 doi:10.20944/preprints202012.0544.v1

Cellular mechanisms participating in brain repair of adult zebrafish and mammals after injury

Batoul Ghaddar[1], Luisa Lübke[2], David Couret[1,3], Sepand Rastegar[2]* and Nicolas Diotel[1]*

Address:

1 Université de La Réunion, INSERM, UMR 1188, Diabète athérothrombose Thérapies Réunion Océan Indien (DéTROI), Saint-Denis de La Réunion, France.

2 Institute of Biological and Chemical Systems-Biological Information Processing (IBCS-BIP), Karlsruhe Institute of Technology (KIT), Postfach 3640, 76021 Karlsruhe, Germany.

3 CHU de La Réunion, Saint-Denis and Saint-Pierre, France

*Correspondence to: nicolas.diotel@univ-reunion.fr and sepand.rastegar@kit.edu

Running title: Adult neurogenesis and cell recruitment after telencephalic injury

Key-words: adult neurogenesis, brain injury, neural stem cell, regeneration, stroke, zebrafish, mice

 doi:10.20944/preprints202012.0544.v1

Abstract

Adult neurogenesis is an evolutionary conserved process occurring in all vertebrates. However, striking differences are observed between the taxa, considering the number of neurogenic niches, the neural stem cell (NSC) identity and brain plasticity under constitutive and injury-induced conditions. Zebrafish has become a popular model for the investigation of the molecular and cellular mechanisms involved in adult neurogenesis. Compared to mammals, the adult zebrafish displays a high number of neurogenic niches distributed throughout the brain. Furthermore, it exhibits a strong regenerative capacity without scar formation or any obvious disabilities. In this review, we will first discuss the similarities and differences regarding (i) the distribution of neurogenic niches in the brain of adult zebrafish and mammals (mainly mouse) and (ii) the nature of the neural stem cells within the main telencephalic niches. In the second part, we will describe the cascade of cellular events occurring after telencephalic injury in zebrafish and mouse. Our study clearly shows that most early events happening right after the brain injury are shared between zebrafish and mouse including cell death, microglia and oligodendrocyte recruitment, as well as injury-induced neurogenesis. In mammals one of the consequences following an injury is the formation of a glial scar that is persistent. This is not the case in zebrafish, which may be one of the main reasons that zebrafish display a higher regenerative capacity.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 December 2020 doi:10.20944/preprints202012.0544.v1

Introduction

Neurogenesis is an important process in which new neurons are formed from a pool of neural stem cells (NSCs). This process is initiated by the proliferation of NSCs leading then to the differentiation, migration and the functional integration of newborn neurons into establishing and/or existing neuronal networks. Until recently, it was believed that neurogenesis only occurs during early embryonic development. However, Altman and Kaplan demonstrated in the 1960s and 1980s, respectively, that new neurons could also be produced in the brain of post-natal and adult rodents as well as monkeys (Altman and Das, 1965; Altman, 1969; Kaplan, 1985). Since this pioneer discovery, an increasing number of works confirmed that indeed adult neurogenesis occurs in the brain of all vertebrates, including mammals (Eriksson et al., 1998; Lindsey and Tropepe, 2006; Boldrini et al., 2018). Under physiological conditions, as well as after brain damage induced by traumatic brain injury (TBI), ischemia or neuro-degeneration, NSCs play key roles in brain plasticity through the genesis of new neurons. Understanding the mechanisms regulating their activation and proliferation during regenerative and constitutive neurogenesis, provides the chance to develop methods for combatting neurodegenerative diseases and disabilities following brain damage.

Adult neurogenesis is an important physiological process that supports brain plasticity and cognitive functions through the continuous generation of new neurons, allowed by the sustained activity of NSCs located in discrete brain regions called neurogenic niches. The persistence of functional neurogenesis during adulthood is evolutionary conserved from invertebrates (i.e crustaceans, insects, etc.) to vertebrates including fish, amphibians, reptiles, birds and mammals. However, the number of neurogenic niches, the proliferation rate of neural stem/progenitor cells, the migration and differentiation of new neurons appears to differ according to species, brain size and lifespan (Lindsey and Tropepe, 2006; Brus et al., 2013; Than-Trong et al., 2018; Diotel et al., 2020). In mammals, the two main neurogenic niches correspond to the subventricular zone of the lateral ventricles (SVZ) and the subgranular zone (SGZ) of the dentate gyrus (DG) of the hippocampus. In striking contrast, the small teleost zebrafish (*Danio rerio*) displays a high number of neurogenic niches distributed throughout its entire encephalon. In addition, while regenerative neurogenesis is imperfect in mammals, teleost fish are able to repair their telencephalon from large injuries without any striking consequences and disabilities (Diotel et al., 2020).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 December 2020 doi:10.20944/preprints202012.0544.v1

Such outstanding regenerative capacities strongly argue for a more comprehensive study of the molecular and cellular mechanisms allowing brain regeneration in teleost fish, in order to translate some important findings to humans.

In this review, we aimed at (i) describing the proliferative areas in the brain of fish and mammals, using mouse as an example (ii) illustrating the spatial and cellular organization of the main telencephalic neurogenic niches in a comparative approach, and (iii) highlighting the similarities and differences regarding the spatio-temporal recruitment of the different cell types involved in brain repair (microglia, oligodendrocytes and their precursors, astrocytes and NSCs). Concerning this last point, we will document the most studied models of brain damage: telencephalic mechanical injury in zebrafish and brain ischemia in mouse. Next, we will review the similarities and differences regarding neurogenic events and molecular mechanisms occurring after brain damage in zebrafish and mouse. Finally, we will highlight the value of zebrafish as a simple model for the analysis of brain repair mechanisms.

Location of neurogenic niches in the brain of adult zebrafish

In the past, pioneer works using BrdU incorporation studies and/or PCNA (Proliferating Cell Nuclear Antigen) immunohistochemistry demonstrated the existence of areas with a strong proliferative activity along the ventricular/periventricular layers in the zebrafish brain (Adolf et al., 2006; Pellegrini et al., 2007; Diotel et al., 2010a). These strongly proliferative areas are widespread and can be detected throughout all the brain subdivisions including the telencephalon, the diencephalon, the mesencephalon and the metencephalon (Figure 1A-C, left column) (Zupanc et al., 2005; Adolf et al., 2006; Grandel et al., 2006; Pellegrini et al., 2007; März et al., 2010).

In the telencephalon, the main proliferative areas are located along the ventricle in the ventral, dorsal, dorsolateral and posterolateral domains. Prominent domains of cell proliferation were also detected in the diencephalon, in the anterior and posterior parts of the preoptic area, as well as in the anterior, mediobasal and caudal hypothalamus. In the posterior part of the encephalon, proliferation was also reported close to the rhombencephalic ventricle (Figure 1A-C). The thalamus, the regions surrounding the habenula, the pretectal periventricular region (a subdomain close to the optic tectum) and the three subdivisions of the cerebellum including the valvula cerebelli, the corpus cerebelli,

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 December 2020 doi:10.20944/preprints202012.0544.v1

and the lobus caudalis cerebelli all harbor substantial proliferation as well (Zupanc et al., 2005; Lindsey and Tropepe, 2006; Pellegrini et al., 2007; Kaslin et al., 2009; Ito et al., 2010). These proliferative regions are highlighted in red in a sagittal zebrafish brain section scheme showing the distribution of neurogenic niches across the brain (Figure 1A).

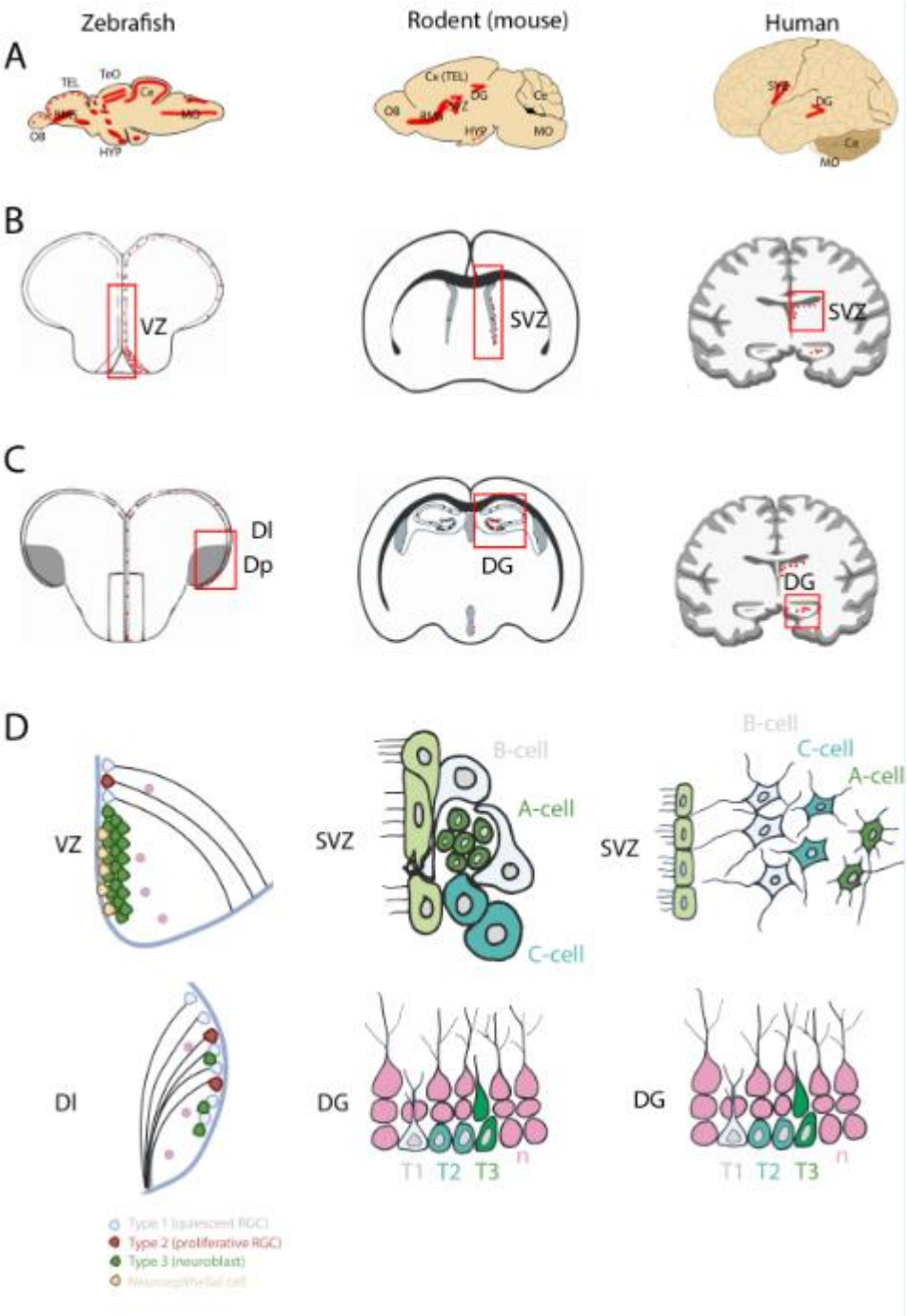

Figure 1: Localization and cellular organization of the main neurogenic niches in the brain of zebrafish, mouse and humans.

A: Sagittal sections of zebrafish (left), mouse (middle) and human (right) brains with the main proliferative regions (neurogenic niches) shown in red. The mammalian brain displays only two main neurogenic niches: the subventricular zone (SVZ) of the lateral ventricles and

 doi:10.20944/preprints202012.0544.v1

subgranular zone of the dentate gyrus (DG) of the hippocampus. The zebrafish brain displays numerous niches throughout the brain.
B and C: Transversal sections through the brain, marking the main neurogenic niches of the respective species shown in A.
D: The main neurogenic niches in the subpallial ventricular zone (VZ), the dorsolateral telencephalon (Dl) in zebrafish and their respective homologues in mammals: the SVZ and the DG of the hippocampus in mouse and humans. In zebrafish, type 1 and type 2 cells are quiescent and proliferative radial glial cells, respectively (quiescent and proliferative NSCs). Type 3 cells are proliferative neuroblasts. The neuroepithelial cells are NSCs from the subpallium. In mammals, the NSCs are shown in grey (B-cells and Type 1 -T1-), the transient amplifying cells in light green (C-Cells and Type -T2-) and the neuroblasts in dark green (A-cells and Type 3 -T3-). Note the hypocellular gap in the human SVZ compared to mice. OB: Olfactory bulbs; TEL: Telencephalon; TeO: Optic Tectum; Ce: Cerebellum; MO: medulla oblongata; HYP: hypothalamus.

All these proliferative areas have been shown to generate new neurons. Consequently, the adult zebrafish exhibits a strong neurogenic capacity due to the high number of active neurogenic niches throughout its brain (Zupanc and Zupanc, 1992; Zupanc et al., 2005; Lindsey and Tropepe, 2006; Pellegrini et al., 2007; Zupanc, 2008; Diotel et al., 2010a; Schmidt et al., 2013).

