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Metabolism and adult neurogenesis: towards an understanding of the role of lipocalin-2 and iron-related oxidative stress

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Highlights

- Nutrient signals and metabolism control quiescent NSCs transition to activate states
- Energy metabolism from glycolysis to mitochondrial phosphorylation regulates NSCs function
- Adult neurogenesis transiently generates oxidative stress

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- LCN2 is an emerging molecular bond of iron-dependent oxidative control for NSCs quiescence

Abstract

The process of generating new functional neurons in the adult mammalian brain occurs from the local neural stem and progenitor cells and requires tight control of the progenitor cell's activity. Several signaling pathways and intrinsic/extrinsic factors have been well studied over the last years, but recent attention has been given to the critical role of cellular metabolism in determining the functional properties of progenitor cells. Here, we review recent advances in the current understanding of when and how metabolism affects neural stem cell (NSC) behavior and subsequent neuronal differentiation and highlight the role of lipocalin-2 (LCN2), a protein involved in the control of oxidative stress, as a recently emerged regulator of NSC activity and neuronal differentiation.

Keywords

Neurogenesis, metabolism, oxidative stress, lipocalin-2

1. Adult neurogenesis

Adult neurogenesis from resident neural stem cells (NSCs) contributes to brain homeostasis, damage/repair, and cognition. The past fifty years of research in the field of adult NSC biology significantly expanded our understanding of its essential characteristics, the environmental conditions and life stages that can alter their properties, and their functional relevance in normal and in diseased brains (Bond et al. 2015). An emerging concept is that adult NSCs are a dynamic population of cells that

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are able to sense and respond to changes in energy homeostasis occurring either locally in the brain and/or systemically in the organism. In this article, we will provide an overview of the current understanding of how energy metabolism influences adult NSC function and neurogenesis. We will give particular attention to the emerging role of redox conditions in the maintenance of the pool of NSC quiescence and its neurogenic activation, as well as the known underlying molecular regulators.

For a long time, neurogenesis was considered to be limited to the embryonic and early postnatal stages, but it is now widely accepted to be active throughout adult mammalian life (Ming and Song 2011). However, a recent intense debate exists on the capacity of the adult brain, particularly in humans, to generate new neurons (Boldrini et al. 2018, Snyder 2018, Sorrells et al. 2018). While some studies suggest that hundreds of new neurons are continuously generated every day (Spalding et al. 2013), then decline with aging (Boldrini et al. 2018), other studies report fewer putative new neurons (Eriksson et al. 1998, Dennis et al. 2016) or undetectable levels (Sorrells et al. 2018). Nevertheless, and despite these discrepancies, it is still generally believed that neurogenesis persists in the adult human brain.

Described as the process of generation and maturation of new neurons from NSCs and their derivate progenitors, neurogenesis in the adult mammalian brain is spatially mostly restricted to the subventricular zone (SVZ) lining of the lateral ventricles and the subgranular zone (SGZ) of the dentate gyrus (DG) in the hippocampus, leading to the formation of new olfactory bulb interneurons and new granule cells, respectively (Figure 1). More recently, a growing number of studies have also identified neurogenesis in other brain regions [see (Gould 2007)], with the hypothalamus recognized as the third relevant neurogenic area in the postnatal murine brain (Kokoeva et al. 2007).

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1.1 Neurogenic areas of the adult mammalian brain

In the adult mammalian brain, pools of neural stem cells (NSCs) reside in specific regions that harbor the ideal microenvironment to support and maintain NSCs. These cells are characterized as self-renewing cells, i.e., they produce at least one stem cell daughter upon division. In addition, as multipotent cells, they produce all three cell types of the brain: neurons, astrocytes, and oligodendrocytes. Considered relatively quiescent (Doetsch et al. 1999), once they are activated and enter the cell cycle, NSCs produce rapid dividing neural progenitors that undergo limited rounds of proliferation to generate more committed progenitors. These neural progenitors ultimately yield differentiated progeny, such as new neurons (neurogenesis), that can then integrate into functional circuits and contribute to brain functioning.

