staining and Gfap immunolabeling. Although this basic description is not wrong it gives an incomplete idea of their morphological appearance. Astrocytes are characterized by the presence of numerous fine processes that are negative for Gfap, which is now known to be visible in approximately 15% of the entire astrocyte volume (205). They are often observed in close proximity to, and interacting with, blood vessels and neurons. Several markers are used to identify astrocytes, even though none of them can be considered specific, ubiquitous or restricted to astrocytes or labels all astrocytes sub-types, between them the ones worth of mention are: Gfap, S100β, aldehyde dehydrogenase 1 family member L1 (Aldh1L1) and Connexin 30 (Cx30). Astrocytes exhibit high heterogeneity in aspects, such as developmental lineage, mitotic control, receptors and ion channel expression, gap junction connectivity, electrophysiological and calcium signaling properties (206).

An in-depth analysis of astrocyte subtypes revealed nine different kinds of astrocytes and their distribution within the mouse brain (207). A more recent study that aimed at characterizing the molecular architecture of the nervous system estimated astrocytes to compose between 6 to 13% of all cells, depending on the region considered (208). In this study, seven distinct subtypes of astrocytes with regionally specialized distribution have been identified. Studies of the interaction between astrocytes domains showed how there is little to no interaction between them and that in the hippocampus and cortex they tend to organize in districts (205,209–212).

Regarding the functions of astrocytes, they are involved in many different processes in both the healthy and the pathological brain. Astrocytes are involved in the maintenance of brain homeostasis, support to neurons, transport and recycling of glutamate and other neurotransmitters, and the formation of the BBB and modulation of synapses plasticity (205,213–220). One of their major functions is glutamate uptake. Astrocytes express proteins

involved in glutamate transport like GLAST and GLT-1. Neurons release the glutamate in the synaptic space and astrocytes uptake it to control the signal transmission between neurons. At the same time, astrocytes are of support to neurons by providing glutamine, so that the glutamate storage in the presynaptic neuron is never depleted.

Astrocytes employ a variety of gliotransmitters to communicate with nearby cells. Studies *in vitro* revealed that Glutamate, ATP, adenosine, d-serine, Tnf-α and eicosanoids are released following increase of intracellular calcium levels (221–225). Nevertheless, the astrocytes' ability to release gliotransmitters to activate neurons has to be proven *in vivo* in physiological conditions.

Astrocytes are also involved in the formation of the glial scar following injury and more about it will be discussed in the following paragraph.

Astrocytes are involved in the regulation of neurogenesis in the niches and they are present in great numbers both in the SVZ and in the DG. Expression of IL-1, IGFBP6, decorin and encephalin inhibits NSCs differentiation, while IL-1β and IL-6 promote it (226,227). Astrocytes also express thrombonspondin 1 to induce NSCs proliferation (228). In the DG astrocytes are able to promote NSCs proliferation and differentiation by providing Wnt (229), EphrinB2 (230). In the SVZ, niche astrocytes express Wnt7a and Bmp ligands to promote neurogenesis (25,231). Moreover, SVZ astrocytes express Notch ligands, like Dlk1 (232), that can bind Notch receptor on the membrane of NSCs and allow the modulation of neurogenesis (233). Astrocytes in which *nestin* has been knocked-out are unable to induce activation of Notch signaling in NSCs, resulting in increased neurogenesis (234).

4.2 ASTROCYTES IN STROKE

Astrocytes have an important role following ischemic stroke, they are able to respond rapidly by expressing different genes, the most important being involved in STAT signaling (235,236), and become reactive. During this process, astrocytes up-regulate GFAP, vimentin and nestin, the number of their processes is reduced and they become thicker and astrocytes themselves start to proliferate (237–239). Within 24h after stroke in the mouse brain, very few astrocytes are positive for the proliferation marker Ki67, but this changes 3 and 5 days after injury, when the percentage of proliferating astrocytes increases to 17 and 29% respectively (240). During the acute and sub-acute phase of stroke, astrocytes within the penumbra participate in the formation of the glial scar, which is a physical barrier that stops infiltration of immune cells in the healthy tissue (241,242). Although the glial scar has an important role in the survival of the tissue and recovery during the first phases after injury, as observed in mice knockout for GFAP and vimentin (243), it later represents a limiting factor for the regenerative process (244). A transcriptome study of reactive astrocytes revealed that they are able to respond differently depending on the stimulus (245). In particular, although both stroke and LPS injection induce gene expression, reactive astrocytes in stroke express more genes than after LPS injection.

Another role of astrocytes in stroke is to avoid the formation of brain edema. Astrocytes respond to the formation of a hypo-osmotic environment in the brain by releasing osmotically active molecules (246–248), which will cause cellular swelling.

Reactive astrocytes may also negatively affect neurons following brain ischemia. In the ischemic cortex, up-regulation of Ephrin-A5 may inhibit axonal sprouting and recovery (249). Moreover, impaired functions of GABA transporter Gat-3/Gat-4 may inhibit synaptic plasticity following stroke (250).

Modern techniques, like single-cell transcriptomics analysis, are allowing us to further characterize cells, both in the healthy and diseased brain. Using these methods, a subpopulation of cells that expresses both astrocyte- and microglia-specific genes has been identified (251). These cells are found in the hippocampus of mice following injury and in the brains of individuals that suffered a stroke or are afflicted by AD, but not in the healthy brain of either species.

Another feature of astrocytes following stroke, and the most interesting one in regards to this thesis, is their ability to become neurogenic. More about it will be discussed during the rest of this chapter.

4.3 ASTROCYTE-DERIVED NEUROGENESIS

In this thesis, the main interest in astrocytes is their ability to generate new neurons, either spontaneously in injury models (8,9) or after manipulation by researchers (4–6,9,201,252,253).

Astrocytes are very similar to NSCs in many aspects, including: marker expression (203), electron microscopy features (254,255), electrophysiological profile (204) and gene expression (256), leaving scientists to wonder whether it would be possible to induce a neurogenic program in astrocytes. By forcing the expression of transcription factors associated with neuronal differentiation it was observed that astrocytes are able to generate new neurons. Transfection to force Neurog2 expression in cortical astrocytes induced direct differentiation into glutamatergic neurons, while Dlx2 determines a GABAergic neuronal identity (4). In the same study it was also proven that isolation of the cortical astrocytes and cultivation in neurospheres increased the efficiency of the reprogramming protocol, suggesting a role of the cortical environment in suppressing the potential of astrocytes to generate new neurons. Moreover, neurospheres derived from cortical astrocytes were not able to generate neurons if isolated from the uninjured cortex, while they were able to do so when isolated from the mouse cortex after stab wound injury.

To understand what may influence the neurogenic potential of astrocytes *in vivo* we have to also keep in mind the role that the environment plays in neurogenesis. Experiments of ectopic transplantation have proven that cells isolated from the hippocampus, spinal cord and substantia nigra can be transplanted in the DG and generate neurons locally (257–259). When

such studies have been repeated in the striatum the results have highlighted that this region may not be as beneficial for the generation of new neurons, compared to the hippocampus (24,260,261). A following study even reported no signs of neurogenesis after transplantation of SVZ derived cells (262). However, when ES cells were used as source material for transplantation, both the striatum and the cortex were able to sustain formation of new neurons (263,264). Results from these studies suggest that, even though there are cell intrinsic neurogenic properties, the environment can be either a limiting or an enhancing factor to the manifestation of the neurogenic potential.

By inducing the expression of NeuroD1, researchers were able to convert astrocytes into neurons both *in vitro* and *in vivo* (4,5). Similar results were obtained with Sox2 and Ascl1 (6,7). Conversion of mouse astrocytes was also obtained by expression of Foxg1, Sox2 and Brn2 (265) and the neuronal subtype specification was modulated by overexpression of specific genes like Lhx8 (cholinergic neurons) and Foxa2 (dopaminergic neurons). Small molecules have also been used for the differentiation of astrocytes into neurons: valproic acid, Chir99021 and Repsox (266).

Independently of external manipulation by scientists, astrocytes have been observed to have an intrinsic potential to generate neurons. The induction of ischemic stroke in the brain of adult mice triggers a latent neurogenic program in astrocytes (9). In this model, astrocytes revert to a transit-amplifying progenitor state, characterized by the expression of Ascl1, and undergo several cycles of cell division before differentiation into Dcx-expressing neuroblasts and finally into neurons. In the same study it was proven that this process is dependent on the down-regulation of Notch signaling and the knockout of *Rbpj* induces astrocyte-derived neurogenesis even in the absence of injury. Unlike in the stroke model, astrocytes in the brains of $Rbpj^{-/-}$ mice generate neuroblasts only in proximity of the SVZ, suggesting that the proximity of the niche supplements for the pro-neurogenic stimuli missing in the healthy brain. A study published soon after also identified striatal astrocytes as a source of new neurons in an injury model based on the injection of quinolinic acid (8). While the reprograming studies mentioned above are based on a direct conversion from astrocyte to neuron, in these studies astrocytes generate new neurons following similar steps to the NSCs, adding another layer of similarities to the one listed at the beginning of this paragraph. This further implies that, if by transdifferentiation it is possible to have a one-to-one conversion rate, by stimulating the endogenous potential of astrocytes each cell could generate several mature neurons.

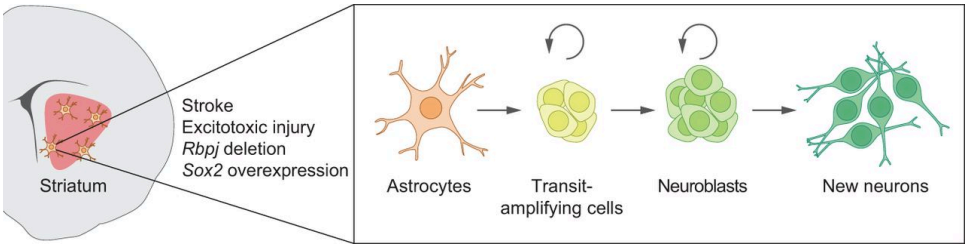


Figure 6: Astrocyte-derived neurogenesis in the mouse striatum. Mature astrocytes in the mouse striatum can become neurogenic following stroke or by blocking Notch signaling. When the neurogenic program is induced, astrocytes lose their typical morphology and become round. At this point they start to express markers typical of transient-amplifying progenitor cells and proliferate, forming clusters. These clusters of Ascl1 positive cells will further mature into Dcx-expressing neuroblasts, which will then separate from each other and mature into neurons. (Image Magnusson JP, Frisén J. Stars from the darkest night: unlocking the neurogenic potential of astrocytes in different brain regions. *Development*. 2016;143(7):1075-1086. doi:10.1242/dev.133975. Used with permission from The Company of Biologists)

The neurogenic potential of astrocytes has been observed not only in the striatum, but also in the cortex. Following stab wound injury, astrocytes are able to proliferate and self renew and, if isolated, show multipotency (267). Other studies proved that the overexpression of Ascl1 in SVZ-derived reactive astrocytes in the cortex following stroke has the ability to transdifferentiate these cells into neurons (201). Similar results in the cortex have been obtained reprogramming both reactive and quiescent astrocytes by overexpressing Ngn2 and Nurr1 (268). Moreover, Notch signaling importance has also been shown in the cortex where, by inducing an injury in *Rbpj* knockout mice, it is possible to trigger astrocyte-derived neurogenesis (269). In this last study, it was shown once again that the injury-induced neurogenic program in astrocytes closely resemble the one from SVZ NSCs when single-cell RNA sequencing is performed (256,269).