Neural stem cells and neural progenitor cells in the adult zebrafish telencephalon

In zebrafish, the main neurogenic niches that have been studied during adulthood are located in the telencephalon, the optic tectum and the cerebellum. The telencephalon remains undoubtedly the most investigated region of the brain, because it shares many features and homologies with the mammalian telencephalon, particularly considering adult neurogenesis (Kizil et al., 2012a; Diotel et al., 2020; Jurisch-Yaksi et al., 2020; Than-Trong et al., 2020). In the telencephalon, several studies have explored the identity and the diversity of the neural/progenitor cells sustaining the strong neurogenic activity observed in the different telencephalic subdomains (Pellegrini et al., 2007; Zupanc, 2008; März et al., 2010; Kizil et al., 2012a; Lindsey et al., 2012; Schmidt et al., 2014). In their initial work, Adolf and colleagues (2006) showed through BrdU incorporation studies and PCNA immunohistochemistry that the telencephalon contains two different types of neural progenitors: (1) slow cycling ones, distributed along the ventricular surface, and (2) fast cycling ones, organized mainly in a subpallial cluster (Adolf et al., 2006) (Figure 2). The slow cycling progenitors were identified as quiescent and proliferative radial glial cells (type 1 and 2 RGCs, respectively). In contrast, the fast-cycling cells were described as neuroblasts (type 3

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 December 2020 doi:10.20944/preprints202012.0544.v1

cells) (Figures 1 and 2) (Zupanc and Clint, 2003; Zupanc et al., 2005; Pellegrini et al., 2007; März et al., 2010; Rothenaigner et al., 2011; Lindsey et al., 2012; Diotel et al., 2016).

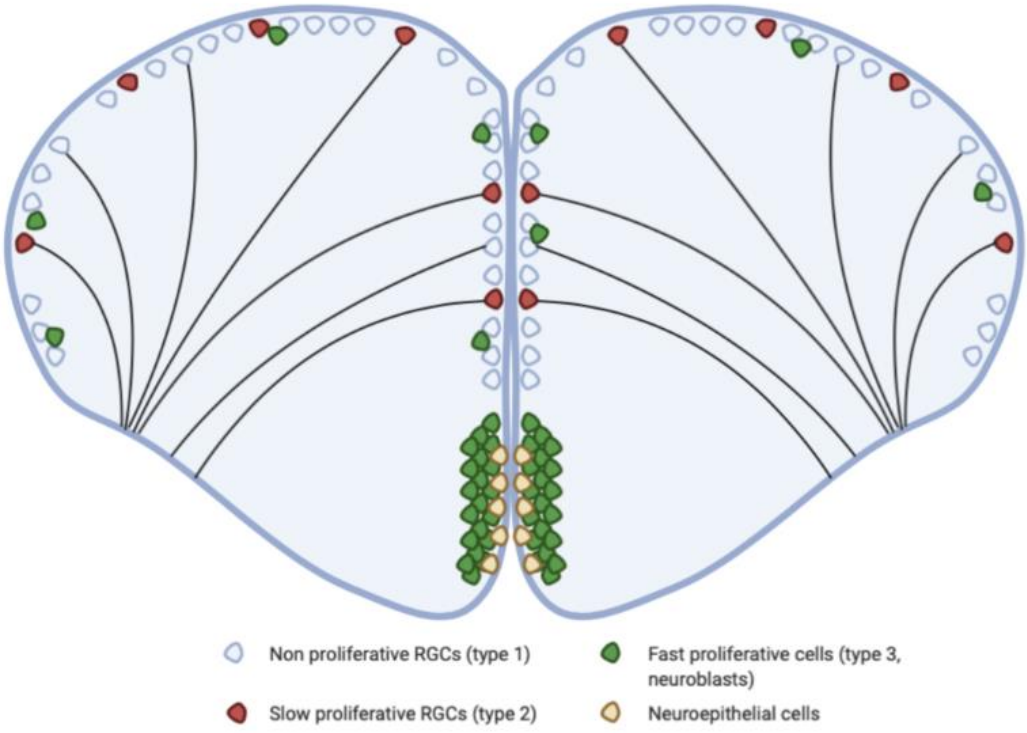

Figure 2: The telencephalon of adult zebrafish contains slow and fast cycling progenitors. The VZ of the dorsal telencephalon (pallium) is mainly composed of quiescent (type 1) or proliferative (type 2) RGCs corresponding to slow cycling progenitors. The ventral part of the telencephalon (subpallium) is composed of fast cycling progenitors (type 3 cells) identified as neuroblasts, grouped within a cluster and forming a rostral migratory like structure (RMS-like). Some neuroblasts are also observed scattered between RGC' soma in the pallium. RGCs were identified as *bona fide* neural stem cells in the pallium and neuroepithelial cells could be neural stem cells in the subpallium.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 December 2020 doi:10.20944/preprints202012.0544.v1

Type 1 and 2 cells: In the dorsal telencephalon, type 1 and type 2 cells correspond to quiescent and proliferative RGCs, respectively (März et al., 2010). These cells are morphologically defined by a small triangular or ovoid soma localized close to the ventricle and extending two cytoplasmic processes: one short process towards the ventricular surface, and one long process crossing the brain parenchyma and reaching the pial surface. In mammals, RGCs were initially described as a scaffold for the migration of newborn neurons during embryonic neurogenesis, and were later shown to behave as neural stem cells, as in zebrafish (Pellegrini et al., 2007; Noctor et al., 2008). At the end of the embryonic development in mammals, the majority of RGCs disappear by transforming into "conventional" astrocytes. However, RGCs persist during adulthood in the brain of adult zebrafish, maintain neurogenic properties and support neuronal migration (Noctor et al., 2002; Merkle et al., 2004; Pellegrini et al., 2007; Pinto and Gotz, 2007; Lam et al., 2009; Than-Trong and Bally-Cuif, 2015; Diotel et al., 2020).

In adult zebrafish, these telencephalic RGCs were shown to perform symmetric and asymmetric division, and also, in some cases, to be able to directly convert into neurons (Rothenaigner et al., 2011; Barbosa et al., 2015; Diotel et al., 2020). In the pallium, lineage tracing and microscopy analyses showed that type 1 cells give rise to type 2 cells, which can give rise to type 3 cells (type 3 = neuroblasts) that are tightly inserted between RGC' soma (Rothenaigner et al., 2011; Lange et al., 2020; Than-Trong et al., 2020). The newborn neurons will migrate radially along the long cytoplasmic RGC processes within the brain parenchyma to leave the ventricular zone (Pellegrini et al., 2007). At their target location they differentiate into mature neurons expressing well-characterized neuronal markers (i.e. HuC/D, Pax6a, PV) and display signs of functional integration such as synaptogenesis (Grandel et al., 2006; Pellegrini et al., 2007; Kroehne et al., 2011; Rothenaigner et al., 2011).

Adult RGCs in zebrafish express a set of well-identified markers (Table 1), including intermediate filaments (Gfap and vimentin), the Brain lipid binding protein (Blbp or fabp7), the calcium binding protein S100β, the estrogen-synthesizing enzyme (Aromatase B or cyp19a1b), and also progenitor markers such as nestin and Sox2 (Pellegrini et al., 2007; März et al., 2010; Lindsey et al., 2012; Than-Trong and Bally-Cuif, 2015; Diotel et al., 2016; Diotel et al., 2020). Recent studies also documented the expression of the inhibitor of DNA binding 1 (Id1), the chemokine receptor cxcr4, *Notch1a/b, Notch3* and *her4* genes in RGCs (Diotel et al., 2010b; Chapouton et al., 2011; Kroehne et al., 2011; Diotel et al., 2015a; Rodriguez Viales

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 December 2020 doi:10.20944/preprints202012.0544.v1

et al., 2015; Zhang et al., 2020). Most of these markers in zebrafish also label embryonic RGCs in mammals or neurogenic astrocytes during adulthood, as reviewed in (Diotel et al., 2020).

Table 1: Main markers expressed by type 1, 2, 3a and 3b cells in the telencephalon of adult zebrafish. The (+/-) means that these markers are expressed at lower levels in the subtype.

Type 1	Type 2	Type 3a	Type 3b
Sox2	Sox2	Sox2	Sox2
Nestin and vimentin	Nestin and vimentin	Nestin	PCNA
GFAP	GFAP	GFAP (-)	PSA-NCAM
S100 beta	S100 beta	S100 beta (+/-)	
GS	GS	BLBP (+/-)	
BLBP	BLBP	AroB (+/-)	
AroB	AroB	PCNA	
Cxcr4	Cxcr4 (+/-)	PSA-NCAM	
Id1	Id1 (+/-)		
Her 4	Her 4		
	PCNA		

Consequently, RGCs (type 1 and 2 cells) have been established as *bona fide* neural stem cells in the telencephalon of adult zebrafish (Rothenaigner et al., 2011; Barbosa et al., 2015).

Type 3 cells: The fast-cycling progenitors correspond to type 3 cells that are considered as neuroblasts. As previously mentioned, these cells can be found tightly inserted between RGC' soma in the pallium, but are mainly localized within a subpallial cluster (März et al., 2010) (Figure 2). These progenitor cells undergo a limited amplification phase before performing symmetric neurogenic divisions (Kishimoto et al., 2011; Rothenaigner et al., 2011). Type 3 cells express committed progenitor markers such as *ascl1a* and PSA-NCAM in addition to progenitor markers such as nestin and sox2 (Chapouton et al., 2010; März et al., 2010; Diotel et al., 2015b). However, in general they do not express, or in some cases only weakly, the RGC markers. The type 3 cells can be divided into two subpopulations of neuroblasts: type 3a and type 3b (Table 1). Type 3a neuroblasts strongly express the commitment marker PSA-NCAM but can also weakly express some of the RGC markers. Type 3b neuroblasts do not express any RGC markers and are PSA-NCAM-positive (März et al., 2010). Both type 3a and 3b express the PCNA proliferation marker.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 December 2020 doi:10.20944/preprints202012.0544.v1

The type 3 cells in the subpallial cluster will actively migrate, reaching the olfactory bulb via a rostral migratory stream-like (RMS) structure to differentiate into GABAergic and TH-positive neurons. The zebrafish RMS-like is reminiscent of the mammalian RMS (Grandel et al., 2006) (see below, part 4; Figure 1B and D)

Cellular events occurring after telencephalic injury in zebrafish

Teleost fish are widely used as a model for the investigation of brain plasticity due to their high constitutive neurogenesis, strong regenerative mechanisms and striking sexual plasticity sustained by important sexual neurobehavioral changes (Grandel et al., 2006; Becker and Becker, 2008; Diotel et al., 2010a; Baumgart et al., 2012; Diotel et al., 2013b; Schmidt et al., 2013; Schmidt et al., 2014). Mechanical injury of the telencephalon by either inserting a small cannula through the skull or through the nasal cavity remains the most investigated model in zebrafish for studying brain regeneration (Kroehne et al., 2011; März et al., 2011; Baumgart et al., 2012; Diotel et al., 2013b). After brain damage in teleost fish, death of damaged cells occurs, followed by the recruitment and/or proliferation of microglia and peripheral immune cells, oligodendrocytes/OPCs, endothelial cells and RGCs (NSCs). As part of the immune response, microglia can be activated and leukocytes can invade the injury site, both of which can release factors required for the activation and proliferation of RGCs, consequently leading to injury-induced neurogenesis.

Cell death after zebrafish telencephalic injury: Very soon after mechanical injury of the telencephalon (from 4 hpl to 6 hpl), numerous TUNEL-positive cells are detected in both brain parenchyma and periventricular zone, while almost no cell death is observed in the contralateral control hemisphere (Kroehne et al., 2011; Kyritsis et al., 2012). The TUNEL-positive cells exhibit features of necrotic and apoptotic cells. In their work, Kroehne et al. showed that cell death could still be observed at 1 dpl in both parenchymal and periventricular regions but returned to control levels at 3 dpl (Kroehne et al., 2011). In contrast, Kyritsis et al. (2012) only observed a decrease in the number of TUNEL-positive cells at 3 dpl in the injured hemisphere when compared to the uninjured control hemisphere. In addition, the injury induced a strong edema, that represented 40% of the volume of the injured telencephalic hemisphere at 1 dpl (Kroehne et al., 2011). At 7 dpl this edema was strongly reduced to only 5% of the total volume of the injured hemisphere.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 December 2020 doi:10.20944/preprints202012.0544.v1

Remarkably, 1 month after the injury, the lesioned hemisphere was almost completely restored regarding tissue morphology and histology. Moreover, no morphological differences could be observed anymore after 1 year (Kroehne et al., 2011). An overview of cell death kinetics occurring after brain damage is shown in Figure 3.