The pools of stem cells are mainly located in the subventricular zone (SVZ) lining of the lateral ventricles and in the subgranular (SGZ) of the dentate gyrus (DG) in the hippocampus. The SVZ is the largest germinal zone present in the adult brain. Within this region, stem cells, designated as type B cells are glial fibrillary acidic protein (GFAP)-expressing astrocyte-like cells displaying functional characteristics that are intermediate between the radial glia and the astrocytes (Doetsch et al. 1997, Doetsch et al. 1999). Type B cells divide slowly and, once activated, generate transit-amplifying progenitors (type C cells) that, in turn, proliferate to produce neuroblasts (type A cells) or glia. Then, neuroblasts migrate anteriorly from the SVZ along the rostral migratory stream (RMS) to the olfactory bulb, where immature neurons migrate radially outward, differentiate into different subtypes of inhibitory interneurons, and integrate into the distinct layers of the OB (Figure 1).

In the adult SGZ of the DG, neurogenesis implies the activation of type 1 radial and

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nonradial NSCs that are placed at the border between the inner granule cell layer and the hilus. These cells, which are mainly quiescent astrocyte-like stem cells that are similar to the type B cells of the SVZ, generate intermediate proliferative type 2 cells (transit-amplifying cells) that can be subdivided into type 2a and type 2b cells. These last types are lineage-committed proliferative neuronal precursors that produce differentiating neuroblasts (type 3 cells). Neuroblasts then migrate into the inner granule cell layer, where they mature into dentate granule neurons that extend dendrites to the molecular layer, prolong axons to the CA3 region (Figure 1) and start to receive GABAergic inputs (Tozuka et al. 2005).

'Noncanonical' adult neurogenesis also occurs in the hypothalamus, a small region in the third ventricle with a key role in the homeostatic regulation of many body functions [reviewed in (Rojczyk-Golebiewska et al. 2014)]. There, tanycytes are hypothalamic stem cells with nonciliated ependymal cells that have radial glia-like features in contact with the cerebrospinal fluid (CSF) and long cell processes that extend into the hypothalamic parenchyma. Tanycytes are classified into four types based on their position along the third ventricle wall (α1, α2-, β1-, and β2-tanycytes); yet, no consensus exists regarding which types of tanycytes are true adult hypothalamic NSCs [reviewed in (Rojczyk-Golebiewska et al. 2014)].

1.2 Functional role of neurogenesis

New adult-born neurons display distinct functions depending on the environmental inputs present during their maturation (Goncalves et al. 2016). Under pathological conditions, increased proliferation and differentiation of NSCs in the neurogenic niches occur as a source of neuronal replacement in damaged cortical regions (Zhang et al. 2004). In addition to roles in cell loss replacement and tissue repair, new neurons generated in adulthood fulfill important functions in plasticity that highly contribute to

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brain physiological functioning and behavior. For instance, in the olfactory bulb, newly born interneurons are involved in olfactory learning (Gheusi et al. 2000). Likewise, neurogenesis in the DG contributes to memory formation and the plasticity of neural circuits, as demonstrated by studies that correlate the increase of neurogenesis with improved behavioral performance (Gage and Temple 2013). Notably, newborn neurons that participate in the formation of new memories in the hippocampus also contribute to the extinction of older memories (Akers et al. 2014). On the other hand, impaired neurogenesis contributes to the pathophysiology of cognitive symptoms in aging (Klempin and Kempermann 2007) and in neuropsychiatric diseases (Snyder et al. 2011). Noticeably, the ablation of neurogenesis precipitates behavioral despair and anhedonia (Snyder et al. 2011), while hippocampal neurogenesis is required for the behavioral effects of antidepressant treatment (Santarelli et al. 2003) and for sustained remission from depressive-like behavior (Mateus-Pinheiro et al. 2013). This reflects the degree of plasticity presented by immature cells, which also include electrophysiological features that are distinct from those of mature granule cells, giving them unique properties that are crucial for their functional role. Newborn neurons are more excitable, present a lower threshold voltage and have more depolarized resting membrane potentials, which allows them to respond to a broader range of stimuli (Ming and Song 2011, Gu et al. 2012). This period of enhanced activity and plasticity is essential for the function of adult-born neurons and for their contribution towards boosting local neural and structural plasticity, which, in turn, influences global brain function.