Human astrocytes have also been reprogrammed into neurons using different protocols, proving their potential for future therapies. The simultaneous inhibition of Notch, BMP, GSK-3β and TGFβ signaling using small molecules converts human fetal astrocytes to neurons *in vitro* and mouse astrocytes *in vivo* (270). Human astrocytes in which NEUROD1, ASCL1, LMX1A and NR4A2 were overexpressed successfully differentiate in dopamine neurons and mouse astrocytes converted in the same fashion can be used to recover the phenotype in a mouse model of PD (271). Similarly to overexpression of transcription factors, small molecules can induce adult human astrocytes to differentiate into neurons (272). In this study, researchers used valproic acid, Chir99021 and Repsox (already efficient in converting mouse astrocytes (266)) in combination with forskolin, i-Bet151 and ISX-9 to differentiate astrocytes into neurons. Although it is suggested in this study that differentiation of astrocytes using this protocol avoids conversion into progenitor cells, Ascl1- and DCX-expressing cells have been observed. This suggests that the conditions mentioned in the study

trigger astrocyte differentiation into transient-amplifying progenitor cells, rather than direct transdifferentiation into neurons, as observed in other studies (9,269,273). Other cocktails of small molecules have proven efficient in differentiating human astrocytes into neurons (274), opening the possibility to use this strategy in the clinic and avoiding the use of viruses for overexpression of transcription factors.

Astrocytes are now considered a promising source of new neurons within the adult mammalian brain and in the rest of this thesis I will show how our most recent work can integrate into this landscape of knowledge and be used to further understand this process.

5 PRESENT INVESTIGATION

5.1 AIMS

Paper I – Understand if previous models aimed at inducing astrocyte-derived neurogenesis can synergize and stimulate astrocytes in non-neurogenic regions to produce neurons.

Paper II – Generate two single-cell RNA sequencing datasets to elucidate the mechanisms behind astrocyte-derived neurogenesis. Use the dataset to compare neurogenic and non-neurogenic astrocytes. Identify and validate signaling pathway with a potential to induce astrocyte to become neurogenic.

5.2 PAPER I – RESULTS AND DISCUSSION

The results previously generated by our lab, prompted us to wonder if stroke and deletion of Notch signaling can have a synergistic effect on neurogenic astrocytes. Moreover, we wanted to test if, by combining the two conditions, we could induce neurogenesis in the lateral striatum, which is not neurogenic in mice knockout for *Rbpj*.

To test our hypothesis, we deleted *Rbpj* specifically in astrocytes, by giving tamoxifen to mice that express CreER under the control of the astrocyte promoter Connexin30 (Cx30) in which the gene encoding for *Rbpj* is flanked by *FloxP* sequences. CreER translocation into the nucleus of astrocytes induces also expression of the reporter Tomato. One week after tamoxifen injection, we induced a stroke by using the Middle Cerebral Artery Occlusion model (MCAO). Animals were sacrificed 7 weeks after stroke and the ischemic hemisphere was compared with the uninjured one and with the ischemic hemisphere from wild-type mice.

We observed an increase in the number of neuroblasts and transit-amplifying progenitor cells in the stroke-injured hemisphere when compared both to the uninjured side and to the ischemic hemisphere of wild-type mice. Neurogenic cells differentiate into mature neurons, as assessed by the presence of Tomato-positive mature neurons (identified by the expression of the marker NeuN), although the number of astrocyte-derived neurons is not significantly higher than in the uninjured side. This lack of difference may be explained by the early time-point for analysis or by the fact that additional neurons are not integrated into the pre-existing neuronal circuits and die.

Two hypotheses may explain the increase in neurogenic cells: astrocytes that entered the neurogenic program due to deletion of *Rbpj* were stimulated by stroke to proliferate more or more astrocytes became neurogenic. To answer this question, we quantified the number of cell clusters. We previously described how each cluster is generated by one neurogenic

astrocyte and further proofs are presented in the supplementary figures of this paper. We observed a significant increase in the number of clusters of transit-amplifying progenitor cells, suggesting that more astrocytes became neurogenic. The position of many of these cells, in very lateral areas of the striatum, where usually they are not observed, further supports this idea. Neuroblast clusters do not seem to be affected, but this may be explained by the migratory nature of neuroblasts, which clusters may dissolve to allow cells to migrate to the site of injury.

Taken together, the data gathered in **Paper I** suggests that many, if not all, striatal astrocytes maintain a neurogenic potential. Notch signaling has an important role in maintaining astrocytes quiescent, but further stimuli are needed to initiate a neurogenic program.

5.3 PAPER II – RESULTS AND DISCUSSION

The results from our previous publication (9) and from **Paper I** suggest that astrocytes' ability to generate neurons is regulated by Notch signaling, which acts as a brake on cell proliferation and differentiation, but further stimulation can enhance the neurogenic program. Also, similarities to SVZ neurogenesis emerged, however it was still unclear to which extent the two processes are similar. To further investigate these two points, we decided to perform single-cell RNA sequencing on astrocytes from *Rbpj* deleted animals.

In particular, we decided to employ two different sequencing techniques: Smart-seq2 and 10X Genomics sequencing. The first method was used to sequence astrocytes from *Rbpj* deleted animals treated with tamoxifen, so the derived dataset is called Cx30-CreER dataset. The second dataset was instead derived from astrocytes isolated from the brain of mice injected with a Cre-expressing Adeno Associated Virus (AAV), hence the name AAV-Cre dataset. This second dataset was also used to prove that there was no contamination from SVZ derived cells in our data.

Comparisons of our two datasets with previously generated ones revealed that striatal astrocyte-derived neurogenesis follows steps more similar to SVZ derived neurogenesis rather than DG-derived neurogenesis (256,275,276). Striatal astrocytes appear to be a form of quiescent stem cells that when activated differentiate into transient-amplifying progenitor cells and later in neuroblasts.

Nevertheless, the majority of astrocytes in the striatum and in the cortex (a non-neurogenic area in our model) appear to activate the neurogenic program following *Rbpj* deletion, but get halted in a pre-division phase and do not generate neuroblasts. To test if administration of external stimuli could trigger these halted astrocytes to commit to differentiation, we screened for the expression of growth factor receptors involved in NSCs differentiation. Egf receptor was highly up-regulated in these astrocytes and administration of Egf in the lateral striatum triggered a two-fold increase in the number of neuroblasts. Nevertheless, injection of Egf in the cortex was not sufficient to increase astrocyte-derived neurogenesis.

In conclusion, the data from **Paper II** show that striatal astrocytes follow a neurogenic trajectory reminiscent of the NSCs from the SVZ. Thus, astrocytes can be considered a form of stem cells in which the default state is quiescence. By blocking the Notch signaling it is possible to reactivate the neurogenic potential of astrocytes, which become receptive to environmental stimuli like Egf. In other areas of the brain like the cortex, the process is similar, but stronger stimuli are needed and this has been further addressed by our group in another publication (269).

6 CONCLUDING REMARKS AND FUTURE PERSPECTIVES

This thesis aims at giving an overview of regenerative medicine of the brain, its application to stroke, the use of astrocytes to generate new neurons and how my work has been relevant to the subject.

The goal of regenerative medicine is to increase the healing ability of the adult human body and potentially step beyond its natural limits. In particular, the aim is to completely recover the functionality of injured tissues and organs. The application of regenerative medicine to the brain attracted interest in the past decades because the complexity of the tissue and its structure makes it particularly vulnerable to injury. Stroke represents one of the most common kinds of injury in the human brain and with substantial repercussions both on the lives of afflicted people and overall on the society. The main strategies that have been adopted until now to overcome the neuronal death induced by an ischemic event rely on the use of stem cells and have been listed in chapter 3. There are two main approaches: the stimulation of endogenous neural stem cells and the transplantation of progenitor cells from exogenous sources. Although discoveries and therapeutics attempts have been carried out following both lines of research with good results, some limitations emerged that are prompting the scientific community to look for alternative solutions. Transplantation of progenitor cells from exogenous sources proved efficient in providing sources of new neurons in the damaged area of animal models. Nevertheless, fetal and ES cells could be problematic from an ethical point of view and the availability of donor cells. Not only is it difficult to obtain donor cells for these particular approaches, but even when tissue is available, the isolation of enough cells is laborious and problematic. The use of iPS cells has been promising to avoid these limitations, but these cells require extra differentiation steps to reduce the possibility to generate tumors. Stimulation of endogenous neurogenesis on the other hand can be difficult because in the adult human brain the stem cells are not as numerous and active as in the developing brain or in the animal models that are currently in use. Moreover, the ability of eventual immature neurons to migrate efficiently over long distances is not confirmed and may represent a considerable limitation. These issues prompted the search for a source of new neurons that could overcome the ethical discussion, the problem of having enough cells, tumorigenicity and require as little and invasive surgeries as possible. Astrocytes have been one of the answers and they seem to be able to bypass most of the above-mentioned limitations.

Several studies addressed the ability of astrocytes to generate neurons and these have been summarized in chapter 4. The two papers presented in this thesis aim at contributing to the knowledge of how striatal astrocytes may have an intrinsic neurogenic ability but, unlike neural stem cells, they are intrinsically quiescent because of Notch signaling. The deletion of *Rbpj* in astrocytes impairs Notch signaling triggering the cell to initiate a pro-neurogenic response. Yet, this is not enough to induce neurogenesis, but a first step to exit the quiescent

default state. More signals need to be provided such as the ones released after an ischemic event in the mouse brain. In the healthy brain, this signals are present nearby the subventricular zone, where *Rbpj* deletion is sufficient to trigger the differentiation into neuroblasts and neurons. The neurogenic process in astrocytes resembles the one from stem cells in the subventricular zone. Moreover, stroke alone is able to induce astrocyte-derived neurogenesis, but the concomitant deletion of Notch signaling allows more astrocytes to actively participate in this process. More importantly, stroke is able to activate neurogenic astrocytes even in the lateral parts of the striatum, where no neurogenesis is observed even when *Rbpj* is deleted. A similar effect can be induced by the injection of Egf, which receptor is up-regulated by astrocytes following *Rbpj* deletion. Cortical astrocytes behave in a similar manner, even if the injection of Egf is not sufficient to induce neurogenesis. Moreover, other studies in the literature showed that the induction of an injury can trigger the neurogenic program in the *Rbpj* deleted cortical astrocytes (269). These data suggests that the environment around astrocytes is also responsible for the expression of their neurogenic potential.