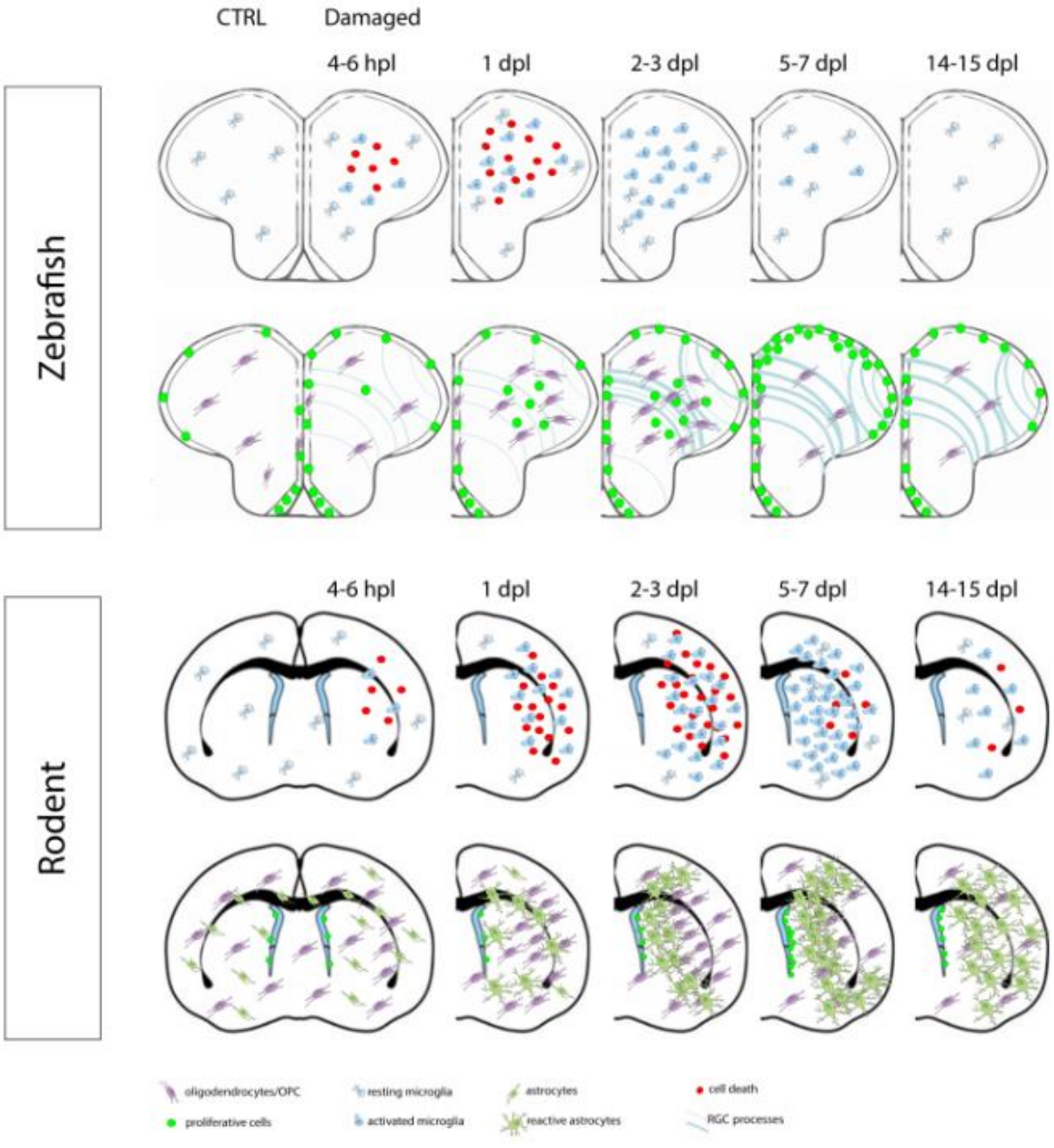

Figure 3: Cellular events occurring after telencephalic injury in zebrafish and stroke in mouse

After brain damage, numerous cells, mainly neurons, die. This process is followed by the activation and recruitment of microglial and other immune cells (leukocytes) in parallel to OPCs. Then an astrogliosis process occurs in mice, while RGCs become reactive and proliferative in zebrafish. Proliferation in the neurogenic niches peak at day 7 after damage in both models.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 December 2020 doi:10.20944/preprints202012.0544.v1

Microglia recruitment and function in response to zebrafish telencephalic injury: Microglia are the resident immune cells of the central nervous system. In contrast to other phagocytotic cells in mammals, microglia display strong interactions with neurons, astrocytes and oligodendrocytes leading to a prominent role of microglia in neuronal development and plasticity (Frost and Schafer, 2016; Hong and Stevens, 2016). As shown in mammals, their most striking characteristic is their high degree of plasticity which enables them to switch from a resting state (quiescent) to a phagocytotic state (ameboid) in response to injury (Davalos et al., 2005; Nimmerjahn et al., 2005; Morrison and Filosa, 2013), a phenomenon also observed in zebrafish after telencephalic injury (Figure 4). As part of the inflammatory response, microglia appear to be among the first cells being recruited and activated following brain injury (März et al., 2011; Kyritsis et al., 2012; Kanagaraj et al., 2020).

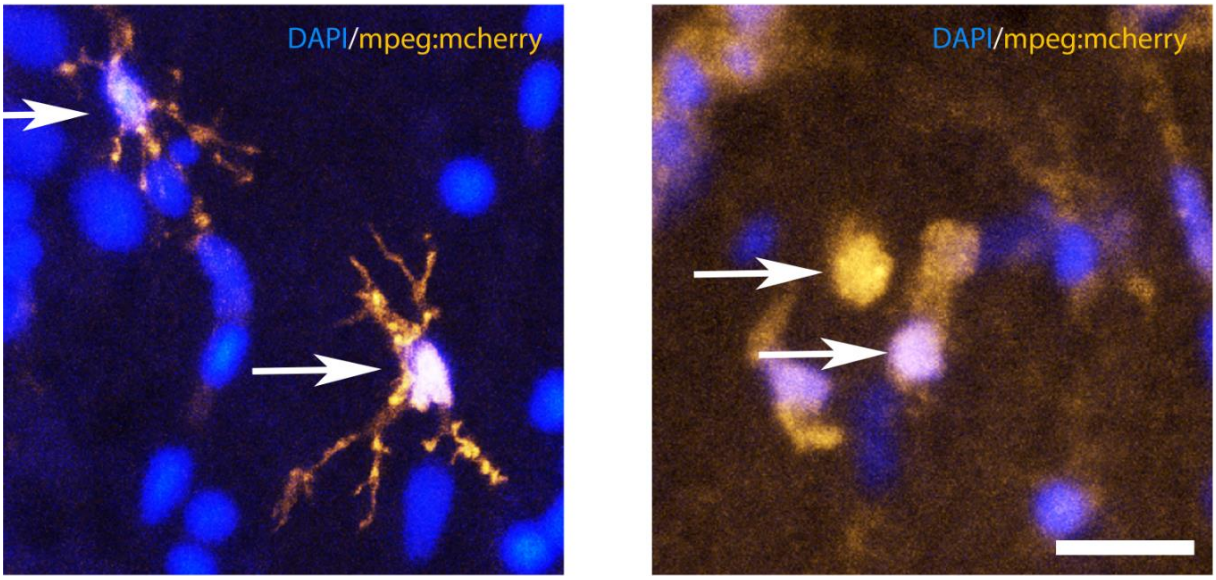

Figure 4: Resting and activated microglia under injured and uninjured (control) conditions in the telencephalon of zebrafish.
Confocal microscopy showing quiescent (resting) microglia (left panel) and ameboid (activated) microglia (right panel) in the adult zebrafish telencephalon. There is an obvious change in the shape of the microglia between injured and uninjured tissue, illustrated by the *mpeg:*mcherry transgenic fish line which labels microglia in the central nervous system.

In mammals, after their activation, they start to secrete chemokines and attract leukocytes to the injury site. This process is then followed by phagocytosis where activated immune cells (including leukocytes) start to remove dying neurons, which helps to control inflammation and aids in tissue repair and functional recovery (Harry and Kraft, 2012).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 December 2020 doi:10.20944/preprints202012.0544.v1

Activated microglia can release pro-inflammatory cytokines including interleukins (IL-1ß and IL-6) and the tumor necrosis factor (TNF-α) (Ransohoff, 2016; Kanazawa et al., 2017) as well as anti-inflammatory factors such as TGF-ß and the cytokines IL-4 and IL-10 (Chu et al., 2015; Xiong et al., 2016; Ma et al., 2017), which are important for the different steps of brain repair.

The exact role of microglia during zebrafish brain repair is still undetermined. Performing L-plastin immunohistochemistry to label both microglia and leukocytes, an increasing number of L-plastin-positive cells can be observed from 6 hpl in the injured telencephalon, peaking at 24 hpl and decreasing slightly from 3 dpl to 5 dpl (Kyritsis et al., 2012). Accordingly, proliferative and non-proliferative microglial cells (ApoE-GFP or L-plastin positive) are shown to be largely increased at 3 and 4 dpl in the lesioned hemisphere compared to the unlesioned ones (Kroehne et al., 2011; März et al., 2011). Taken together, these data demonstrate that in order to aid with brain recovery, microglial and potential peripheral immune cells are quickly recruited after brain injury, starting at 6 hpl before returning back to basal levels at 7 dpl. The general recruitment of microglia/immune cells after stab wound is shown in Figure 3. Recently, it was confirmed in zebrafish that microglia recruitment peaks at 1 dpl before it declines, remaining still significantly up-regulated at 4 dpl (Kanagaraj et al., 2020). As in mammals, inflammatory molecules secreted, in part, by microglia have an impact on neural stem cell plasticity, regeneration and neuronal repair, namely in injury-induced neurogenesis in zebrafish (Kyritsis et al., 2012; Kanagaraj et al., 2020). Indeed, the inhibition of microglia activation during brain injury results in impaired injury-induced neurogenesis (Kanagaraj et al., 2020). The phagocytotic activity of microglia and probably of other immune cells fortify the beneficial impact of inflammation on regeneration after telencephalic injury in the teleost fish. However, the precise function of these factors during zebrafish brain regeneration needs to be further investigated.

Oligodendrocyte/oligodendrocyte progenitor cell recruitment after zebrafish telencephalic damage: Oligodendrocytes are among the most important cells within the central nervous system as they participate in the development and maintenance of the myelin sheath. In mammals, mature oligodendrocytes lose their proliferative capacity and newly generated oligodendrocytes derived from non-myelinated oligodendrocyte precursor cells (OPCs) (Gensert and Goldman, 1997). To investigate the recruitment of oligodendrocytes and OPCs

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 December 2020 doi:10.20944/preprints202012.0544.v1

in the zebrafish CNS, März et al., (2011) used an *olig2*:EGFP transgenic line. They observed an increased number of OPCs and mature oligodendrocytes at 1 dpl (in 50% of the studied brains). This accumulation of OPCs is more prominent between 2 dpl and 14 dpl and is detected in almost all the studied brains (94%). Interestingly, at 35 dpl, the *olig2*:EGFP clusters are almost not observed anymore in the injured hemisphere (März et al., 2011). Surprisingly, in contrast to mammals, there is no increase in the proliferation rate of olig2-positive cells in the injured hemisphere, compared to the uninjured hemisphere (März et al., 2011, Baumgart et al., 2012). Consequently, the proliferation of the olig2-positive cells appears to be moderate after stab wound injury of the zebrafish telencephalon. The general recruitment of olig2-positive cells after injury is shown in Figure 3.

In summary, immune cells (microglia and peripheral cells) and oligodendrocytes/OPCs are among the first cells recruited and activated after stab wound injury in zebrafish. At 2 dpl, around 50% of the proliferative cells within the damaged brain parenchyma could be identified as endothelial cells, Olig2-GFP positive cells and microglial-like/immune cells (Kroehne et al., 2011).

Injury-induced proliferation and neurogenesis in the zebrafish telencephalon after telencephalic damage: After telencephalic lesion, brain cell proliferation occurs with different kinetics within the brain parenchyma and in neurogenic niches (Diotel et al., 2013a). Simultaneous to the recruitment of immune cells and the accumulation of OPCs, starting between 1 and 2 dpl, a higher number of PCNA-positive cells can be detected in the injured hemisphere. After 48 hpl, this proliferation is especially observed along the ventricular layer where RGCs reside. This number peaks between 5 and 8 dpl and slowly decreases until 15 dpl to reach the normal proliferation rate at 35 dpl (Diotel et al., 2010a; März et al., 2011; Baumgart et al., 2012; Kishimoto et al., 2012; Kizil et al., 2012b; Diotel et al., 2013b). Double immunohistochemistry against proliferation and RGC markers, as well as the use of transgenic fish, have shown that the reactive proliferative cells localized along the ventricle correspond to RGCs expressing S100ß, BLBP, GFAP and also vimentin (Kroehne et al., 2011; März et al., 2011; Baumgart et al., 2012; Diotel et al., 2013b). This injury-induced proliferation of RGCs has been shown to produce newborn neurons (HuC/D-, parvalbumin-positive) which persist for more than 2 to 3 months after brain injury (Kroehne et al., 2011; Baumgart et al., 2012). They also exhibit the MAP2(a+b) dendritic marker, the synaptic

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 December 2020 doi:10.20944/preprints202012.0544.v1

vesicle marker SV2 and the synaptic marker metabotropic glutamate receptor 2 (mGlu2) proving their functional maturation (Kroehne et al., 2011). Consequently, after telencephalic injury, RGCs switch from a quiescent to a proliferative state and generate newborn neurons to replace neurons which have been lost due to the damage.

An important aspect to consider in injury-induced NSC proliferation is the influence of the pro-inflammatory cytokines transiently upregulated after telencephalic injury and shown to be necessary for NSC activation (Kyritsis et al., 2012). Furthermore, the transcription factor Gata3 is required for reactive proliferation of RGCs and the subsequent regenerative neurogenesis (Kizil et al., 2012b). Interestingly, the Gata3 transcription factor is mainly expressed by RGCs but is also detected in L-plastin positive microglia (Kizil et al., 2012b), pointing again to an important role of the inflammatory response (leukotriene/gata 3 (Kizil et al., 2012b)).

Characterization of neurogenic niches in the telencephalon of adult rodents

In mammals, the *bona fide* RGCs do not persist during adulthood (Noctor et al., 2001; Noctor et al., 2008). However, in some discrete regions, RGCs transform into cells which display astrocytic features and maintain NSC properties during adulthood. The two main neurogenic regions observed in the brain of adult mammals are the subventricular zone (SVZ) of the lateral ventricle and the subgranular zone (SGZ) of the dentate gyrus (DG) in the hippocampus (Lindsey and Tropepe, 2006; Grandel and Brand, 2013). The SVZ and SGZ of the DG are both localized in the telencephalon but produce different types of neurons and are composed of different subsets of progenitors.

Organization of the SVZ neurogenic niche in mice: In the SVZ, astrocyte-like cells (called B cells, *bone fide* stem cells) have been shown to self-renew and generate transit-amplifying cells (C cells) that give birth to neuroblasts (A cells) (Figure 1B and 1D) (Ming and Song, 2005; Obernier and Alvarez-Buylla, 2019). These neuroblasts will then migrate in chain following the rostral migratory stream (RMS) to reach the olfactory bulbs. They will differentiate into GABAergic, glutamatergic and dopaminergic neurons in the periglomerular layer of the olfactory bulbs and into GABAergic interneurons in the granular cell layer of the olfactory bulbs (Grandel and Brand, 2013).

 doi:10.20944/preprints202012.0544.v1

Organization of the SGZ neurogenic niche in mice: In the SGZ of the DG, radial glial-like/astrocyte cells (type 1) can self-renew and generate type 2a and 2b progenitors, which are also able to self-renew, and finally type 3 cells (Figure 1C and 1D) (Ming and Song, 2005; Obernier and Alvarez-Buylla, 2019). The latter will give rise to glutamatergic granular neurons. Interestingly, neurogenesis in the dentate gyrus has been shown to be linked to learning, environmental enrichment and social interactions (Kempermann, 2011; Grandel and Brand, 2013). Newborn neurons in the SGZ migrate only short distances, in contrast to the new neurons from the SVZ that migrate for longer distances through the rostral migratory stream (RMS) (Gould, 2007).

Organization of the SVZ and SGZ neurogenic niches in humans: In humans, adult neurogenesis, or at least its functional relevance is still under debate, especially when it comes to the neurogenic activity in the mammalian hippocampus (Eriksson et al., 1998; Spalding et al., 2013; Dennis et al., 2016; Kempermann et al., 2018).

In the SVZ, post-mortem studies in adult human brains have revealed that GFAP-positive astrocytes are separated from the ependymal wall by a hypocellular gap (Figure 1D). Only some of these astrocytes seem to proliferate and therefore the adult human SVZ appears devoid of newborn neurons that migrate in chain (no RMS). Supporting this notion, only very few new neurons displaying a migratory phenotype were observed in the anterior SVZ (Quiñones-Hinojosa et al., 2006). In contrast to rodents in which the newborn neurons from the SVZ migrate towards the olfactory bulb, they appear to migrate within the adjacent striatum in humans to become medium spiny neurons (Ernst et al., 2014). Consequently, the SVZ neurogenic niche differs greatly between humans and rodents in the cellular organization of the niche and in the newborn neuron migration (Quiñones-Hinojosa et al., 2006).

In the hippocampus, the neurogenic niche is very similar to the rodent one. However, from a functional point of view, post-mortem studies supported the hypothesis that hippocampal neurogenesis strongly decreases during childhood to become almost undetectable at adulthood (Sorrells et al., 2018). In contrast to that, in the same year, the work of Boldrini and collaborators showed through autopsy of hippocampi from healthy humans at different ages, that many immature neurons could be detected in the dentate gyrus, suggesting that healthy older individuals maintain functional neurogenesis (Boldrini et

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 December 2020 doi:10.20944/preprints202012.0544.v1

al., 2018). In conclusion, the hippocampal neurogenic niche shares many similarities with the one in rodents by locally generating neurons from neural precursors close to the niche (Spalding et al., 2013) (Figure 1D).

Taken together, these data demonstrate that zebrafish, rodents and humans share similar features in the maintenance of adult neurogenesis with some homologies between the main telencephalic neurogenic niches and the type of newborn cells that are generated. The Figure 1 highlights these similarities, as well as some differences between zebrafish and mammals. However, the functional relevance of adult neurogenesis in humans remains under debate.

Cellular events occurring after brain damage in rodents

Compared to zebrafish, mammals have a reduced ability to regenerate their brain and to fully recover sensory and motor functions. Understanding the cellular and molecular events occurring during brain regeneration is a challenging field of research but nevertheless important for the fight against disabilities resulting from brain damage. In the following section, we will discuss the cascade of cellular events occurring after brain damage such as brain ischemia and/or traumatic brain injury in the brain of rodents. Importantly, even if the injury models developed in zebrafish are closer to traumatic brain injury (TBI) models in mammals, we decided to mainly focus on stroke models in rodents because (i) stroke is much more studied than TBI (Pubmed research: “stroke rodent” → 31.011 articles versus “traumatic brain injury rodent” → 7.847, the 21th of December 2020), (ii) TBI and stroke lead to almost similar cellular events and disorders such as cognitive, neurological and psychological disorders (Castor and El Massioui, 2018), (iii) TBI is a risk factor for stroke (Albrecht et al., 2015). Last but not least, TBI and stroke share common molecular and cellular events including, among others, increased BBB permeability, pro-inflammatory cytokine release, metabolic stress, glial reactivity, neuronal degeneration, axon damage, infarct formation, glial scar formation, nervous tissue atrophy and functional deficits (Bramlett and Dietrich, 2004).

Although the brain represents only 2% of the total body weight, it consumes around 20% of the total body dioxygen and is highly active from a metabolic point of view using around 25% of the body’s glucose (Quastel and Wheatley, 1932; Magistretti and Pellerin, 1996; Belanger et al., 2011). Thus, brain damage will strongly impact brain homeostasis

 doi:10.20944/preprints202012.0544.v1

through a decreased supply of nutrients (i.e glucose and dioxygen) leading to severe outcomes, particularly in the case of stroke.

Cell death after brain damage in rodents: Briefly after brain ischemia, cell death will progressively occur within the first hours, as presented in zebrafish, but will persist for several days. This was shown through different methods using standard coloration, Fluoro-Jade C probes, and triphenyl tetrazolium chloride stainings (Liszczak et al., 1984; Chen et al., 2009; Popp et al., 2009; Zille et al., 2012). Additionally, numerous TUNEL-positive cells are detected after 1h, peaking at 24 hours and are still detected after 28 days in stroke models in rodents (Zhang et al., 2010). In rat, subjected to a 90 min ischemia by the MCAO method (Middle Cerebral Artery Occlusion), the number of TUNEL-positive cells peaks at 48 hours post-ischemia and returns to basal levels only 6 days post-stroke (Luo et al., 2009). Several studies have shown that the processes/mechanisms promoting cell death are irreversibly initiated between 3 and 12 hours post injury (Moskowitz et al., 2010; Sims and Yew, 2017). An overview of cell death kinetics occurring after stroke in mouse is shown in Figure 3.

Considering traumatic brain injury in mice, primary cell death occurs after injury followed by a second wave of neuronal cell death resulting from both biochemical and physiological disruptions, induced by the insult in a way similar to stroke (Stoica and Faden, 2010; Yang et al., 2016).

Consequently, after brain damage, it seems that cell death is very severe and persists for several weeks including a secondary wave of neurodegeneration (Zhang et al., 2012). This differs greatly from the situation documented in zebrafish for which cell death is solved between 1 and 3 dpl. This important process of cell death occurring in mammals could trigger a chronic neuroinflammatory state that could be inhibitory for regenerative mechanisms.

Microglia recruitment after brain damage in rodents: As discussed for zebrafish, in healthy brains, microglial cells remain stable and only a few of them are proliferating (Askew et al., 2017; Boareto et al., 2017). Microglia are among the first cells responding to brain injury/ischemia: they actively migrate to the injured site, switch from resting to ameboid states, and proliferate (Xing et al., 2012; Zhang, 2019). As nicely reviewed by Lourbopoulos and Benakis, within the first 24 hours post stroke (hps), activated microglia are detected in

both infarct and peri-infarct regions (Benakis et al., 2014; Lourbopoulos et al., 2015). Between 2 and 7 days after stroke, microglia are further activated within the ischemic core (Lourbopoulos et al., 2015; Chen et al., 2019). Then, in the two following weeks, the number of microglia decreases in the peri-infarct and core regions. Interestingly, a substantial number of peripheral immune cells (neutrophils and macrophages) also invade the infarct and peri-infarct regions from day 1 post-stroke, due to the leakage of the blood-brain barrier and to chemoattractant factors. Their number is increased between day 3 and day 7 post-stroke but remains quite significant 7-14 days after ischemia (Benakis et al., 2014). Together, resident and peripheral immune cells play key roles in the removal of dead cell debris and potentially participate in limiting the damage to the surrounding nervous tissue. Of interest, microglia and macrophages will also accumulate around the damaged area, in a region where the glial scar will develop. Interestingly, it also appears that the sensitivity of microglial cells to brain ischemia is dependent on the regions (Zhang, 2019). The recruitment of microglia during and after brain ischemia is highlighted in Figure 3. Similar to stroke, TBI also induces microglia activation from 1 to 3 days post-injury, that can persist until 28 days after the trauma (Bye et al., 2007; Perego et al., 2011; Patel et al., 2013; Donat et al., 2017).

Compared to zebrafish, microglial recruitment and activation is prolonged which is possibly linked to the persistent cell death occurring within the damaged hemisphere. Such a persistent cell death, as well as microglia/peripheral immune cells recruitment could induce chronic disruption of brain homeostasis, impairing consequent brain repair mechanisms.

Oligodendrocyte/oligodendrocyte progenitor cell recruitment after brain damage in rodents: In mammals, oligodendrocytes are sensitive to cerebral ischemia (Pantoni et al., 1996; Dewar et al., 2003; Zhang et al., 2013), and their death, as well as the loss of the myelin sheath strongly impairs neuronal function. After brain ischemia, lineage tracing showed that OPCs are generated from NSCs located in the SVZ, and provide new oligodendrocytes (Zawadzka et al., 2010; Rafalski et al., 2013; Zhang et al., 2013). Thus, a significant increase in OPCs is observed, giving rise to mature myelinating oligodendrocytes in the peri-infarct gray and white matter where sprouting axons are located (Gregersen et al., 2001; Ueno et al., 2012a; Ueno et al., 2012b; Zhang et al., 2013). This oligodendrogenesis has been shown to improve brain repair processes and neurological scores (Zhang et al., 2013). After brain ischemia, OPCs also seem to be involved in post-stroke angiogenesis

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 December 2020 doi:10.20944/preprints202012.0544.v1

(Kishida et al., 2019), a process linked to neurogenesis (Xiong et al., 2010). Indeed, OPCs in the cerebral cortex shift from a parenchymal to a perivascular subtype. The recruitment of oligodendrocytes/OPCs during and after brain ischemia is highlighted in Figure 3. In the TBI model, mature oligodendrocytes undergo apoptosis occurring from 2 days to 2 weeks after the insult. In parallel, Olig2-positive cell proliferation is observed starting at 48h and can persist until 21 days after the injury (Dent et al., 2015; Flygt et al., 2017). These data show that OPCs respond to brain injury in a way similar to what was shown for stroke. Such a proliferation may lead to the genesis of new oligodendrocytes contributing to remyelination.

Consequently, the situation is very different in mammals compared to zebrafish, as the number of OPCs is significantly increased, and they actively proliferate providing numerous new oligodendrocytes. In zebrafish, the proliferation rate of olig2-positive cells remains low and their number is unchanged during the regenerative process, although olig2 clusters are observed in close vicinity to the lesion (März et al., 2011). Therefore, oligodendrogenesis appears to be vastly different between zebrafish and rodents. The role of olig2-positive cells at the lesion site remains largely unknown in zebrafish, but could be linked with regenerative neurogenesis, axonogenesis and synaptogenesis.