The future of astrocyte-derived neurogenesis relies on the identification of the pathways that are important to regulate this process and which stimuli are needed to induce neuronal differentiation. It will be important to verify if the astrocyte-derived neurons can compare to the stem cell-derived one. Moreover, these observations are still limited to animal models and human astrocytes have more layers of complexity.

7 ACKNOWLEDGEMENTS

Good job to myself!

…just joking:

I would like to thank my supervisor, **Jonas Frisén**, for giving me the chance to join his lab and work on my PhD project. I am thankful to both my co-supervisors: **Konstantinos Meletis** and, in particular, **Jens Magnusson** who guided me during my first steps.

All of the past and present (…and some of the future, maybe) people from the Frisén lab: it has been amazing to work and share the science life with you all. **Marta Paterlini**, I appreciated our moments for venting out the frustrations about Italian politics. **Sarantis Giatrellis**, thank you for the fun chats and the free-of-charge fishing lessons. **Helena Lönnqvist**, you are the soul and propulsion engine of the entire lab…and don't think you got rid of me: I will still bug you with questions on how to save my plants from myself! **Moa Stenudd**, **Johanna Classon**, **Embla Steiner**, **Camilla Engblom**, **Joanna Hård** and **Kanar Alkass**, I cannot even count how many times we have talked about the most different topics; you have created a beautiful environment where one can grow not only scientifically, but as a person. **Carl-Johan Eriksson**, **Julia Reinius**, **Joachim Vist** and **Björn Diemer** you guys have already proven motivation and great potential, keep it going! **Sue Park**, you are new to the group, but I am sure you are going to have a great time. **Michael Ratz**, now that your football player career is over, I am sure your science will become even better (…I had to make one last joke about it!). **Leonie Von Berlin**, I am looking forward to read the papers you will publish during your PhD, keep bullying Mathew every once in a while. **Jeff Mold**, you are the source of 90% of the chaos in the office, yet you contribute in equal percentage to the fun…working in a quiet office from now on will feel, somehow, wrong. **Ilke Demirci**, I have never seen anyone so committed to work and partying at the same time as much as you, I will miss the inflamed discussions about politics and life during lunch. **Enric Llorens-Bobadilla**, it has been great to be able to talk about science with you, a bit less great to always get defeated at squash (but soon I will win a game against you, that's a promise), but if I would "accidentally" end up lost somewhere in Turkey at 3am, I know I could count on you to find our way back (maybe by driving someone else's car…). **Ionuț Dumitru**, our provider of cookies, you always made sure none of us will collapse due to hypoglycemia during the working day; you are the best partner for board games and exploration of Stockholm's food scene. **Mathew Tata**, for someone who is often complaining, you are extremely talented in showing the bright side of things and lift the mood during really bad days; I always appreciate the valuable suggestions and points of view you share during our discussions. **Margherita Zamboni**, during the past 5 years we have always been working side by side and I will sincerely miss you in my daily life as a colleague, but most importantly as a friend.

Marion, **Enikő**, **Áron**, **Johannes** and **JP**, you are great friends and a wonderful group to be part of and to share so many moments; I am happy that I will spend some more years in

Sweden, because honestly I wasn't ready to leave you all! **Carmen**, I miss our philosophical discussions about life (and I miss the amounts of ice-cream associated with them!), but I am so happy for you and your bundle of joy; I am looking forward for the next pub-quiz! **Ana and Robin** (yes, the two of you for me must be mentioned together!), you are two wonderful people and I cannot believe how much support you gave me when I needed it; you are so genuine and kind.

All the people I crossed my path with from **CMB, Biomedicum** and **KI** in general, you are so many I cannot mention everybody (and I really need to send the thesis to the printer) and I hope you will forgive me if I forget someone. Here we go:

The **CMB Pub Crew**: **Isabelle, Pedro, Miloš, Stefina, Yildiz, Gonçalo, Milind, Helena, Christina** and all the **past members**, we shared a lot of tough work and so much fun and I am grateful that, even if the pubs are part of the past, we are still in touch. **Tatiana, Alberto, Mauricio, Carlos, Simona, Tiago, Shahul, Antonio (pistola), Dominika, David, Katrin, Nikola, Manuela, Giorgia, Daniel, David** and **Anna** you all made old CMB such a fun department that Biomedicum felt like a downgrade.

All the **KICC** people, I am happy I could share with you such great moments trying to create a better working environment.

The friends from the **rest of KI and all the people I met because of them: Paula, Theresa and Tom**, **Marco**, **Jorge**, **Michele and Laura**, **Alex**, **Teresa**, **Igor**, **Milana**, **Marin**, **Elena**, **Andrei**, **Henna**, **Ewoud**, **Sunjay**, **Niyaz and Shalina**, **Michael and Eliane**, **Natalie**, **Maria**, **Mino**, **Dörte**, **Manideep and Miila**, **Viktoria**, **Elisa**, **Francesca and Mike**, **Katarina** and **Joanna**.

All the people in the B6 quarter: **Simon's group** and **Sandberg** lab.

The best climbing team out there: **Susanne**, I still can't believe that you are not even done with the PhD and started medical school, I'd love to make fun of you as usual, but this time I just can't, I wish I would be as brave as you are! **Joanne**, you are rocking in the climbing gym, but you and **Jarda** are rocking also on the real walls; keep it going and I am looking forward to hear (or read…wink wink) about your adventures in Norway. **Jana**, you might have joined for a short time, but you made the climbing sessions so much fun and brought a

lot of motivation; thank you for your friendship. **Heather**, you are one of the most energetic and adrenaline-filled person I have ever met; I am looking forward for the return of summer so that we can all go bouldering outdoor again! **Mirela**, we miss you so much in the climbing gym, come back!!! **Renata**, I know what you are thinking, but we miss you as well! It is not the same without you making us feel like losers when we cannot finish a "simple" problem ("Come on, it's just a 6C+++!!!").

All my **friends and relatives in Italy**...sorry, you are really too many to go one-by-one, but I love you all!

Thank you also to all the people from the **Goldman lab** in Rochester, it was wonderful to know you, you made me feel so welcome even if I was there for such a short time.

Alla mia famiglia: **Daniela**, **Enzo** ed **Anna**, voi mi avete sempre sempre supportato qualunque strada decidessi di intraprendere nella mia vita. Mi siete stati vicino e sopportato con tanta pazienza (non sono una persona facile e ne sono consapevole). Ma volevo dirvi che questa vittoria è mia tanto quanto vostra, non sarei mai arrivato dove sono se non fosse stato per voi; non solo per il supporto morale, ma soprattutto per l'ambiente che avete creato, per le esperienze formative e per avermi sempre incoraggiato ad usare la testa.

A great thank you goes to the person that has been the closest to me in the past 2 years: **Alexandra**, I really don't know how I would have managed the last times without you; to be honest, I really don't care about knowing it. You are a continuous source of inspiration and motivation for me. One chapter of my life is ending, and I am happy that you have been here for me during the end of it, but I am even happier that you are gonna be here now that a new one will start!

I would also like to thank Alexandra's parents, **Ieremia** and **Dora**, who welcomed me so warmly into the family.

PhD is a journey and I am so happy I could share it with so many wonderful people. **All of you** enriched my life.

8 REFERENCES

1. Altman, J. Are New Neurons Formed in the Brains of Adult Mammals? *Science (80-.).* **135**, 1127–1128 (1962).

2. Doetsch, F., Caille, I., Lim, D. A., Garcı, J. M. & Alvarez-buylla, A. Subventricular Zone Astrocytes Are Neural Stem Cells in the Adult Mammalian Brain. **97**, 703–716 (1999).

3. Kempermann, G. & Gage, F. H. New Nerve Cells for the Adult Brain. *Sci. Am.* **280**, 48–53 (1999).

4. Heinrich, C. *et al.* Directing astroglia from the cerebral cortex into subtype specific functional neurons. *PLoS Biol.* **8**, (2010).

5. Guo, Z. *et al.* Article In Vivo Direct Reprogramming of Reactive Glial Cells into Functional Neurons after Brain Injury and in an Alzheimer ' s Disease Model. *Stem Cell* **14**, 188–202 (2014).

6. Heinrich, C. *et al.* Sox2-mediated conversion of NG2 glia into induced neurons in the injured adult cerebral cortex. *Stem cell reports* **3**, 1000–14 (2014).

7. Liu, Y. *et al.* Ascl1 Converts Dorsal Midbrain Astrocytes into Functional Neurons In Vivo. *J. Neurosci.* **35**, 9336–55 (2015).

8. Nato, G. *et al.* Striatal astrocytes produce neuroblasts in an excitotoxic model of Huntington's disease. *Development* **142**, 840–5 (2015).

9. Magnusson, J. P. *et al.* A latent neurogenic program in astrocytes regulated by Notch signaling in the mouse. *Science (80-.).* **346**, 237–241 (2014).

10. Reynolds, B. A. & Weiss, S. Generation of Neurons and Astrocytes from Isolated Cells of the Adult Mammalian Central Nervous System. (1992).

11. Amrein, I. Adult Hippocampal Neurogenesis in Natural Populations of Mammals. 1–20 (2015).

12. Morshead, C. M., Garcia, A. D., Sofroniew, M. V. & van der Kooy, D. The ablation of glial fibrillary acidic protein-positive cells from the adult central nervous system results in the loss of forebrain neural stem cells but not retinal stem cells. *Eur. J. Neurosci.* **18**, 76–84 (2003).

13. Lois, C. & Alvarez-Buylla, A. Long-distance neuronal migration in the adult mammalian brain. *Science (80-.).* **264**, 1145–1148 (1994).

14. Lois, C., García-Verdugo, J. M. & Alvarez-Buylla, A. Chain migration of neuronal precursors. *Science (80-.).* **271**, 978–981 (1996).

15. Merkle, F. T., Mirzadeh, Z. & Alvarez-Buylla, A. Mosaic organization of neural stem cells in the adult brain. *Science* **317**, 381–4 (2007).