Reactive astrogliosis after brain injury and injury-induced neurogenesis in rodents: After any type of brain damage (i.e. stroke, traumatic brain injury, neurotoxic drug exposure, neurodegenerative disease), astrocytes surrounding the damaged region will react and undergo important morphological and/or functional changes such as hypertrophy, overexpression of some genes or astrocytic markers such as GFAP and nestin, which will progressively modify their function (Pekny and Nilsson, 2005; Sofroniew, 2005; 2009; Liu and Chopp, 2016; Sims and Yew, 2017). Although all the astrocytes surrounding the damaged area react, they do not constitute a homogenous population; at least 2 different types of reactive astrocytes have been described. The astrocytes in close vicinity to the lesioned site will start to proliferate and migrate surrounding the injured territory. These astrocytes are of peculiar importance for the establishment of the well-known glial scar (mainly composed of extracellular matrix and numerous processes from astrocytes). The astrocytes that are further away from the lesion site will also react but will stay resident and maintain their connection to the neighboring cells.

 doi:10.20944/preprints202012.0544.v1

Under stroke conditions, proliferation of astrocytes starts in the first days and remains restricted to an area 200 micrometers around the infarcted site (Barreto et al., 2011; Shimada et al., 2011). Interestingly, the inhibition of astrocyte proliferation increases the size of the injury and worsens neurological scores, correlated with a higher neutrophil infiltration and impaired BBB regeneration, as shown for TBI (Myer et al., 2006; Sofroniew, 2015; Burda et al., 2016). New astrocytes are also generated from NSCs that could migrate from the SVZ a few days after stroke onset, and could survive until several weeks after the stroke (Faiz et al., 2015).

Reactive astrocytes become hypertrophic with thicker and bushier/ramified processes; they also upregulate many astrocytic markers, such as GFAP (Wilhelmsson et al., 2006). GFAP upregulation after brain ischemia will be weak at 24h post-injury but will increase rapidly during the first week (Al Ahmad et al., 2011; Zamanian et al., 2012), while the number of GFAP-positive astrocytes increases within the first two weeks (Ding, 2014; Li et al., 2014) . Vimentin and nestin, two other intermediate filaments are also upregulated after ischemia and their expression levels correlate with those of GFAP (Schroeter et al., 1995; Zamanian et al., 2012). Interestingly, a single knock-out of GFAP or vimentin has no real impact on reactive gliosis and glial scar formation while a double KO severely impacts reactive gliosis, as shown by a decrease in astrocyte hypertrophia and glial scar formation. Under stroke conditions, the double KO of these intermediate filaments increases the size of the infarct and leads to more acute neurological outcomes (Li et al., 2008; de Pablo et al., 2013). Similarly, in TBI models, astrogliosis also occurs through structural and functional changes including hypertrophy and overexpression of intermediate filaments (nestin, vimentin, and GFAP) (Ben-Gigi et al., 2015; Zhou et al., 2020).

Considering injury-induced neurogenesis, newborn neurons are produced from the SVZ and migrate within the injured striatum and cortex of rodents after stroke. During their migration, they will progressively differentiate and express neuronal markers (i.e. DCX, PSA-NCAM, Hu and NeuN) (Arvidsson et al., 2002; Parent et al., 2002; Yamashita et al., 2006). Interestingly, these newborn neurons do not reach the olfactory bulbs through the RMS, as during constitutive neurogenesis, but reach the damaged areas due to attractive factors (Yamashita et al., 2006; Lindvall and Kokaia, 2015). These new neurons are highly detectable between 14 and 28 days after stroke (Yamashita et al., 2006). Consequently, after stroke, NSCs from the SVZ give rise to neuroblasts that migrate towards the damaged regions

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 December 2020 doi:10.20944/preprints202012.0544.v1

(striatum and cortex), where they differentiate into mature neurons (Lindvall and Kokaia, 2015). New migrating neuroblasts can still be observed 1 year after stroke (Osman et al., 2011). In addition, after stroke, hippocampal neurogenesis is also detected but remains imperfect (Woitke et al., 2017). In rodents, TBI models also display injury-induced neurogenesis (Dash et al., 2001; Chirumamilla et al., 2002; Ngwenya and Danzer, 2018). The similarities and differences regarding cell activation and cell recruitment after brain injuries in mammals and zebrafish are highlighted in Table 2.

Table 2: Comparison of events after brain damage in zebrafish and mammals

	Zebrafish	Mammals
Glia reactivity/hypertrophy	+	+
Microglia recruitment	+	+
Microglia proliferation	+	+
Oligodendrocytes recruitment	+	+
Oligodendrocytes proliferation	+/-	+
Astrocyte/RGC recruitment	- (RGC)	+ (astrocyte)
Astrocyte/RGC proliferation	+ (RGC)	+ (astrocyte)
GFAP/vimentin up-regulation	+	+
Glial scar formation	-	+
Glial scar persistence	-	+
Regenerative capacities	+++	+/-

In summary, even if it is difficult to have a realistic view of the cellular events occurring during stroke due to the diversity of protocols (permanent or transient ischemia, models of MCAO - Longa *vs* Koizumi -, duration of the stroke, age and sex of animals, species, regions studied and time-points analyzed…), an integrative work has been realized showing cell death, astrocyte, oligodendrocyte and endothelium cell behavior from 3h post stroke to 1 week after a 30 min brain MCAO (Buscemi et al., 2019).

Brain damage: what about humans?

When it comes to the close investigation of the consequences of brain damage, for example due to stroke, unfortunately in humans, studies are highly limited due to the incapacity of collecting post mortem tissue after the onset of stroke. However, it was shown that apoptosis occurs quickly in the human brain after ischemia with cell death being delayed for several days (Sairanen et al., 2006; Radak et al., 2017). Similar to the situation in rodents, microglia are recruited and proliferate at the periphery of the damaged area in post-mortem human brain tissue of stroke patients (Otxoa-de-Amezaga et al., 2019), and peripheral immune cells are

 doi:10.20944/preprints202012.0544.v1

attracted as well (Rosell et al., 2008; Perez-de-Puig et al., 2015). Furthermore, in the peri-infarct region after ischemic stroke in humans, the development of a glial scar can be observed (Huang et al., 2014), as well as injury-induced neurogenesis (Jin et al., 2006; Lindvall and Kokaia, 2015).

Glial scar: a paradigm for understanding the difference between zebrafish and mammalian regeneration? After brain damage in mammals, reactive gliosis takes place involving microglia, oligodendrocyte and astrocyte cells. Activation of astrocytes will lead to the formation of the glial scar. The glial scar is supposed to protect the central nervous system and to participate in the healing process. During the glial scar formation, reactive astrocytes secrete many extracellular matrix components such as laminin, fibronectin, tenascin C, and proteoglycans (Sofroniew, 2009; Buffo et al., 2010). In zebrafish, no astrocyte-like structures were observed in the telencephalon under homeostatic or regenerative conditions. However, RGCs are suggested to sustain many astrocytic features and functions, such as typical marker expression, steroidogenesis, blood-brain barrier establishment and neurogenic properties (Diotel et al., 2018; Jurisch-Yaksi et al., 2020). Although, no astrogliosis was observed in zebrafish after telencephalic injury, reactive RGC gliosis occurs, as shown by the up-regulation of GFAP and vimentin as well as the hypertrophy of RGC glial processes (Kroehne et al., 2011). The upregulation of RGC markers (vimentin, GFAP, BLBP and S100β) and the hypertrophy of RGC processes is observed quickly after brain damage and can remain visible up to 1 month after injury. In addition, numerous studies also demonstrate an increase in RGC number following brain injury in the telencephalon (Kroehne et al., 2011; März et al., 2011; Kishimoto et al., 2012; Diotel et al., 2013b; Rodriguez Viales et al., 2015). Interestingly, collagen Acid-Fuchsin-Orange G staining confirms the transient accumulation of collagen at 14 dpl at the injury site (Kroehne et al., 2011). However, this fibrotic scar formation, including reactive glial cell accumulation, hypertrophy of glial processes, persistence of inflammatory cells and ectopic extracellular matrix deposition are not detected later or just occasionally in a small number of brains. Remarkably, the work from Baumgart and colleagues reports that RGC hypertrophy and reactivity is observed for large lesions but not for small ones. Therefore, RGC reactivity could be linked to the severity of the damage (Baumgart et al., 2012), as astrogliosis in mammals. Furthermore, discrete lesions will only allow the proliferation of RGCs, while larger lesions

 doi:10.20944/preprints202012.0544.v1

could potentially initiate the additional migration of some RGCs within the brain parenchyma. However, such migration should remain quite discrete as the analysis of stab wounded brain sections did not demonstrate any migration processes in the past.

Consequently, it appears that RGC reactivity mimics, in part, astrogliosis with respect to the mammalian situation through (1) increased expression of glial markers, (2) increased proliferation and hypertrophy, (3) potential migration of RGCs in some cases and (4) the increased extracellular matrix deposition. However, unlike in mammals, there is no evidence for permanent scar formation in the zebrafish brain as there is no persistent extracellular matrix deposition. An overview of proliferation and RGC reactivity occurring during brain lesion is shown in Figure 3.

Conclusion

Brain ischemia, traumatic brain injuries and neurodegenerative diseases are of major concerns worldwide and constitute main health issues. Following such brain damage or neurological disorders, the massive death of neurons and their subsequent consequences can lead to severe disabilities including cognitive, sensorimotor and even personality dysfunctions. Rodents are very interesting and useful models to study brain repair mechanisms, because they are mammals and therefore share a high degree of genetic similarity with humans. However, as for humans, their brain plasticity and regenerative capacities are strongly limited. For these reasons, the use of highly regenerative species is important for cross-comparison and for a better understanding of the molecular and cellular mechanisms that enable these organisms to regenerate and replace the damaged nervous tissue. Thus, the key mechanisms sustaining these regenerative capacities could be used to modify and promote brain regeneration in mammals (Zambusi and Ninkovic, 2020). Several hypotheses have been advanced in order to explain the vast plasticity of the central nervous system of animals like the zebrafish, compared to mammals. Among them, zebrafish appear to respond to injury by turning on genes that are not activated in other models and to additionally activate epigenetic programs. Furthermore, they display an immune response allowing an enhanced regeneration (Marques et al., 2019). The blunted regeneration observed in mammals is largely attributed to inflammatory processes inducing the formation of a glial scar. Last but not least, zebrafish are still growing during their entire lifespan, and

 doi:10.20944/preprints202012.0544.v1

their brain seems to retain some embryonic features that could explain, in part, their strong regenerative capacities (Diotel et al., 2010a).

One first interesting aspect to be considered is that neural stem/progenitor cells react similarly by increasing their proliferation after brain injuries in zebrafish and mammals. In both taxa, proliferation of neural stem/progenitor cells peaks at around 7 days post injury. However, the vast majority of freshly generated newborn neurons after injury in mammals fails to reach the damaged site due to the formation of the glial scar. This scar gliosis provides an extracellular environment that does not allow the integration of newborn cells and is a potential inhibitor of neurogenesis (i.e. chondroitin sulfate proteoglycans and myelin components) (Muramatsu et al., 2012). As a result, new neurons are unable to cross the glial scar, will degenerate and can therefore not compensate the functions lost during the massive neuronal death induced by brain ischemia or injury. In contrast, although brain lesion in zebrafish strongly induces the proliferation of neural stem cells, as in mammals, it will not lead to the formation of a strong and persistent glial scar. This will consequently allow the migration of new neurons to the injured site and lead to their functional integration and to the recovery of impaired functions. These data comfort the general idea that the glial scar has a negative impact on newborn neuron integration in the brain of mammals. However, recent data suggest that considering the glial scar as good or bad for CNS recovery is not as simple as suggested (Tang, 2016; Bradbury and Burnside, 2019).

Another interesting aspect is that the reactivity of the brain, following brain damage, in mammals and zebrafish is quite similar. It involves the death of parenchymal and periventricular cells (neurons and glia) that will lead to the recruitment and the activation of microglia followed by oligodendrocytes and OPCs. Then, astrogliosis will occur in the brain of mammals characterized by the up-regulation of well-known markers (i.e. GFAP, vimentin, nestin) and the formation of the glial scar. In fish, RGCs (the presumed equivalent of astrocytes in mammals) will also react, by over-expressing astrocytic markers (GFAP, vimentin, nestin) and becoming hypertrophic, like astrocytes in mammals. A small and discrete glial scar will develop that is resolved quickly. However, cell death, microglia and oligodendrocyte reactivity persist longer in the brain of mammals than in zebrafish. Such prolonged neuroinflammatory mechanisms and oligodendrocyte reactivity could also participate in the inefficient neurogenesis occurring after brain damage in mammals, in sharp contrast to zebrafish.

 doi:10.20944/preprints202012.0544.v1

Of note, another important event to consider when discussing brain recovery is angiogenesis. In addition to serving as an important scaffold for neuroblast migration, blood vessels can secrete important factors (i.e. prostacyclin) promoting axonal growth and subsequent recovery (Muramatsu et al., 2012). Actually, it is more and more admitted that improving angiogenesis could favor neurogenesis and brain recovery (Xiong et al., 2010). Thus, neurogenesis cannot be considered anymore as the only way to improve functional recovery from stroke or brain damage, but should be examined in connection with angiogenesis, as it seems that angiogenesis and neurogenesis are coupled (Ruan et al., 2015).