16. Palmer, T. D., Takahashi, J. & Gage, F. H. The adult rat hippocampus contains primordial neural stem cells. *Mol. Cell. Neurosci.* **8**, 389–404 (1997).

17. Kim, E. J., Battiste, J., Nakagawa, Y. & Johnson, J. E. Ascl1 (Mash1) lineage cells contribute to discrete cell populations in CNS architecture. *Mol. Cell. Neurosci.* **38**, 595–606 (2008).

18. Calzolari, F. *et al.* Fast clonal expansion and limited neural stem cell self-renewal in the adult subependymal zone. *Nat. Neurosci.* **18**, 490–2 (2015).

19. Ortega, F. *et al.* Oligodendrogliogenic and neurogenic adult subependymal zone neural stem cells constitute distinct lineages and exhibit differential responsiveness to Wnt signalling. *Nat. Cell Biol.* **15**, 602–13 (2013).

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

21. Mirzadeh, Z., Merkle, F. T., Soriano-Navarro, M., Garcia-Verdugo, J. M. & Alvarez-Buylla, A. Neural Stem Cells Confer Unique Pinwheel Architecture to the Ventricular Surface in Neurogenic Regions of the Adult Brain. *Cell Stem Cell* **3**, 265–278 (2008).

22. Sawamoto, K. New Neurons Follow the Flow of Cerebrospinal Fluid in the Adult Brain. *Science (80-.).* **311**, 629–632 (2006).

23. Lehtinen, M. K. *et al.* The Cerebrospinal Fluid Provides a Proliferative Niche for Neural Progenitor Cells. *Neuron* **69**, 893–905 (2011).

24. Lim, D. A., Tramontin, A. D., Trevejo, J. M., Herrera, D. G. & Alvarez-buylla, A. Noggin Antagonizes BMP Signaling to Create a Niche for Adult Neurogenesis. **28**, 713–726 (2000).

25. Peretto, P. *et al.* Expression of the secreted factors noggin and bone morphogenetic proteins in the subependymal layer and olfactory bulb of the adult mouse brain. *Neuroscience* **128**, 685–696 (2004).

26. Huang, X. *et al.* Transventricular delivery of Sonic hedgehog is essential to cerebellar ventricular zone development. *Proc. Natl. Acad. Sci. U. S. A.* **107**, 8422–7 (2010).

27. Lehtinen, M. K. *et al.* The Cerebrospinal Fluid Provides a Proliferative Niche for Neural Progenitor Cells. *Neuron* **69**, 893–905 (2011).

28. Grandbarbe, L. *et al.* Delta-Notch signaling controls the generation of neurons/glia from neural stem cells in a stepwise process. *Development* **130**, 1391–402 (2003).

29. Nelson, B. R. *et al.* Acheate-scute like 1 (Ascl1) is required for normal delta-like (Dll) gene expression and notch signaling during retinal development. *Dev. Dyn.* **238**, 2163–2178 (2009).

30. Sueda, R., Imayoshi, I., Harima, Y. & Kageyama, R. High Hes1 expression and resultant Ascl1 suppression regulate quiescent vs. active neural stem cells in the adult mouse brain. *Genes Dev.* **33**, 511–523 (2019).

31. Tavazoie, M. *et al.* A specialized vascular niche for adult neural stem cells. *Cell Stem Cell* **3**, 279–88 (2008).

32. Cao, L. *et al.* VEGF links hippocampal activity with neurogenesis, learning and memory. *Nat. Genet.* **36**, 827–835 (2004).

33. Kuhn, H. G., Winkler, J., Kempermann, G., Thal, L. J. & Gage, F. H. Epidermal growth factor and fibroblast growth factor-2 have different effects on neural progenitors in the adult rat brain. *J. Neurosci.* **17**, 5820–5829 (1997).

34. Doetsch, F., Petreanu, L., Caille, I., Garcia-Verdugo, J. M. & Alvarez-Buylla, A. EGF converts transit-amplifying neurogenic precursors in the adult brain into multipotent

stem cells. *Neuron* **36**, 1021–1034 (2002).

35. Ghashghaei, H. T. *et al.* Reinduction of ErbB2 in astrocytes promotes radial glial progenitor identity in adult cerebral cortex. *Genes Dev.* **21**, 3258–3271 (2007).

36. Shi, Y. *et al.* Expression and function of orphan nuclear receptor TLX in adult neural stem cells. *Nature* **427**, 78–83 (2004).

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

38. Hack, M. A. *et al.* Neuronal fate determinants of adult olfactory bulb neurogenesis. *Nat. Neurosci.* **8**, 865–872 (2005).

39. Marshall, C. A. G., Novitch, B. G. & Goldman, J. E. Olig2 directs astrocyte and oligodendrocyte formation in postnatal subventricular zone cells. *J. Neurosci.* **25**, 7289–7298 (2005).

40. Encinas, J. M., Vaahtokari, A. & Enikolopov, G. Fluoxetine targets early progenitor cells in the adult brain. *Proc. Natl. Acad. Sci. U. S. A.* **103**, 8233–8 (2006).

41. Kronenberg, G. *et al.* Subpopulations of Proliferating Cells of the Adult Hippocampus Respond Differently to Physiologic Neurogenic Stimuli. **463**, 455–463 (2003).

42. Seri, B., Garcı, J. M., Mcewen, B. S. & Alvarez-buylla, A. Astrocytes Give Rise to New Neurons in the Adult Mammalian Hippocampus. **21**, 7153–7160 (2001).

43. Shin, J. *et al.* Single-Cell RNA-Seq with Waterfall Reveals Molecular Cascades underlying Adult Neurogenesis. *Cell Stem Cell* **17**, 360–72 (2015).

44. Song, J. *et al.* Neuronal circuitry mechanism regulating adult quiescent neural stem-cell fate decision. *Nature* **489**, 150–4 (2012).

45. Jang, M.-H. *et al.* Secreted frizzled-related protein 3 regulates activity-dependent adult hippocampal neurogenesis. *Cell Stem Cell* **12**, 215–23 (2013).

46. Cheung, T. H. & Rando, T. A. Molecular regulation of stem cell quiescence. *Nat. Rev. Mol. Cell Biol.* **14**, 329–40 (2013).

47. Mira, H. *et al.* Signaling through BMPR-IA regulates quiescence and long-term activity of neural stem cells in the adult hippocampus. *Cell Stem Cell* **7**, 78–89 (2010).

48. Ahn, S. & Joyner, A. L. In vivo analysis of quiescent adult neural stem cells responding to Sonic hedgehog. *Nature* **437**, 894–897 (2005).

49. Lugert, S. *et al.* Quiescent and Active Hippocampal Neural Stem Cells with Distinct Morphologies Respond Selectively to Physiological and Pathological Stimuli and Aging. *Cell Stem Cell* **6**, 445–456 (2010).

50. Venere, M. *et al.* Sox1 marks an activated neural stem/progenitor cell in the hippocampus. *Development* **139**, 3938–49 (2012).

51. Sierra, A. *et al.* Microglia shape adult hippocampal neurogenesis through apoptosis-coupled phagocytosis. *Cell Stem Cell* **7**, 483–495 (2010).

52. Tashiro, A., Sandler, V. M., Toni, N., Zhao, C. & Gage, F. H. NMDA-receptor-

mediated, cell-specific integration of new neurons in adult dentate gyrus. *Nature* **442**, 929–933 (2006).

53. Ge, S., Sailor, K. A., Ming, G. & Song, H. Synaptic integration and plasticity of new neurons in the adult hippocampus. *J. Physiol.* **586**, 3759–65 (2008).

54. Christian, K. M., Song, H. & Ming, G. Functions and dysfunctions of adult hippocampal neurogenesis. *Annu. Rev. Neurosci.* **37**, 243–62 (2014).

55. Deng, W., Aimone, J. B. & Gage, F. H. New neurons and new memories: how does adult hippocampal neurogenesis affect learning and memory? *Nat. Rev. Neurosci.* **11**, 339–50 (2010).

56. Sahay, A., Wilson, D. A. & Hen, R. Pattern separation: a common function for new neurons in hippocampus and olfactory bulb. *Neuron* **70**, 582–8 (2011).

57. Clelland, C. D. *et al.* A functional role for adult hippocampal neurogenesis in spatial pattern separation. *Science (80-.).* **325**, 210–213 (2009).

58. Chambers, R. A., Potenza, M. N., Hoffman, R. E. & Miranker, W. Simulated apoptosis/neurogenesis regulates learning and memory capabilities of adaptive neural networks. *Neuropsychopharmacology* **29**, 747–758 (2004).

59. Deisseroth, K. *et al.* Excitation-neurogenesis coupling in adult neural stem/progenitor cells. *Neuron* **42**, 535–52 (2004).

60. Nissant, A., Bardy, C., Katagiri, H., Murray, K. & Lledo, P. M. Adult neurogenesis promotes synaptic plasticity in the olfactory bulb. *Nat. Neurosci.* **12**, 728–730 (2009).

61. Feliciano, D. M. & Bonfanti, L. Noncanonical Sites of Adult Neurogenesis in the Mammalian Brain. (2015).

62. Luzzati, F. *et al.* Quiescent neuronal progenitors are activated in the juvenile guinea pig lateral striatum and give rise to transient neurons. *Development* **141**, 4065–75 (2014).

63. Luzzati, F., De Marchis, S., Fasolo, A. & Peretto, P. Neurogenesis in the caudate nucleus of the adult rabbit. *J. Neurosci.* **26**, 609–21 (2006).

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

65. Dayer, A. G., Cleaver, K. M., Abouantoun, T. & Cameron, H. A. New GABAergic interneurons in the adult neocortex and striatum are generated from different precursors. (2005). doi:10.1083/jcb.200407053

66. Bédard, A., Cossette, M., Lévesque, M. & Parent, A. Proliferating cells can differentiate into neurons in the striatum of normal adult monkey. *Neurosci. Lett.* **328**, 213–216 (2002).

67. Benraiss, A., Chmielnicki, E., Lerner, K., Roh, D. & Goldman, S. A. Adenoviral Brain-Derived Neurotrophic Factor Induces Both Neostriatal and Olfactory Neuronal Recruitment from Endogenous Progenitor Cells in the Adult Forebrain. **21**, 6718–6731 (2001).

68. Wang, C. *et al.* Human and monkey striatal interneurons are derived from the medial

ganglionic eminence but not from the adult subventricular zone. *J. Neurosci.* **34**, 10906–23 (2014).

69. Sanai, N. *et al.* Unique astrocyte ribbon in adult human brain contains neural stem cells but lacks chain migration. *Nature* **427**, 740–744 (2004).

70. Quiñones-Hinojosa, A. *et al.* Cellular composition and cytoarchitecture of the adult human subventricular zone: a niche of neural stem cells. *J. Comp. Neurol.* **494**, 415–34 (2006).

71. Klempin, F., Kronenberg, G., Cheung, G., Kettenmann, H. & Kempermann, G. Properties of Doublecortin-(DCX)-Expressing Cells in the Piriform Cortex Compared to the Neurogenic Dentate Gyrus of Adult Mice. *PLoS One* **6**, e25760 (2011).

72. Wang, C. *et al.* Identification and characterization of neuroblasts in the subventricular zone and rostral migratory stream of the adult human brain. *Cell Res.* **21**, 1534–50 (2011).

73. Sanai, N. *et al.* Corridors of migrating neurons in the human brain and their decline during infancy. *Nature* **478**, 382–386 (2011).

74. Curtis, M. A. *et al.* Human neuroblasts migrate to the olfactory bulb via a lateral ventricular extension. *Science* **315**, 1243–9 (2007).

75. Libby, W. F., Berger, R., Mead, J. F., Alexander, G. V. & Ross, J. F. Replacement Rates for Human Tissue from Atmospheric Radiocarbon. *Science (80-.).* **146**, 1170–1172 (1964).

76. Harkness, D. D. Further investigations of the transfer of bomb 14 C to man. *Nature* **240**, 302–3 (1972).

77. Spalding, K. L., Buchholz, B. A., Bergman, L.-E., Druid, H. & Frisén, J. Age written in teeth by nuclear tests. *Nature* **437**, 333–334 (2005).

78. Spalding, K. L., Bhardwaj, R. D., Buchholz, B. a., Druid, H. & Frisén, J. Retrospective birth dating of cells in humans. *Cell* **122**, 133–43 (2005).

79. Spalding, K. L. *et al.* Dynamics of fat cell turnover in humans. *Nature* **453**, 783–7 (2008).

80. Bhardwaj, R. D. *et al.* Neocortical neurogenesis in humans is restricted to development. *Proc. Natl. Acad. Sci. U. S. A.* **103**, 12564–8 (2006).

81. Bergmann, O. *et al.* Evidence for cardiomyocyte renewal in humans. *Science* **324**, 98–102 (2009).

82. Bergmann, O. *et al.* The Age of Olfactory Bulb Neurons in Humans. *Neuron* **74**, 634–639 (2012).

83. Ernst, A. *et al.* Neurogenesis in the striatum of the adult human brain. *Cell* **156**, 1072–1083 (2014).

84. Eriksson, P. S. *et al.* Neurogenesis in the adult human hippocampus. *Nat. Med.* **4**, 1313–1317 (1998).

85. Knoth, R. *et al.* Murine features of neurogenesis in the human hippocampus across the lifespan from 0 to 100 years. *PLoS One* **5**, (2010).

86. Kempermann, G. *et al.* Human Adult Neurogenesis: Evidence and Remaining Questions. *Cell Stem Cell* **23**, 25–30 (2018).

87. Spalding, K. L. *et al.* Dynamics of hippocampal neurogenesis in adult humans. *Cell* **153**, 1219–1227 (2013).

88. Boldrini, M. *et al.* Short Article Human Hippocampal Neurogenesis Persists throughout Aging Short Article Human Hippocampal Neurogenesis Persists throughout Aging. 589–599 (2018). doi:10.1016/j.stem.2018.03.015

89. Sorrells, S. F. *et al.* children to undetectable levels in adults. *Nat. Publ. Gr.* **555**, 377–381 (2018).

90. Boekhoorn, K., Joels, M. & Lucassen, P. J. Increased proliferation reflects glial and vascular-associated changes, but not neurogenesis in the presenile Alzheimer hippocampus. *Neurobiol. Dis.* **24**, 1–14 (2006).

91. Parent, J. M. *et al.* Dentate granule cell neurogenesis is increased by seizures and contributes to aberrant network reorganization in the adult rat hippocampus. *J. Neurosci.* **17**, 3727–3738 (1997).

92. Gray, W. P. & Sundstrom, L. E. Kainic acid increases the proliferation of granule cell progenitors in the dentate gyrus of the adult rat. *Brain Res.* **790**, 52–9 (1998).

93. Jessberger, S., Römer, B., Babu, H. & Kempermann, G. Seizures induce proliferation and dispersion of doublecortin-positive hippocampal progenitor cells. *Exp. Neurol.* **196**, 342–51 (2005).

94. Parent, J. M., Valentin, V. V. & Lowenstein, D. H. Prolonged Seizures Increase Proliferating Neuroblasts in the Adult Rat Subventricular Zone–Olfactory Bulb Pathway. *J. Neurosci.* **22**, 3174–3188 (2002).

95. Gould, E., Cameron, H. A. & McEwen, B. S. Blockade of NMDA receptors increases cell death and birth in the developing rat dentate gyrus. *J. Comp. Neurol.* **340**, 551–65 (1994).

96. Tozuka, Y., Fukuda, S., Namba, T., Seki, T. & Hisatsune, T. GABAergic excitation promotes neuronal differentiation in adult hippocampal progenitor cells. *Neuron* **47**, 803–15 (2005).

97. Huang, Y., Doherty, J. J. & Dingledine, R. Altered histone acetylation at glutamate receptor 2 and brain-derived neurotrophic factor genes is an early event triggered by status epilepticus. *J. Neurosci.* **22**, 8422–8 (2002).

98. Jessberger, S. *et al.* Epigenetic modulation of seizure-induced neurogenesis and cognitive decline. *J. Neurosci.* **27**, 5967–5975 (2007).

99. Ribak, C. E., Tran, P. H., Spigelman, I., Okazaki, M. M. & Nadler, J. V. Status epilepticus-induced hilar basal dendrites on rodent granule cells contribute to recurrent excitatory circuitry. *J. Comp. Neurol.* **428**, 240–53 (2000).

100. Dashtipour, K., Wong, A. M., Obenaus, A., Spigelman, I. & Ribak, C. E. Temporal profile of hilar basal dendrite formation on dentate granule cells after status epilepticus. *Epilepsy Res.* **54**, 141–51 (2003).

101. Shapiro, L. A., Korn, M. J. & Ribak, C. E. Newly generated dentate granule cells from

epileptic rats exhibit elongated hilar basal dendrites that align along GFAP-immunolabeled processes. *Neuroscience* **136**, 823–31 (2005).

102. Jin, K. *et al.* Increased hippocampal neurogenesis in Alzheimer's disease. *Proc. Natl. Acad. Sci. U. S. A.* **101**, 343–7 (2004).

103. Moreno-Jiménez, E. P. *et al.* Adult hippocampal neurogenesis is abundant in neurologically healthy subjects and drops sharply in patients with Alzheimer's disease. *Nat. Med.* **25**, 554–560 (2019).

104. Tobin, M. K. *et al.* Human Hippocampal Neurogenesis Persists in Aged Adults and Alzheimer's Disease Patients. *Cell Stem Cell* **24**, 974-982.e3 (2019).

105. Wen, P. H. *et al.* Overexpression of wild type but not an FAD mutant presenilin-1 promotes neurogenesis in the hippocampus of adult mice. *Neurobiol. Dis.* **10**, 8–19 (2002).

106. Wang, R., Dineley, K. T., Sweatt, J. D. & Zheng, H. Presenilin 1 familial Alzheimer's disease mutation leads to defective associative learning and impaired adult neurogenesis. *Neuroscience* **126**, 305–12 (2004).

107. Chevallier, N. L. *et al.* Perturbed neurogenesis in the adult hippocampus associated with presenilin-1 A246E mutation. *Am. J. Pathol.* **167**, 151–9 (2005).

108. Dong, H. *et al.* Modulation of hippocampal cell proliferation, memory, and amyloid plaque deposition in APPsw (Tg2576) mutant mice by isolation stress. *Neuroscience* **127**, 601–9 (2004).

109. Haughey, N. J. *et al.* Disruption of neurogenesis by amyloid beta-peptide, and perturbed neural progenitor cell homeostasis, in models of Alzheimer's disease. *J. Neurochem.* **83**, 1509–24 (2002).

110. Donovan, M. H. *et al.* Decreased adult hippocampal neurogenesis in the PDAPP mouse model of Alzheimer's disease. *J. Comp. Neurol.* **495**, 70–83 (2006).

111. Jin, K. *et al.* Enhanced neurogenesis in Alzheimer's disease transgenic (PDGF-APPSw,Ind) mice. *Proc. Natl. Acad. Sci. U. S. A.* **101**, 13363–7 (2004).

112. Winner, B. *et al.* Mutant alpha-synuclein exacerbates age-related decrease of neurogenesis. *Neurobiol. Aging* **29**, 913–25 (2008).

113. Johnson, C. O. *et al.* Global, regional, and national burden of stroke, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol.* **18**, 439–458 (2019).

114. Go, A. S. *et al. Heart Disease and Stroke Statistics - 2014 Update: A report from the American Heart Association*. *Circulation* **129**, (2014).

115. Nakanishi, N. *et al.* Neuroprotection by the NR3A subunit of the NMDA receptor. *J. Neurosci.* **29**, 5260–5265 (2009).

116. Joza, N. *et al.* Essential role of the mitochondrial apoptosis-inducing factor in programmed cell death. *Nature* **410**, 549–554 (2001).

117. Mihara, M. *et al.* P53 Has a Direct Apoptogenic Role At the Mitochondria. *Mol. Cell* **11**, 577–590 (2003).

118. Gupta, S., Campbell, D., Dérijard, B. & Davis, R. J. Transcription factor ATF2 regulation by the JNK signal transduction pathway. *Science (80-.).* **267**, 389–393 (1995).

119. Green, S. L., Kulp, K. S. & Vulliet, R. Cyclin-dependent protein kinase 5 activity increases in rat brain following ischemia. *Neurochem. Int.* **31**, 617–23 (1997).

120. Saft, M. *et al.* Stem Cell Repair of the Microvascular Damage in Stroke. 1–22 (2020).

121. Lee, J. S. *et al.* A long-term follow-up study of intravenous autologous mesenchymal stem cell transplantation in patients with ischemic stroke. *Stem Cells* **28**, 1099–1106 (2010).

122. Jin, K. *et al.* Directed migration of neuronal precursors into the ischemic cerebral cortex and striatum. *Mol. Cell. Neurosci.* **24**, 171–189 (2003).

123. Parent, J. M., Vexler, Z. S., Gong, C., Derugin, N. & Ferriero, D. M. Rat forebrain neurogenesis and striatal neuron replacement after focal stroke. *Ann. Neurol.* **52**, 802–813 (2002).