Finally, from an evolutionary point of view, a remaining question is whether the mammalian brain lost its regenerative capacities or if it inhibits the regenerative capacities. Another possibility is that the teleost fish have developed such capabilities independently of mammals. One of the main differences between these taxa remains that, in zebrafish, neural stem cells retain a part of their embryonic features allowing their high reactivity and plasticity and continuing growth of the brain during adulthood (Diotel et al., 2010a). Last but not least, a comprehensive understanding of the mechanisms by which the glial scar is transiently generated and resolved in zebrafish could open a way for promoting brain regeneration in mammals and avoiding the consequences of brain damage. In addition, it could also be argued that mammals lost the ability to drive the expression of key genes involved in the regenerative process, due to a major regulatory change in their expression following injury (Wang et al., 2020).

In summary, it seems that proposing multifactorial therapeutic approaches targeting cell death, microglia, OPCs, astrocytes and NSCs could be more efficient for improving regeneration than targeting only one mechanism.

Funding

The research in the lab of Nicolas Diotel is supported by grants from FEDER RE0022527 (ZEBRATOX) and EU-Région Réunion-French State national counterpart. Sepand Rastegar is supported by the Helmholtz Association BioInterfaces in Technology and Medicine Program and the Deutsche Forschungsgemeinschaft training group (GRK2039).

Author Contributions

SR, ND, BG, DC and LL wrote the manuscript together. All authors contributed to the article and approved the submitted version.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

REFERENCES

Adolf B, Chapouton P, Lam CS, Topp S, Tannhauser B, Strahle U, Gotz M, Bally-Cuif L. 2006. Conserved and acquired features of adult neurogenesis in the zebrafish telencephalon. Dev Biol 295(1):278-293.

Al Ahmad A, Taboada CB, Gassmann M, Ogunshola OO. 2011. Astrocytes and pericytes differentially modulate blood-brain barrier characteristics during development and hypoxic insult. J Cereb Blood Flow Metab 31(2):693-705.

Albrecht JS, Liu X, Smith GS, Baumgarten M, Rattinger GB, Gambert SR, Langenberg P, Zuckerman IH. 2015. Stroke incidence following traumatic brain injury in older adults. J Head Trauma Rehabil 30(2):E62-67.

Altman J. 1969. Autoradiographic and histological studies of postnatal neurogenesis. IV. Cell proliferation and migration in the anterior forebrain, with special reference to persisting neurogenesis in the olfactory bulb. J Comp Neurol 137(4):433-457.

Altman J, Das GD. 1965. Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. J Comp Neurol 124(3):319-335.

Arvidsson A, Collin T, Kirik D, Kokaia Z, Lindvall O. 2002. Neuronal replacement from endogenous precursors in the adult brain after stroke. Nat Med 8(9):963-970.

Askew K, Li K, Olmos-Alonso A, Garcia-Moreno F, Liang Y, Richardson P, Tipton T, Chapman MA, Riecken K, Beccari S, Sierra A, Molnar Z, Cragg MS, Garaschuk O, Perry VH, Gomez-Nicola D. 2017. Coupled Proliferation and Apoptosis Maintain the Rapid Turnover of Microglia in the Adult Brain. Cell reports 18(2):391-405.

Barbosa JS, Sanchez-Gonzalez R, Di Giaimo R, Baumgart EV, Theis FJ, Gotz M, Ninkovic J. 2015. Neurodevelopment. Live imaging of adult neural stem cell behavior in the intact and injured zebrafish brain. Science 348(6236):789-793.

Barreto GE, Sun X, Xu L, Giffard RG. 2011. Astrocyte proliferation following stroke in the mouse depends on distance from the infarct. PLoS One 6(11):e27881.

Baumgart EV, Barbosa JS, Bally-Cuif L, Gotz M, Ninkovic J. 2012. Stab wound injury of the zebrafish telencephalon: a model for comparative analysis of reactive gliosis. Glia 60(3):343-357.

Becker CG, Becker T. 2008. Adult zebrafish as a model for successful central nervous system regeneration. Restor Neurol Neurosci 26(2-3):71-80.

Belanger M, Allaman I, Magistretti PJ. 2011. Brain energy metabolism: focus on astrocyte-neuron metabolic cooperation. Cell metabolism 14(6):724-738.

Ben-Gigi L, Sweetat S, Besser E, Fellig Y, Wiederhold T, Polakiewicz RD, Behar O. 2015. Astrogliosis Induced by Brain Injury Is Regulated by Sema4B Phosphorylation. eNeuro 2(3).

Benakis C, Garcia-Bonilla L, Iadecola C, Anrather J. 2014. The role of microglia and myeloid immune cells in acute cerebral ischemia. Front Cell Neurosci 8:461.

Boareto M, Iber D, Taylor V. 2017. Differential interactions between Notch and ID factors control neurogenesis by modulating Hes factor autoregulation. Development 144(19):3465-3474.

Boldrini M, Fulmore CA, Tartt AN, Simeon LR, Pavlova I, Poposka V, Rosoklija GB, Stankov A, Arango V, Dwork AJ, Hen R, Mann JJ. 2018. Human Hippocampal Neurogenesis Persists throughout Aging. Cell Stem Cell 22(4):589-599 e585.

Bradbury EJ, Burnside ER. 2019. Moving beyond the glial scar for spinal cord repair. Nat Commun 10(1):3879.

Bramlett HM, Dietrich WD. 2004. Pathophysiology of cerebral ischemia and brain trauma: similarities and differences. J Cereb Blood Flow Metab 24(2):133-150.

Brus M, Keller M, Levy F. 2013. Temporal features of adult neurogenesis: differences and similarities across mammalian species. Front Neurosci 7:135.

Buffo A, Rolando C, Ceruti S. 2010. Astrocytes in the damaged brain: molecular and cellular insights into their reactive response and healing potential. Biochem Pharmacol 79(2):77-89.

Burda JE, Bernstein AM, Sofroniew MV. 2016. Astrocyte roles in traumatic brain injury. Exp Neurol 275 Pt 3:305-315.

Buscemi L, Price M, Bezzi P, Hirt L. 2019. Spatio-temporal overview of neuroinflammation in an experimental mouse stroke model. Scientific reports 9(1):507.

Bye N, Habgood MD, Callaway JK, Malakooti N, Potter A, Kossmann T, Morganti-Kossmann MC. 2007. Transient neuroprotection by minocycline following traumatic brain injury is associated with attenuated microglial activation but no changes in cell apoptosis or neutrophil infiltration. Exp Neurol 204(1):220-233.

Castor N, El Massioui F. 2018. Traumatic brain injury and stroke: does recovery differ? Brain Inj 32(13-14):1803-1810.

Chapouton P, Skupien P, Hesl B, Coolen M, Moore JC, Madelaine R, Kremmer E, Faus-Kessler T, Blader P, Lawson ND, Bally-Cuif L. 2010. Notch activity levels control the balance between quiescence and recruitment of adult neural stem cells. J Neurosci 30(23):7961-7974.

Chapouton P, Webb KJ, Stigloher C, Alunni A, Adolf B, Hesl B, Topp S, Kremmer E, Bally-Cuif L. 2011. Expression of hairy/enhancer of split genes in neural progenitors and neurogenesis domains of the adult zebrafish brain. J Comp Neurol 519(9):1748-1769.

Chen B, Friedman B, Cheng Q, Tsai P, Schim E, Kleinfeld D, Lyden PD. 2009. Severe blood-brain barrier disruption and surrounding tissue injury. Stroke 40(12):e666-674.

Chen J, Zhang M, Zhang X, Fan L, Liu P, Yu L, Cao X, Qiu S, Xu Y. 2019. EZH2 inhibitor DZNep modulates microglial activation and protects against ischaemic brain injury after experimental stroke. Eur J Pharmacol 857:172452.

Chirumamilla S, Sun D, Bullock MR, Colello RJ. 2002. Traumatic brain injury induced cell proliferation in the adult mammalian central nervous system. J Neurotrauma 19(6):693-703.

Chu HX, Broughton BR, Kim HA, Lee S, Drummond GR, Sobey CG. 2015. Evidence That Ly6C(hi) Monocytes are Protective in Acute Ischemic Stroke by Promoting M2 Macrophage Polarization. Stroke 46(7):1929-1937.

Dash PK, Mach SA, Moore AN. 2001. Enhanced neurogenesis in the rodent hippocampus following traumatic brain injury. J Neurosci Res 63(4):313-319.

Davalos D, Grutzendler J, Yang G, Kim JV, Zuo Y, Jung S, Littman DR, Dustin ML, Gan WB. 2005. ATP mediates rapid microglial response to local brain injury in vivo. Nat Neurosci 8(6):752-758.

de Pablo Y, Nilsson M, Pekna M, Pekny M. 2013. Intermediate filaments are important for astrocyte response to oxidative stress induced by oxygen-glucose deprivation and reperfusion. Histochem Cell Biol 140(1):81-91.

Dennis CV, Suh LS, Rodriguez ML, Kril JJ, Sutherland GT. 2016. Human adult neurogenesis across the ages: An immunohistochemical study. Neuropathol Appl Neurobiol 42(7):621-638.

Dent KA, Christie KJ, Bye N, Basrai HS, Turbic A, Habgood M, Cate HS, Turnley AM. 2015. Oligodendrocyte birth and death following traumatic brain injury in adult mice. PLoS One 10(3):e0121541.

Dewar D, Underhill SM, Goldberg MP. 2003. Oligodendrocytes and ischemic brain injury. J Cereb Blood Flow Metab 23(3):263-274.

Ding S. 2014. Dynamic reactive astrocytes after focal ischemia. Neural regeneration research 9(23):2048-2052.

Diotel N, Beil T, Strahle U, Rastegar S. 2015a. Differential expression of id genes and their potential regulator znf238 in zebrafish adult neural progenitor cells and neurons suggests distinct functions in adult neurogenesis. Gene Expr Patterns 19(1-2):1-13.

Diotel N, Charlier TD, Lefebvre d'Hellencourt C, Couret D, Trudeau VL, Nicolau JC, Meilhac O, Kah O, Pellegrini E. 2018. Steroid Transport, Local Synthesis, and Signaling within the Brain: Roles in Neurogenesis, Neuroprotection, and Sexual Behaviors. Front Neurosci 12:84.

Diotel N, Le Page Y, Mouriec K, Tong SK, Pellegrini E, Vaillant C, Anglade I, Brion F, Pakdel F, Chung BC, Kah O. 2010a. Aromatase in the brain of teleost fish: expression, regulation and putative functions. Front Neuroendocrinol 31(2):172-192.

Diotel N, Lubke L, Strahle U, Rastegar S. 2020. Common and Distinct Features of Adult Neurogenesis and Regeneration in the Telencephalon of Zebrafish and Mammals. Front Neurosci 14:568930.

Diotel N, Rodriguez Viales R, Armant O, Marz M, Ferg M, Rastegar S, Strahle U. 2015b. Comprehensive expression map of transcription regulators in the adult zebrafish telencephalon reveals distinct neurogenic niches. J Comp Neurol 523(8):1202-1221.

Diotel N, Vaillant C, Gabbero C, Mironov S, Fostier A, Gueguen MM, Anglade I, Kah O, Pellegrini E. 2013a. Effects of estradiol in adult neurogenesis and brain repair in zebrafish. Hormones and Behavior 63(2):193-207.

Diotel N, Vaillant C, Gabbero C, Mironov S, Fostier A, Gueguen MM, Anglade I, Kah O, Pellegrini E. 2013b. Effects of estradiol in adult neurogenesis and brain repair in zebrafish. Horm Behav 63(2):193-207.

Diotel N, Vaillant C, Gueguen MM, Mironov S, Anglade I, Servili A, Pellegrini E, Kah O. 2010b. Cxcr4 and Cxcl12 expression in radial glial cells of the brain of adult zebrafish. J Comp Neurol 518(24):4855-4876.

Diotel N, Vaillant C, Kah O, Pellegrini E. 2016. Mapping of brain lipid binding protein (Blbp) in the brain of adult zebrafish, co-expression with aromatase B and links with proliferation. Gene Expr Patterns 20(1):42-54.

Donat CK, Scott G, Gentleman SM, Sastre M. 2017. Microglial Activation in Traumatic Brain Injury. Frontiers in aging neuroscience 9:208.

Eriksson PS, Perfilieva E, Bjork-Eriksson T, Alborn AM, Nordborg C, Peterson DA, Gage FH. 1998. Neurogenesis in the adult human hippocampus. Nat Med 4(11):1313-1317.

Ernst A, Alkass K, Bernard S, Salehpour M, Perl S, Tisdale J, Possnert G, Druid H, Frisen J. 2014. Neurogenesis in the striatum of the adult human brain. Cell 156(5):1072-1083.