124. Yamashita, T. *et al.* Subventricular zone-derived neuroblasts migrate and differentiate into mature neurons in the post-stroke adult striatum. *J. Neurosci.* **26**, 6627–36 (2006).

125. Thored, P. *et al.* Persistent production of neurons from adult brain stem cells during recovery after stroke. *Stem Cells* **24**, 739–47 (2006).

126. Thored, P. *et al.* Long-term neuroblast migration along blood vessels in an area with transient angiogenesis and increased vascularization after stroke. *Stroke* **38**, 3032–3039 (2007).

127. Kojima, T. *et al.* Subventricular zone-derived neural progenitor cells migrate along a blood vessel scaffold toward the post-stroke striatum. *Stem Cells* **28**, 545–554 (2010).

128. Ohab, J. J., Fleming, S., Blesch, A. & Carmichael, S. T. A neurovascular niche for neurogenesis after stroke. *J. Neurosci.* **26**, 13007–13016 (2006).

129. Kaneko, N. *et al.* New neurons use slit-robo signaling to migrate through the glial meshwork and approach a lesion for functional regeneration. *Sci. Adv.* **4**, 1–14 (2018).

130. Robin, A. M. *et al.* Stromal cell-derived factor 1α mediates neural progenitor cell motility after focal cerebral ischemia. *J. Cereb. Blood Flow Metab.* **26**, 125–134 (2006).

131. Yan, Y. P. *et al.* Monocyte chemoattractant protein-1 plays a critical role in neuroblast migration after focal cerebral ischemia. *J. Cereb. Blood Flow Metab.* **27**, 1213–1224 (2007).

132. Lee, S. R. *et al.* Involvement of matrix metalloproteinase in neuroblast cell migration from the subventricular zone after stroke. *J. Neurosci.* **26**, 3491–3495 (2006).

133. Liu, X. S. *et al.* Angiopoietin 2 mediates the differentiation and migration of neural progenitor cells in the subventricular zone after stroke. *J. Biol. Chem.* **284**, 22680–9 (2009).

134. Butti, E. *et al.* Subventricular zone neural progenitors protect striatal neurons from glutamatergic excitotoxicity. 3320–3335 (2012). doi:10.1093/brain/aws194

135. Hoehn, B. D., Palmer, T. D. & Steinberg, G. K. Neurogenesis in rats after focal cerebral ischemia is enhanced by indomethacin. *Stroke* **36**, 2718–24 (2005).

136. Iosif, R. E. *et al.* Suppression of stroke-induced progenitor proliferation in adult subventricular zone by tumor necrosis factor receptor 1. *J. Cereb. Blood Flow Metab.* **28**, 1574–1587 (2008).

137. Thored, P. *et al.* Long-term accumulation of microglia with proneurogenic phenotype concomitant with persistent neurogenesis in adult subventricular zone after stroke. *Glia* **57**, 835–849 (2009).

138. Lindvall, O. & Björklund, A. Cell Therapy in Parkinson's Disease. *NeuroRx* **1**, 382–393 (2004).

139. Lindvall, O. & Björklund, A. Cell Therapeutics in Parkinson's Disease. *Neurotherapeutics* **8**, 539–548 (2011).

140. Andres, R. H. *et al.* Human neural stem cells enhance structural plasticity and axonal transport in the ischaemic brain. *Brain* **134**, 1777–1789 (2011).

141. Darsalia, V., Kallur, T. & Kokaia, Z. Survival, migration and neuronal differentiation of human fetal striatal and cortical neural stem cells grafted in stroke-damaged rat striatum. *Eur. J. Neurosci.* **26**, 605–614 (2007).

142. Horie, N. *et al.* Transplanted stem cell-secreted vascular endothelial growth factor effects poststroke recovery, inflammation, and vascular repair. *Stem Cells* **29**, 274–285 (2011).

143. Kelly, S. *et al.* Transplanted human fetal neural stem cells survive , migrate , and differentiate in ischemic rat cerebral cortex. **101**, 11839–11844 (2004).

144. Oki, K. *et al.* Human-induced pluripotent stem cells form functional neurons and improve recovery after grafting in stroke-damaged brain. *Stem Cells* **30**, 1120–1133 (2012).

145. Shen, C.-C. *et al.* Intravenous implanted neural stem cells migrate to injury site, reduce infarct volume, and improve behavior after cerebral ischemia. *Curr. Neurovasc. Res.* **7**, 167–79 (2010).

146. Abeysinghe, H. C. S. *et al.* Pre-differentiation of human neural stem cells into GABAergic neurons prior to transplant results in greater repopulation of the damaged brain and accelerates functional recovery after transient ischemic stroke. *Stem Cell Res. Ther.* **6**, 1–19 (2015).

147. Darsalia, V. *et al.* Cell number and timing of transplantation determine survival of human neural stem cell grafts in stroke-damaged rat brain. *J. Cereb. Blood Flow Metab.* **31**, 235–242 (2011).

148. Roitberg, B. Z. *et al.* Survival and early differentiation of human neural stem cells transplanted in a nonhuman primate model of stroke. *J. Neurosurg.* **105**, 96–102 (2006).

149. Chu, K. *et al.* Human neural stem cells improve sensorimotor deficits in the adult rat brain with experimental focal ischemia. *Brain Res.* **1016**, 145–153 (2004).

150. Jiang, Q. *et al.* MRI detects white matter reorganization after neural progenitor cell

treatment of stroke. *Neuroimage* **32**, 1080–1089 (2006).

151. Pollock, K. *et al.* A conditionally immortal clonal stem cell line from human cortical neuroepithelium for the treatment of ischemic stroke. *Exp. Neurol.* **199**, 143–155 (2006).

152. Zhu, J. de, Wang, J. jie, Ge, G. & Kang, C. sheng. Effects of Noggin-Transfected Neural Stem Cells on Neural Functional Recovery and Underlying Mechanism in Rats with Cerebral Ischemia Reperfusion Injury. *J. Stroke Cerebrovasc. Dis.* **26**, 1547–1559 (2017).

153. Bacigaluppi, M. *et al.* Neural stem cell transplantation induces stroke recovery by upregulating glutamate transporter GLT-1 in astrocytes. *J. Neurosci.* **36**, 10529–10544 (2016).

154. Mine, Y. *et al.* Grafted human neural stem cells enhance several steps of endogenous neurogenesis and improve behavioral recovery after middle cerebral artery occlusion in rats. *Neurobiol. Dis.* **52**, 191–203 (2013).

155. Kim, M. *et al.* TNF-α Pretreatment Improves the Survival and Function of Transplanted Human Neural Progenitor Cells Following Hypoxic-Ischemic Brain Injury. *Cells* **9**, 1195 (2020).

156. Sakata, H. *et al.* Neural stem cells genetically modified to overexpress Cu/Zn-superoxide dismutase enhance amelioration of ischemic stroke in mice. *Stroke* **43**, 2423–2429 (2012).

157. Sakata, H. *et al.* Interleukin 6-preconditioned neural stem cells reduce ischaemic injury in stroke mice. *Brain* **135**, 3298–3310 (2012).

158. Kalladka, D. *et al.* Human neural stem cells in patients with chronic ischaemic stroke (PISCES): a phase 1, first-in-man study. *Lancet* **388**, 787–796 (2016).

159. Muir, K. W. *et al.* Intracerebral implantation of human neural stem cells and motor recovery after stroke: Multicentre prospective single-arm study (PISCES-2). *J. Neurol. Neurosurg. Psychiatry* 396–401 (2020). doi:10.1136/jnnp-2019-322515

160. Daadi, M. M., Maag, A. L. & Steinberg, G. K. Adherent self-renewable human embryonic stem cell-derived neural stem cell line: Functional engraftment in experiment stroke model. *PLoS One* **3**, (2008).

161. Daadi, M. M. *et al.* Molecular and Magnetic Resonance Imaging of Human Embryonic Stem Cell – Derived Neural Stem Cell Grafts in Ischemic Rat Brain. *Mol. Ther.* **17**, 1282–1291 (2009).

162. Bühnemann, C. *et al.* Neuronal differentiation of transplanted embryonic stem cell-derived precursors in stroke lesions of adult rats. *Brain* **129**, 3238–3248 (2006).

163. Steinbeck, J. A., Koch, P., Derouiche, A. & Brüstle, O. Human embryonic stem cell-derived neurons establish region-specific, long-range projections in the adult brain. *Cell. Mol. Life Sci.* **69**, 461–470 (2012).

164. Rosenblum, S. *et al.* BDNF pretreatment of human embryonic-derived neural stem cells improves cell survival and functional recovery after transplantation in hypoxic-ischemic stroke. *Cell Transplant.* **24**, 2449–2461 (2015).

165. Seminatore, C. *et al.* The postischemic environment differentially impacts teratoma or tumor formation after transplantation of human embryonic stem cell-derived neural progenitors. *Stroke* **41**, 153–159 (2010).

166. Yao, H. *et al.* Transdifferentiation-induced neural stem cells promote recovery of middle cerebral artery stroke rats. *PLoS One* **10**, 1–19 (2015).

167. Jiang, M. *et al.* Induction of pluripotent stem cells transplantation therapy for ischemic stroke. *Mol. Cell. Biochem.* **354**, 67–75 (2011).

168. Jensen, M. B., Yan, H., Krishnaney-Davison, R., Al Sawaf, A. & Zhang, S. C. Survival and differentiation of transplanted neural stem cells derived from human induced pluripotent stem cells in a rat stroke model. *J. Stroke Cerebrovasc. Dis.* **22**, 304–308 (2013).

169. Kawai, H. *et al.* Tridermal tumorigenesis of induced pluripotent stem cells transplanted in ischemic brain. *J. Cereb. Blood Flow Metab.* **30**, 1487–1493 (2010).

170. Yamashita, T., Kawai, H., Tian, F., Ohta, Y. & Abe, K. Tumorigenic development of induced pluripotent stem cells in ischemic mouse brain. *Cell Transplant.* **20**, 883–891 (2011).

171. Nakagawa, M. *et al.* A novel efficient feeder-Free culture system for the derivation of human induced pluripotent stem cells. *Sci. Rep.* **4**, 1–7 (2014).

172. Okita, K. *et al.* An efficient nonviral method to generate integration-free human-induced pluripotent stem cells from cord blood and peripheral blood cells. *Stem Cells* **31**, 458–466 (2013).

173. Trokovic, R. *et al.* Advanced Feeder-Free Generation of Induced Pluripotent Stem Cells Directly From Blood Cells. *Stem Cells Transl. Med.* **3**, 1402–1409 (2014).