Faiz M, Sachewsky N, Gascon S, Bang KW, Morshead CM, Nagy A. 2015. Adult Neural Stem Cells from the Subventricular Zone Give Rise to Reactive Astrocytes in the Cortex after Stroke. Cell Stem Cell 17(5):624-634.

Flygt J, Clausen F, Marklund N. 2017. Diffuse traumatic brain injury in the mouse induces a transient proliferation of oligodendrocyte progenitor cells in injured white matter tracts. Restor Neurol Neurosci 35(2):251-263.

Frost JL, Schafer DP. 2016. Microglia: Architects of the Developing Nervous System. Trends in cell biology 26(8):587-597.

Gensert JM, Goldman JE. 1997. Endogenous progenitors remyelinate demyelinated axons in the adult CNS. Neuron 19(1):197-203.

Gould E. 2007. How widespread is adult neurogenesis in mammals? Nat Rev Neurosci 8(6):481-488.

Grandel H, Brand M. 2013. Comparative aspects of adult neural stem cell activity in vertebrates. Development genes and evolution 223(1-2):131-147.

Grandel H, Kaslin J, Ganz J, Wenzel I, Brand M. 2006. Neural stem cells and neurogenesis in the adult zebrafish brain: origin, proliferation dynamics, migration and cell fate. Dev Biol 295(1):263-277.

Gregersen R, Christensen T, Lehrmann E, Diemer NH, Finsen B. 2001. Focal cerebral ischemia induces increased myelin basic protein and growth-associated protein-43 gene transcription in peri-infarct areas in the rat brain. Exp Brain Res 138(3):384-392.

Harry GJ, Kraft AD. 2012. Microglia in the developing brain: a potential target with lifetime effects. Neurotoxicology 33(2):191-206.

Hong S, Stevens B. 2016. Microglia: Phagocytosing to Clear, Sculpt, and Eliminate. Dev Cell 38(2):126-128.

Huang L, Wu ZB, Zhuge Q, Zheng W, Shao B, Wang B, Sun F, Jin K. 2014. Glial scar formation occurs in the human brain after ischemic stroke. Int J Med Sci 11(4):344-348.

Ito Y, Tanaka H, Okamoto H, Ohshima T. 2010. Characterization of neural stem cells and their progeny in the adult zebrafish optic tectum. Dev Biol 342(1):26-38.

Jin K, Wang X, Xie L, Mao XO, Zhu W, Wang Y, Shen J, Mao Y, Banwait S, Greenberg DA. 2006. Evidence for stroke-induced neurogenesis in the human brain. Proc Natl Acad Sci U S A 103(35):13198-13202.

Jurisch-Yaksi N, Yaksi E, Kizil C. 2020. Radial glia in the zebrafish brain: Functional, structural, and physiological comparison with the mammalian glia. Glia.

Kanagaraj P, Chen JY, Skaggs K, Qadeer Y, Conner M, Cutler N, Richmond J, Kommidi V, Poles A, Affrunti D, Powell C, Goldman D, Parent J. 2020. Microglia Stimulate Zebrafish Brain Repair Via a Specific Inflammatory Cascade. BioRXiv 79(3):268-280.

Kanazawa M, Ninomiya I, Hatakeyama M, Takahashi T, Shimohata T. 2017. Microglia and Monocytes/Macrophages Polarization Reveal Novel Therapeutic Mechanism against Stroke. International journal of molecular sciences 18(10).

Kaplan MS. 1985. Formation and turnover of neurons in young and senescent animals: an electronmicroscopic and morphometric analysis. Ann N Y Acad Sci 457:173-192.

Kaslin J, Ganz J, Geffarth M, Grandel H, Hans S, Brand M. 2009. Stem cells in the adult zebrafish cerebellum: initiation and maintenance of a novel stem cell niche. J Neurosci 29(19):6142-6153.

Kempermann G. 2011. Seven principles in the regulation of adult neurogenesis. Eur J Neurosci 33(6):1018-1024.

Kempermann G, Gage FH, Aigner L, Song H, Curtis MA, Thuret S, Kuhn HG, Jessberger S, Frankland PW, Cameron HA, Gould E, Hen R, Abrous DN, Toni N, Schinder AF, Zhao X, Lucassen PJ, Frisen J. 2018. Human Adult Neurogenesis: Evidence and Remaining Questions. Cell Stem Cell 23(1):25-30.

Kishida N, Maki T, Takagi Y, Yasuda K, Kinoshita H, Ayaki T, Noro T, Kinoshita Y, Ono Y, Kataoka H, Yoshida K, Lo EH, Arai K, Miyamoto S, Takahashi R. 2019. Role of Perivascular Oligodendrocyte Precursor Cells in Angiogenesis After Brain Ischemia. J Am Heart Assoc 8(9):e011824.

Kishimoto N, Alfaro-Cervello C, Shimizu K, Asakawa K, Urasaki A, Nonaka S, Kawakami K, Garcia-Verdugo JM, Sawamoto K. 2011. Migration of neuronal precursors from the telencephalic ventricular zone into the olfactory bulb in adult zebrafish. J Comp Neurol 519(17):3549-3565.

Kishimoto N, Shimizu K, Sawamoto K. 2012. Neuronal regeneration in a zebrafish model of adult brain injury. Disease models & mechanisms 5(2):200-209.

Kizil C, Kaslin J, Kroehne V, Brand M. 2012a. Adult neurogenesis and brain regeneration in zebrafish. Dev Neurobiol 72(3):429-461.

Kizil C, Kyritsis N, Dudczig S, Kroehne V, Freudenreich D, Kaslin J, Brand M. 2012b. Regenerative neurogenesis from neural progenitor cells requires injury-induced expression of Gata3. Dev Cell 23(6):1230-1237.

Kroehne V, Freudenreich D, Hans S, Kaslin J, Brand M. 2011. Regeneration of the adult zebrafish brain from neurogenic radial glia-type progenitors. Development 138(22):4831-4841.

Kyritsis N, Kizil C, Zocher S, Kroehne V, Kaslin J, Freudenreich D, Iltzsche A, Brand M. 2012. Acute inflammation initiates the regenerative response in the adult zebrafish brain. Science 338(6112):1353-1356.

Lam CS, Marz M, Strahle U. 2009. gfap and nestin reporter lines reveal characteristics of neural progenitors in the adult zebrafish brain. Dev Dyn 238(2):475-486.

Lange C, Rost F, Machate A, Reinhardt S, Lesche M, Weber A, Kuscha V, Dahl A, Rulands S, Brand M. 2020. Single cell sequencing of radial glia progeny reveals the diversity of newborn neurons in the adult zebrafish brain. Development 147(1).

Li H, Zhang N, Lin HY, Yu Y, Cai QY, Ma L, Ding S. 2014. Histological, cellular and behavioral assessments of stroke outcomes after photothrombosis-induced ischemia in adult mice. BMC Neurosci 15:58.

Li L, Lundkvist A, Andersson D, Wilhelmsson U, Nagai N, Pardo AC, Nodin C, Stahlberg A, Aprico K, Larsson K, Yabe T, Moons L, Fotheringham A, Davies I, Carmeliet P, Schwartz JP, Pekna M, Kubista M, Blomstrand F, Maragakis N, Nilsson M, Pekny M. 2008. Protective role of reactive astrocytes in brain ischemia. J Cereb Blood Flow Metab 28(3):468-481.

Lindsey BW, Darabie A, Tropepe V. 2012. The cellular composition of neurogenic periventricular zones in the adult zebrafish forebrain. J Comp Neurol 520(10):2275-2316.

Lindsey BW, Tropepe V. 2006. A comparative framework for understanding the biological principles of adult neurogenesis. Prog Neurobiol 80(6):281-307.

Lindvall O, Kokaia Z. 2015. Neurogenesis following Stroke Affecting the Adult Brain. Cold Spring Harbor perspectives in biology 7(11).

Liszczak TM, Hedley-Whyte ET, Adams JF, Han DH, Kolluri VS, Vacanti FX, Heros RC, Zervas NT. 1984. Limitations of tetrazolium salts in delineating infarcted brain. Acta neuropathologica 65(2):150-157.

Liu Z, Chopp M. 2016. Astrocytes, therapeutic targets for neuroprotection and neurorestoration in ischemic stroke. Prog Neurobiol 144:103-120.

Lourbopoulos A, Erturk A, Hellal F. 2015. Microglia in action: how aging and injury can change the brain's guardians. Front Cell Neurosci 9:54.

Luo Y, Kuo CC, Shen H, Chou J, Greig NH, Hoffer BJ, Wang Y. 2009. Delayed treatment with a p53 inhibitor enhances recovery in stroke brain. Ann Neurol 65(5):520-530.

Ma Y, Wang J, Wang Y, Yang GY. 2017. The biphasic function of microglia in ischemic stroke. Prog Neurobiol 157:247-272.

Magistretti PJ, Pellerin L. 1996. Cellular mechanisms of brain energy metabolism. Relevance to functional brain imaging and to neurodegenerative disorders. Ann N Y Acad Sci 777:380-387.

Marques IJ, Lupi E, Mercader N. 2019. Model systems for regeneration: zebrafish. Development 146(18).

März M, Chapouton P, Diotel N, Vaillant C, Hesl B, Takamiya M, Lam CS, Kah O, Bally-Cuif L, Strahle U. 2010. Heterogeneity in progenitor cell subtypes in the ventricular zone of the zebrafish adult telencephalon. Glia 58(7):870-888.

März M, Schmidt R, Rastegar S, Strahle U. 2011. Regenerative response following stab injury in the adult zebrafish telencephalon. Dev Dyn 240(9):2221-2231.

Merkle FT, Tramontin AD, Garcia-Verdugo JM, Alvarez-Buylla A. 2004. Radial glia give rise to adult neural stem cells in the subventricular zone. Proc Natl Acad Sci U S A 101(50):17528-17532.

Ming GL, Song H. 2005. Adult neurogenesis in the mammalian central nervous system. Annual review of neuroscience 28:223-250.

Morrison HW, Filosa JA. 2013. A quantitative spatiotemporal analysis of microglia morphology during ischemic stroke and reperfusion. J Neuroinflammation 10:4.

Moskowitz MA, Lo EH, Iadecola C. 2010. The science of stroke: mechanisms in search of treatments. Neuron 67(2):181-198.

Muramatsu R, Takahashi C, Miyake S, Fujimura H, Mochizuki H, Yamashita T. 2012. Angiogenesis induced by CNS inflammation promotes neuronal remodeling through vessel-derived prostacyclin. Nat Med 18(11):1658-1664.

Myer DJ, Gurkoff GG, Lee SM, Hovda DA, Sofroniew MV. 2006. Essential protective roles of reactive astrocytes in traumatic brain injury. Brain : a journal of neurology 129(Pt 10):2761-2772.

Ngwenya LB, Danzer SC. 2018. Impact of Traumatic Brain Injury on Neurogenesis. Front Neurosci 12:1014.

Nimmerjahn A, Kirchhoff F, Helmchen F. 2005. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science 308(5726):1314-1318.

Noctor SC, Flint AC, Weissman TA, Dammerman RS, Kriegstein AR. 2001. Neurons derived from radial glial cells establish radial units in neocortex. Nature 409(6821):714-720.

Noctor SC, Flint AC, Weissman TA, Wong WS, Clinton BK, Kriegstein AR. 2002. Dividing precursor cells of the embryonic cortical ventricular zone have morphological and molecular characteristics of radial glia. J Neurosci 22(8):3161-3173.

Noctor SC, Martinez-Cerdeno V, Kriegstein AR. 2008. Distinct behaviors of neural stem and progenitor cells underlie cortical neurogenesis. J Comp Neurol 508(1):28-44.

Obernier K, Alvarez-Buylla A. 2019. Neural stem cells: origin, heterogeneity and regulation in the adult mammalian brain. Development 146(4).

Osman AM, Porritt MJ, Nilsson M, Kuhn HG. 2011. Long-term stimulation of neural progenitor cell migration after cortical ischemia in mice. Stroke 42(12):3559-3565.

Otxoa-de-Amezaga A, Miro-Mur F, Pedragosa J, Gallizioli M, Justicia C, Gaja-Capdevila N, Ruiz-Jaen F, Salas-Perdomo A, Bosch A, Calvo M, Marquez-Kisinousky L, Denes A, Gunzer M, Planas AM. 2019. Microglial cell loss after ischemic stroke favors brain neutrophil accumulation. Acta neuropathologica 137(2):321-341.

Pantoni L, Garcia JH, Gutierrez JA. 1996. Cerebral white matter is highly vulnerable to ischemia. Stroke 27(9):1641-1646; discussion 1647.

Parent JM, Vexler ZS, Gong C, Derugin N, Ferriero DM. 2002. Rat forebrain neurogenesis and striatal neuron replacement after focal stroke. Ann Neurol 52(6):802-813.

Patel AR, Ritzel R, McCullough LD, Liu F. 2013. Microglia and ischemic stroke: a double-edged sword. Int J Physiol Pathophysiol Pharmacol 5(2):73-90.

Pekny M, Nilsson M. 2005. Astrocyte activation and reactive gliosis. Glia 50(4):427-434.