174. Oh, S. H. *et al.* Multimodal Therapeutic Effects of Neural Precursor Cells Derived from Human-Induced Pluripotent Stem Cells through Episomal Plasmid-Based Reprogramming in a Rodent Model of Ischemic Stroke. *Stem Cells Int.* **2020**, (2020).

175. Tornero, D. *et al.* Human induced pluripotent stem cell-derived cortical neurons integrate in stroke-injured cortex and improve functional recovery. *Brain* **136**, 3561–3577 (2013).

176. Tornero, D. *et al.* Synaptic inputs from stroke-injured brain to grafted human stem cell-derived neurons activated by sensory stimuli. *Brain* **140**, 692–706 (2017).

177. Yuan, T. *et al.* Human induced pluripotent stem cell-derived neural stem cells survive, migrate, differentiate, and improve neurologic function in a rat model of middle cerebral artery occlusion. *Stem Cell Res. Ther.* **4**, (2013).

178. Hermanto, Y. *et al.* Transplantation of feeder-free human induced pluripotent stem cell–derived cortical neuron progenitors in adult male Wistar rats with focal brain ischemia. *J. Neurosci. Res.* **96**, 863–874 (2018).

179. Gomi, M. *et al.* Functional recovery of the murine brain ischemia model using human induced pluripotent stem cell-derived telencephalic progenitors. *Brain Res.* **1459**, 52–60 (2012).

180. Polentes, J. *et al.* Human induced pluripotent stem cells improve stroke outcome and

reduce secondary degeneration in the recipient brain. *Cell Transplant.* **21**, 2587–2602 (2012).

181. Chang, D. J. *et al.* Therapeutic potential of human induced pluripotent stem cells in experimental stroke. *Cell Transplant.* **22**, 1427–1440 (2013).

182. Laterza, C. *et al.* Attenuation of reactive gliosis in stroke-injured mouse brain does not affect neurogenesis from grafted human iPSC-derived neural progenitors. *PLoS One* **13**, 1–15 (2018).

183. Tatarishvili, J. *et al.* Human induced pluripotent stem cells improve recovery in stroke-injured aged rats. *Restor. Neurol. Neurosci.* **32**, 547–558 (2014).

184. Baker, E. W. *et al.* Induced Pluripotent Stem Cell-Derived Neural Stem Cell Therapy Enhances Recovery in an Ischemic Stroke Pig Model. *Sci. Rep.* **7**, 1–15 (2017).

185. Lau, V. W., Platt, S. R., Grace, H. E., Baker, E. W. & West, F. D. Human iNPC therapy leads to improvement in functional neurologic outcomes in a pig ischemic stroke model. *Brain Behav.* **8**, 1–11 (2018).

186. Kuroda, S. & Houkin, K. Translational Challenge for Bone Marrow Stroma Cell Therapy after Stroke. in **32**, 62–68 (2013).

187. Li, G. *et al.* Bone marrow mesenchymal stem cell therapy in ischemic stroke: mechanisms of action and treatment optimization strategies. *Neural Regen. Res.* **11**, 1015–24 (2016).

188. Wang, J. *et al.* Bone marrow mononuclear cells exert long-term neuroprotection in a rat model of ischemic stroke by promoting arteriogenesis and angiogenesis. *Brain. Behav. Immun.* **34**, 56–66 (2013).

189. Wang, J. *et al.* Bone marrow mononuclear cell transplantation promotes therapeutic angiogenesis via upregulation of the VEGF-VEGFR2 signaling pathway in a rat model of vascular dementia. *Behav. Brain Res.* **265**, 171–180 (2014).

190. Kikuchi-Taura, A. *et al.* Bone Marrow Mononuclear Cells Activate Angiogenesis via Gap Junction-Mediated Cell-Cell Interaction. *Stroke* 1279–1289 (2020). doi:10.1161/STROKEAHA.119.028072

191. Savitz, S. I. *et al.* Intravenous autologous bone marrow mononuclear cells for ischemic stroke. *Ann. Neurol.* **70**, 59–69 (2011).

192. Taguchi, A. *et al.* Intravenous Autologous Bone Marrow Mononuclear Cell Transplantation for Stroke: Phase1/2a Clinical Trial in a Homogeneous Group of Stroke Patients. *Stem Cells Dev.* **24**, 2207–2218 (2015).

193. Prasad, K. *et al.* Intravenous autologous bone marrow mononuclear stem cell therapy for ischemic stroke: A multicentric, randomized trial. *Stroke* **45**, 3618–3624 (2014).

194. Calvo, C. F. *et al.* Vascular endothelial growth factor receptor 3 directly regulates murine neurogenesis. *Genes Dev.* **25**, 831–844 (2011).

195. Decressac, M. *et al.* Neuropeptide Y stimulates proliferation, migration and differentiation of neural precursors from the subventricular zone in adult mice. *Neurobiol. Dis.* **34**, 441–449 (2009).

196. Shingo, T., Todd Sorokan, S., Shimazaki, T. & Weiss, S. Erythropoietin regulates the

in vitro and in vivo production of neuronal progenitors by mammalian forebrain neural stem cells. *J. Neurosci.* **21**, 9733–9743 (2001).

197. Kolb, B. *et al.* 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 (2007).

198. Kluska, M. M., Witte, O. W., Bolz, J. & Redecker, C. Neurogenesis in the adult dentate gyrus after cortical infarcts: Effects of infarct location, N-methyl-D-aspartate receptor blockade and anti-inflammatory treatment. *Neuroscience* **135**, 723–735 (2005).

199. Doeppner, T. R. *et al.* TAT-Hsp70-mediated neuroprotection and increased survival of neuronal precursor cells after focal cerebral ischemia in mice. *J. Cereb. Blood Flow Metab.* **29**, 1187–1196 (2009).

200. Kobayashi, Y. *et al.* The role of NADRIN, a Rho GTPase-activating protein, in the morphological differentiation of astrocytes. *J. Biochem.* **153**, 389–398 (2013).

201. Faiz, M. *et al.* Adult Neural Stem Cells from the Subventricular Zone Give Rise to Reactive Astrocytes in the Cortex after Stroke. *Cell Stem Cell* **17**, 624–634 (2015).

202. Sun, W. *et al.* SOX9 is an astrocyte-specific nuclear marker in the adult brain outside the neurogenic regions. *J. Neurosci.* **37**, 4493–4507 (2017).

203. Sirko, S. *et al.* Reactive glia in the injured brain acquire stem cell properties in response to sonic hedgehog glia. *Cell Stem Cell* **12**, 426–439 (2013).

204. Fukuda, S. *et al.* Two Distinct Subpopulations of Nestin-Positive Cells in Adult Mouse Dentate Gyrus. **23**, 9357–9366 (2003).

205. Bushong, E. A., Martone, M. E., Jones, Y. Z. & Ellisman, M. H. Protoplasmic astrocytes in CA1 stratum radiatum occupy separate anatomical domains. *J. Neurosci.* **22**, 183–92 (2002).

206. Matyash, V. & Kettenmann, H. Heterogeneity in astrocyte morphology and physiology. *Brain Res. Rev.* **63**, 2–10 (2010).

207. Emsley, J. G. & Macklis, J. D. Astroglial heterogeneity closely reflects the neuronal-defined anatomy of the adult murine CNS. *Neuron Glia Biol.* **2**, 175–186 (2006).

208. Zeisel, A. *et al.* Molecular Architecture of the Mouse Nervous System. *Cell* **174**, 999-1014.e22 (2018).

209. Ogata, K. & Kosaka, T. Structural and quantitative analysis of astrocytes in the mouse hippocampus. *Neuroscience* **113**, 221–33 (2002).

210. Livet, J. *et al.* Transgenic strategies for combinatorial expression of fluorescent proteins in the nervous system. *Nature* **450**, 56–62 (2007).

211. Halassa, M. M., Fellin, T., Takano, H., Dong, J. H. & Haydon, P. G. Synaptic islands defined by the territory of a single astrocyte. *J. Neurosci.* **27**, 6473–6477 (2007).

212. Oberheim, N. A. *et al.* Loss of astrocytic domain organization in the epileptic brain. *J. Neurosci.* **28**, 3264–3276 (2008).

213. Koehler, R. C., Roman, R. J. & Harder, D. R. Astrocytes and the regulation of cerebral

blood flow. *Trends Neurosci.* **32**, 160–169 (2009).

214. Mulligan, S. J. & MacVicar, B. A. Calcium transients in astrocyte endfeet cause cerebrovascular constrictions. *Nature* **431**, 195–9 (2004).

215. Christopherson, K. S. *et al.* Thrombospondins are astrocyte-secreted proteins that promote CNS synaptogenesis. *Cell* **120**, 421–433 (2005).

216. Eroglu, C. & Barres, B. A. Regulation of synaptic connectivity by glia. *Nature* **468**, 223–231 (2010).

217. Ullian, E. M., Sapperstein, S. K., Christopherson, K. S. & Barres, B. A. Control of synapse number by glia. *Science (80-.).* **291**, 657–661 (2001).

218. Allen, N. J. *et al.* Astrocyte glypicans 4 and 6 promote formation of excitatory synapses via GluA1 AMPA receptors. *Nature* **486**, 410–414 (2012).

219. Kucukdereli, H. *et al.* Control of excitatory CNS synaptogenesis by astrocyte-secreted proteins hevin and SPARC. *Proc. Natl. Acad. Sci. U. S. A.* **108**, (2011).

220. Stogsdill, J. A. *et al.* Astrocytic neuroligins control astrocyte morphogenesis and synaptogenesis. *Nature* **551**, 192–197 (2017).

221. Beattie, E. C. *et al.* Control of synaptic strength by glial TNFα. *Science (80-.).* **295**, 2282–2285 (2002).

222. Cotrina, M. L., Lin, J. H. C. & Nedergaard, M. Cytoskeletal assembly and ATP release regulate astrocytic calcium signaling. *J. Neurosci.* **18**, 8794–8804 (1998).

223. Parpura, V. *et al.* Glutamate-mediated astrocyte-neuron signalling. *Nature* **369**, 744–747 (1994).

224. Zonta, M. *et al.* Neuron-to-astrocyte signaling is central to the dynamic control of brain microcirculation. *Nat. Neurosci.* **6**, 43–50 (2003).

225. Schell, M. J., Molliver, M. E. & Snyder, S. H. D-serine, an endogenous synaptic modulator: Localization to astrocytes and glutamate-stimulated release. *Proc. Natl. Acad. Sci. U. S. A.* **92**, 3948–3952 (1995).

226. Barkho, B. Z. *et al.* Identification of astrocyte-expressed factors that modulate neural stem/progenitor cell differentiation. *Stem Cells Dev.* **15**, 407–421 (2006).

227. Garber, C. *et al.* Astrocytes decrease adult neurogenesis during virus-induced memory dysfunction via IL-1. *Nat. Immunol.* **19**, 151–161 (2018).

228. Lu, Z. & Kipnis, J. Thrombospondin 1—a key astrocyte‐derived neurogenic factor. *FASEB J.* **24**, 1925–1934 (2010).

229. Lie, D.-C. *et al.* Wnt signalling regulates adult hippocampal neurogenesis. *Nature* **437**, 1370–5 (2005).

230. Ashton, R. S. *et al.* Astrocytes regulate adult hippocampal neurogenesis through ephrin-B signaling. *Nat. Neurosci.* **15**, 1399–1406 (2012).

231. Moreno-Estellés, M. *et al.* Symmetric expansion of neural stem cells from the adult olfactory bulb is driven by astrocytes via WNT7A. *Stem Cells* **30**, 2796–2809 (2012).

232. Ferrón, S. R. *et al.* Postnatal loss of Dlk1 imprinting in stem cells and niche astrocytes regulates neurogenesis. *Nature* **475**, 381–387 (2011).

233. Basak, O., Giachino, C., Fiorini, E., MacDonald, H. R. & Taylor, V. Neurogenic subventricular zone stem/progenitor cells are Notch1-dependent in their active but not quiescent state. *J. Neurosci.* **32**, 5654–5666 (2012).

234. Wilhelmsson, U. *et al.* Nestin Regulates Neurogenesis in Mice Through Notch Signaling from Astrocytes to Neural Stem Cells. *Cereb. Cortex* **29**, 4050–4066 (2019).

235. Choi, J. S. *et al.* Upregulation of gp130 and STAT3 activation in the rat hippocampus following transient forebrain ischemia. *Glia* **41**, 237–246 (2003).

236. Justicia, C., Gabriel, C. & Planas, A. M. Activation of the JAK/STAT pathway following transients focal cerebral ischemia: Signaling through Jak1 and Stat3 in astrocytes. *Glia* **30**, 253–270 (2000).

237. Bardehle, S. *et al.* Live imaging of astrocyte responses to acute injury reveals selective juxtavascular proliferation. *Nat. Neurosci.* **16**, 580–586 (2013).

238. Pekny, M. & Pekna, M. Astrocyte reactivity and reactive astrogliosis: Costs and benefits. *Physiol. Rev.* **94**, 1077–1098 (2014).

239. Lange Canhos, L. *et al.* Repetitive injury and absence of monocytes promote astrocyte self-renewal and neurological recovery. *Glia* 1–17 (2020). doi:10.1002/glia.23893

240. Frik, J. *et al.* Cross‐talk between monocyte invasion and astrocyte proliferation regulates scarring in brain injury. *EMBO Rep.* **19**, 1–20 (2018).

241. Bush, T. G. *et al.* Leukocyte Infiltration, Neuronal Degeneration, and Neurite Outgrowth after Ablation of Scar-Forming, Reactive Astrocytes in Adult Transgenic Mice provision of metabolic substrates for neurons, and inter-actions with endothelia to create and maintain the b. *Neuron* **23**, 297–308 (1999).

242. Myer, D. J., Gurkoff, G. G., Lee, S. M., Hovda, D. A. & Sofroniew, M. V. Essential protective roles of reactive astrocytes in traumatic brain injury. *Brain* **129**, 2761–2772 (2006).

243. Wilhelmsson, U. *et al.* Absence of glial fibrillary acidic protein and vimentin prevents hypertrophy of astrocytic processes and improves post-traumatic regeneration. *J. Neurosci.* **24**, 5016–5021 (2004).

244. Pekny, M. & Pekna, M. Reactive gliosis in the pathogenesis of CNS diseases. *Biochim. Biophys. Acta - Mol. Basis Dis.* **1862**, 483–491 (2016).

245. Zamanian, J. L. *et al.* Genomic analysis of reactive astrogliosis. *J. Neurosci.* **32**, 6391–6410 (2012).

246. Morán, J., Maar, T. & Pasantes-Morales, H. Cell volume regulation in taurine deficient cultured astrocytes. *Adv. Exp. Med. Biol.* **359**, 361–7 (1994).

247. Vitarella, D., DiRisio, D. J., Kimelberg, H. K. & Aschner, M. Potassium and taurine release are highly correlated with regulatory volume decrease in neonatal primary rat astrocyte cultures. *J. Neurochem.* **63**, 1143–1149 (1994).

248. Pasantes‐Morale, H., Moran, J. & Schousboe, A. Volume‐sensitive release of taurine

from cultured astrocytes: Properties and mechanism. *Glia* **3**, 427–432 (1990).

249. Overman, J. J. *et al.* A role for ephrin-A5 in axonal sprouting, recovery, and activity-dependent plasticity after stroke. *Proc. Natl. Acad. Sci. U. S. A.* **109**, E2230-9 (2012).

250. Clarkson, A. N., Huang, B. S., MacIsaac, S. E., Mody, I. & Carmichael, S. T. Reducing excessive GABA-mediated tonic inhibition promotes functional recovery after stroke. *Nature* **468**, 305–309 (2010).

251. Wilhelmsson, U. *et al.* Injury Leads to the Appearance of Cells with Characteristics of Both Microglia and Astrocytes in Mouse and Human Brain. *Cereb. Cortex* **27**, 3360–3377 (2017).

252. Duan, C.-L. *et al.* Striatal astrocytes transdifferentiate into functional mature neurons following ischemic brain injury. *Glia* n/a-n/a (2015). doi:10.1002/glia.22837

253. Berninger, B. *et al.* Functional properties of neurons derived from in vitro reprogrammed postnatal astroglia. *J. Neurosci.* **27**, 8654–8664 (2007).

254. Doetsch, F., García-Verdugo, J. M. & Alvarez-Buylla, A. Cellular composition and three-dimensional organization of the subventricular germinal zone in the adult mammalian brain. *J. Neurosci.* **17**, 5046–61 (1997).

255. Seri, B., García-Verdugo, J. M., McEwen, B. S. & Alvarez-Buylla, A. Astrocytes Give Rise to New Neurons in the Adult Mammalian Hippocampus. *J. Neurosci.* **21**, 7153–7160 (2001).

256. Llorens-bobadilla, E. *et al.* Single-Cell Transcriptomics Reveals a Population of Dormant Neural Stem Cells that Become Activated upon Brain Injury Article Single-Cell Transcriptomics Reveals a Population of Dormant Neural Stem Cells that Become Activated upon Brain Injury. *Stem Cell* **17**, 329–340 (2015).

257. Gage, F. H. *et al.* Survival and differentiation of adult neuronal progenitor cells transplanted to the adult brain. **92**, 11879–11883 (1995).

258. Shihabuddin, L. S., Horner, P. J., Ray, J. & Gage, F. H. Adult Spinal Cord Stem Cells Generate Neurons after Transplantation in the Adult Dentate Gyrus. **20**, 8727–8735 (2000).

259. Lie, D. C. *et al.* The Adult Substantia Nigra Contains Progenitor Cells with Neurogenic Potential. **22**, 6639–6649 (2002).

260. Herrera, D. G., Garcia-verdugo, J. M. & Alvarez-buylla, A. Adult-Derived Neural Precursors Transplanted into Multiple Regions in the Adult Brain. 867–877 (1999).

261. Zhang, R. L. *et al.* MIGRATION AND DIFFERENTIATION OF ADULT RAT SUBVENTRICULAR ZONE PROGENITOR CELLS TRANSPLANTED INTO THE ADULT RAT. **116**, 373–382 (2003).

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

263. Gaillard, A. *et al.* Reestablishment of damaged adult motor pathways by grafted embryonic cortical neurons. **10**, 1294–1299 (2007).

264. Martínez-Cerdeño, V. *et al.* Embryonic MGE Precursor Cells Grafted into Adult Rat

Striatum Integrate and Ameliorate Motor Symptoms in 6-OHDA-Lesioned Rats. *cell s* 238–250 (2010). doi:10.1016

265. Ma, K. *et al.* Direct conversion of mouse astrocytes into neural progenitor cells and specific lineages of neurons. *Transl. Neurodegener.* **7**, 1–15 (2018).

266. Cheng, L. *et al.* Direct conversion of astrocytes into neuronal cells by drug cocktail. *Cell Res.* **25**, 1269–1272 (2015).

267. Buffo, A. *et al.* Origin and progeny of reactive gliosis: A source of multipotent cells in the injured brain. *Proc. Natl. Acad. Sci. U. S. A.* **105**, 3581–3586 (2008).

268. Mattugini, N. *et al.* Inducing Different Neuronal Subtypes from Astrocytes in the Injured Mouse Cerebral Cortex. *Neuron* **103**, 1086-1095.e5 (2019).

269. Zamboni, M., Llorens-Bobadilla, E., Magnusson, J. P. & Frisén, J. A Widespread Neurogenic Potential of Neocortical Astrocytes Is Induced by Injury. *Cell Stem Cell* (2020). doi:10.1016/j.stem.2020.07.006

270. Yin, J.-C. *et al.* Chemical Conversion of Human Fetal Astrocytes into Neurons through Modulation of Multiple Signaling Pathways. *Stem Cell Reports* **12**, 488–501 (2019).

271. Rivetti Di Val Cervo, P. *et al.* Induction of functional dopamine neurons from human astrocytes in vitro and mouse astrocytes in a Parkinson's disease model. *Nat. Biotechnol.* **35**, 444–452 (2017).

272. Gao, L. *et al.* Direct Generation of Human Neuronal Cells from Adult Astrocytes by Small Molecules. *Stem Cell Reports* **8**, 538–547 (2017).

273. Nakajima-Koyama, M., Lee, J., Ohta, S., Yamamoto, T. & Nishida, E. Induction of pluripotency in astrocytes through a neural stem cell-like state. *J. Biol. Chem.* **290**, 31173–31188 (2015).

274. Zhang, L. *et al.* Small Molecules Efficiently Reprogram Human Astroglial Cells into Functional Neurons. *Cell Stem Cell* **17**, 735–747 (2015).

275. Zywitza, V., Misios, A., Bunatyan, L., Willnow, T. E. & Rajewsky, N. Single-Cell Transcriptomics Characterizes Cell Types in the Subventricular Zone and Uncovers Molecular Defects Impairing Adult Neurogenesis. *Cell Rep.* **25**, 2457-2469.e8 (2018).

276. Hochgerner, H., Zeisel, A., Lönnerberg, P. & Linnarsson, S. Conserved properties of dentate gyrus neurogenesis across postnatal development revealed by single-cell RNA sequencing. *Nat. Neurosci.* **21**, 290–299 (2018).