Pellegrini E, Mouriec K, Anglade I, Menuet A, Le Page Y, Gueguen MM, Marmignon MH, Brion F, Pakdel F, Kah O. 2007. Identification of aromatase-positive radial glial cells as progenitor cells in the ventricular layer of the forebrain in zebrafish. J Comp Neurol 501(1):150-167.

Perego C, Fumagalli S, De Simoni MG. 2011. Temporal pattern of expression and colocalization of microglia/macrophage phenotype markers following brain ischemic injury in mice. J Neuroinflammation 8:174.

Perez-de-Puig I, Miro-Mur F, Ferrer-Ferrer M, Gelpi E, Pedragosa J, Justicia C, Urra X, Chamorro A, Planas AM. 2015. Neutrophil recruitment to the brain in mouse and human ischemic stroke. Acta neuropathologica 129(2):239-257.

Pinto L, Gotz M. 2007. Radial glial cell heterogeneity--the source of diverse progeny in the CNS. Prog Neurobiol 83(1):2-23.

Popp A, Jaenisch N, Witte OW, Frahm C. 2009. Identification of ischemic regions in a rat model of stroke. PLoS One 4(3):e4764.

Quastel JH, Wheatley AH. 1932. Oxidations by the brain. Biochem J 26(3):725-744.

Quiñones-Hinojosa A, Sanai N, Soriano-Navarro M, Gonzalez-Perez O, Mirzadeh Z, Gil-Perotin S, Romero-Rodriguez R, Berger MS, Garcia-Verdugo JM, Alvarez-Buylla A. 2006. Cellular composition and cytoarchitecture of the adult human subventricular zone: a niche of neural stem cells. J Comp Neurol 494(3):415-434.

Radak D, Katsiki N, Resanovic I, Jovanovic A, Sudar-Milovanovic E, Zafirovic S, Mousad SA, Isenovic ER. 2017. Apoptosis and Acute Brain Ischemia in Ischemic Stroke. Current vascular pharmacology 15(2):115-122.

Rafalski VA, Ho PP, Brett JO, Ucar D, Dugas JC, Pollina EA, Chow LM, Ibrahim A, Baker SJ, Barres BA, Steinman L, Brunet A. 2013. Expansion of oligodendrocyte progenitor cells following SIRT1 inactivation in the adult brain. Nature cell biology 15(6):614-624.

Ransohoff RM. 2016. How neuroinflammation contributes to neurodegeneration. Science 353(6301):777-783.

Rodriguez Viales R, Diotel N, Ferg M, Armant O, Eich J, Alunni A, Marz M, Bally-Cuif L, Rastegar S, Strahle U. 2015. The helix-loop-helix protein id1 controls stem cell proliferation during regenerative neurogenesis in the adult zebrafish telencephalon. Stem Cells 33(3):892-903.

Rosell A, Cuadrado E, Ortega-Aznar A, Hernandez-Guillamon M, Lo EH, Montaner J. 2008. MMP-9-positive neutrophil infiltration is associated to blood-brain barrier breakdown and basal lamina type IV collagen degradation during hemorrhagic transformation after human ischemic stroke. Stroke 39(4):1121-1126.

Rothenaigner I, Krecsmarik M, Hayes JA, Bahn B, Lepier A, Fortin G, Gotz M, Jagasia R, Bally-Cuif L. 2011. Clonal analysis by distinct viral vectors identifies bona fide neural stem cells in the adult zebrafish telencephalon and characterizes their division properties and fate. Development 138(8):1459-1469.

Ruan L, Wang B, ZhuGe Q, Jin K. 2015. Coupling of neurogenesis and angiogenesis after ischemic stroke. Brain Res 1623:166-173.

Sairanen T, Karjalainen-Lindsberg ML, Paetau A, Ijas P, Lindsberg PJ. 2006. Apoptosis dominant in the periinfarct area of human ischaemic stroke--a possible target of antiapoptotic treatments. Brain : a journal of neurology 129(Pt 1):189-199.

Schmidt R, Beil T, Strähle U, Rastegar S. 2014. Stab wound injury of the zebrafish adult telencephalon: a method to investigate vertebrate brain neurogenesis and regeneration. J Vis Exp(90):e51753.

Schmidt R, Strähle U, Scholpp S. 2013. Neurogenesis in zebrafish - from embryo to adult. Neural Dev 8:3.

Schroeter M, Schiene K, Kraemer M, Hagemann G, Weigel H, Eysel UT, Witte OW, Stoll G. 1995. Astroglial responses in photochemically induced focal ischemia of the rat cortex. Exp Brain Res 106(1):1-6.

Shimada IS, Borders A, Aronshtam A, Spees JL. 2011. Proliferating reactive astrocytes are regulated by Notch-1 in the peri-infarct area after stroke. Stroke 42(11):3231-3237.

Sims NR, Yew WP. 2017. Reactive astrogliosis in stroke: Contributions of astrocytes to recovery of neurological function. Neurochem Int 107:88-103.

Sofroniew MV. 2005. Reactive astrocytes in neural repair and protection. Neuroscientist 11(5):400-407.

Sofroniew MV. 2009. Molecular dissection of reactive astrogliosis and glial scar formation. Trends Neurosci 32(12):638-647.

Sofroniew MV. 2015. Astrocyte barriers to neurotoxic inflammation. Nat Rev Neurosci 16(5):249-263.

Sorrells SF, Paredes MF, Cebrian-Silla A, Sandoval K, Qi D, Kelley KW, James D, Mayer S, Chang J, Auguste KI, Chang EF, Gutierrez AJ, Kriegstein AR, Mathern GW, Oldham MC, Huang EJ, Garcia-Verdugo JM, Yang Z, Alvarez-Buylla A. 2018. Human hippocampal neurogenesis drops sharply in children to undetectable levels in adults. Nature 555(7696):377-381.

Spalding KL, Bergmann O, Alkass K, Bernard S, Salehpour M, Huttner HB, Bostrom E, Westerlund I, Vial C, Buchholz BA, Possnert G, Mash DC, Druid H, Frisen J. 2013. Dynamics of hippocampal neurogenesis in adult humans. Cell 153(6):1219-1227.

Stoica BA, Faden AI. 2010. Cell death mechanisms and modulation in traumatic brain injury. Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics 7(1):3-12.

Tang BL. 2016. The astrocyte scar - not so inhibitory after all? Neural regeneration research 11(7):1054-1055.

Than-Trong E, Bally-Cuif L. 2015. Radial glia and neural progenitors in the adult zebrafish central nervous system. Glia 63(8):1406-1428.

Than-Trong E, Kiani B, Dray N, Ortica S, Simons B, Rulands S, Alunni A, Bally-Cuif L. 2020. Lineage hierarchies and stochasticity ensure the long-term maintenance of adult neural stem cells. Sci Adv 6(18):eaaz5424.

Than-Trong E, Ortica-Gatti S, Mella S, Nepal C, Alunni A, Bally-Cuif L. 2018. Neural stem cell quiescence and stemness are molecularly distinct outputs of the Notch3 signalling cascade in the vertebrate adult brain. Development 145(10).

Ueno T, Ito J, Hoshikawa S, Ohori Y, Fujiwara S, Yamamoto S, Ohtsuka T, Kageyama R, Akai M, Nakamura K, Ogata T. 2012a. The identification of transcriptional targets of Ascl1 in oligodendrocyte development. Glia 60(10):1495-1505.

Ueno Y, Chopp M, Zhang L, Buller B, Liu Z, Lehman NL, Liu XS, Zhang Y, Roberts C, Zhang ZG. 2012b. Axonal outgrowth and dendritic plasticity in the cortical peri-infarct area after experimental stroke. Stroke 43(8):2221-2228.

Wang W, Hu CK, Zeng A, Alegre D, Hu D, Gotting K, Ortega Granillo A, Wang Y, Robb S, Schnittker R, Zhang S, Alegre D, Li H, Ross E, Zhang N, Brunet A, Sanchez Alvarado A. 2020. Changes in regeneration-responsive enhancers shape regenerative capacities in vertebrates. Science 369(6508).

Wilhelmsson U, Bushong EA, Price DL, Smarr BL, Phung V, Terada M, Ellisman MH, Pekny M. 2006. Redefining the concept of reactive astrocytes as cells that remain within their unique domains upon reaction to injury. Proc Natl Acad Sci U S A 103(46):17513-17518.

Woitke F, Ceanga M, Rudolph M, Niv F, Witte OW, Redecker C, Kunze A, Keiner S. 2017. Adult hippocampal neurogenesis poststroke: More new granule cells but aberrant morphology and impaired spatial memory. PLoS One 12(9):e0183463.

Xing C, Arai K, Lo EH, Hommel M. 2012. Pathophysiologic cascades in ischemic stroke. Int J Stroke 7(5):378-385.

Xiong XY, Liu L, Yang QW. 2016. Functions and mechanisms of microglia/macrophages in neuroinflammation and neurogenesis after stroke. Prog Neurobiol 142:23-44.

Xiong Y, Mahmood A, Chopp M. 2010. Angiogenesis, neurogenesis and brain recovery of function following injury. Curr Opin Investig Drugs 11(3):298-308.

Yamashita T, Ninomiya M, Hernandez Acosta P, Garcia-Verdugo JM, Sunabori T, Sakaguchi M, Adachi K, Kojima T, Hirota Y, Kawase T, Araki N, Abe K, Okano H, Sawamoto K. 2006. Subventricular zone-derived neuroblasts migrate and differentiate into mature neurons in the post-stroke adult striatum. J Neurosci 26(24):6627-6636.

Yang Y, Wang H, Li L, Li X, Wang Q, Ding H, Wang X, Ye Z, Wu L, Zhang X, Zhou M, Pan H. 2016. Sinomenine Provides Neuroprotection in Model of Traumatic Brain Injury via the Nrf2-ARE Pathway. Front Neurosci 10:580.

Zamanian JL, Xu L, Foo LC, Nouri N, Zhou L, Giffard RG, Barres BA. 2012. Genomic analysis of reactive astrogliosis. J Neurosci 32(18):6391-6410.

Zambusi A, Ninkovic J. 2020. Regeneration of the central nervous system-principles from brain regeneration in adult zebrafish. World J Stem Cells 12(1):8-24.

Zawadzka M, Rivers LE, Fancy SP, Zhao C, Tripathi R, Jamen F, Young K, Goncharevich A, Pohl H, Rizzi M, Rowitch DH, Kessaris N, Suter U, Richardson WD, Franklin RJ. 2010. CNS-

 doi:10.20944/preprints202012.0544.v1

resident glial progenitor/stem cells produce Schwann cells as well as oligodendrocytes during repair of CNS demyelination. Cell Stem Cell 6(6):578-590.

Zhang C, Chopp M, Cui Y, Wang L, Zhang R, Zhang L, Lu M, Szalad A, Doppler E, Hitzl M, Zhang ZG. 2010. Cerebrolysin enhances neurogenesis in the ischemic brain and improves functional outcome after stroke. J Neurosci Res 88(15):3275-3281.

Zhang G, Ferg M, Lubke L, Takamiya M, Beil T, Gourain V, Diotel N, Strahle U, Rastegar S. 2020. Bone morphogenetic protein signaling regulates Id1 mediated neural stem cell quiescence in the adult zebrafish brain via a phylogenetically conserved enhancer module. Stem Cells.

Zhang J, Zhang Y, Xing S, Liang Z, Zeng J. 2012. Secondary neurodegeneration in remote regions after focal cerebral infarction: a new target for stroke management? Stroke 43(6):1700-1705.

Zhang R, Chopp M, Zhang ZG. 2013. Oligodendrogenesis after cerebral ischemia. Front Cell Neurosci 7:201.

Zhang S. 2019. Microglial activation after ischaemic stroke. Stroke Vasc Neurol 4(2):71-74.

Zhou Y, Shao A, Yao Y, Tu S, Deng Y, Zhang J. 2020. Dual roles of astrocytes in plasticity and reconstruction after traumatic brain injury. Cell Commun Signal 18(1):62.

Zille M, Farr TD, Przesdzing I, Muller J, Sommer C, Dirnagl U, Wunder A. 2012. Visualizing cell death in experimental focal cerebral ischemia: promises, problems, and perspectives. J Cereb Blood Flow Metab 32(2):213-231.

Zupanc GK. 2008. Adult neurogenesis and neuronal regeneration in the brain of teleost fish. J Physiol Paris 102(4-6):357-373.

Zupanc GK, Clint SC. 2003. Potential role of radial glia in adult neurogenesis of teleost fish. Glia 43(1):77-86.

Zupanc GK, Hinsch K, Gage FH. 2005. Proliferation, migration, neuronal differentiation, and long-term survival of new cells in the adult zebrafish brain. J Comp Neurol 488(3):290-319.

Zupanc GK, Zupanc MM. 1992. Birth and migration of neurons in the central posterior/prepacemaker nucleus during adulthood in weakly electric knifefish (Eigenmannia sp.). Proc Natl Acad Sci U S A 89(20):9539-9543.