1.3 Modulation of neurogenesis

Neurogenesis from quiescent NSCs occurs through a stereotypic developmental sequence that is controlled by the complex interplay between intrinsic and extrinsic

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signals (Ming and Song 2011). Numerous studies identified several key factors and signaling mechanisms, including hormones, neurotrophins and growth factors, as well as cell-extrinsic cues, such as stress, aging and physical activity (Table 1). The cellular composition of the niche where NSCs reside highly contributes to the maintenance of the required microenvironment of the signaling gradients of growth factors, neurotransmitters and transcription factors that are necessary for the neurogenic process and the preservation of NSCs self-renewal capacity. For instance, adult NSCs release factors that contribute to autocrine and paracrine signaling and, in both neurogenic niches, NSCs concentrate in dense clusters near blood vessels that allow their constant contact with circulating molecules and nutrients (Tavazoie et al. 2008). In this sense, the identification of novel mechanisms and factors governing NSC homeostasis should be restricted not only to those that are locally produced in the brain but also to those that are delivered from the periphery. For example, the recent identification of the blood-derived iron trafficking protein lipocalin-2 (LCN2) as a novel regulator of NSC quiescence and self-renewal (Ferreira et al. 2018) opened new perspectives on the crosstalk between the periphery and the brain (as we will review later).

2. Regulation of adult neural stem cell activity and neurogenesis by cellular metabolism

Until very recently, the role of cellular metabolism in determining the functional properties of NSCs and in the generation of new brain cells was disregarded. Nevertheless, it seems noticeable that aspects such as the cell's energy status are linked to NSC activity and differentiation processes (Ito and Suda 2014). In fact, the metabolic requirements of differentiated cells are drastically different from those of self-renewing,

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multipotent NSCs (Rafalski and Brunet 2011). From development through adulthood, the transition from neural progenitor cell to a differentiated neuron, astrocyte, or oligodendrocyte is associated with numerous transcriptional changes, including genes that are associated with metabolism and energy sensing (Bonnert et al. 2006). Moreover, the process of differentiation is associated with an increase in cell volume and requires substantial amounts of energy for DNA replication and organelle synthesis, thus being highly dependent on cellular metabolic shifts, mitochondrial function and oxygen levels (Ito and Suda 2014, Almeida and Vieira 2016). Accordingly, cell metabolism is an essential indicator of general cell function. In fact, impaired neurogenesis has been described to occur in metabolic disorders, such as diabetes (Han et al. 2016) and is even suggested to be one of the risk factors associated with neurological and psychiatric diseases. However, the precise identity of stage-specific metabolic programs and their impact on adult neurogenesis is only now starting to be elucidated. In accordance, a recent single-cell RNAseq analysis of adult NSCs provided the first evidence that lineage progression in neurogenesis is functionally coupled to the activity of specific metabolic states (Shin et al. 2015).

2.1 How neural stem cells sense energy

Evidence that different forms of energy metabolism modulate neurogenesis implies the activity of specific molecules and signaling pathways that are capable of sensing environmental metabolic alterations in the regulation of NSC maintenance, proliferation and differentiation. These include insulin/insulin-like growth factor-1 (IGF-1) signaling, forkhead box O (FoxO) transcription factors, the mammalian target of rapamycin (mTOR), AMP-activated protein kinase (AMPK) and sirtuins [see also (Rafalski and Brunet 2011)]. Specifically, these metabolic and lifespan regulators were linked to the

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redox state of NSCs, with mTOR signaling also acting as a sensor of nutrient availability. Importantly, these signaling modules may cooperate with other pathways involved in stem cell maintenance and differentiation.

Table 2 provides a summarized view of the effects of energy-sensing molecular mechanisms in NSC maintenance and neurogenesis.

2.2 Lipid metabolism as an emerging regulator of neural stem cells and neurogenesis

Fatty acids from either *de novo* synthesis or storage in fat can function as secondary messengers for specific signal transduction pathways (Ito and Suda 2014). In fact, mechanisms regulating lipid metabolism provide alternative routes to support cancer cell proliferation and survival (Schafer et al. 2009). Interestingly, similar mechanisms were recently were described to be important for adult neurogenesis (Knobloch et al. 2013). The discovery that high levels of lipogenesis are required for the activation of quiescent NSCs and their proper proliferation (Knobloch et al. 2013) provided the first direct evidence that lineage progression in adult hippocampal neurogenesis is functionally coupled with the activity of a specific metabolic program. In fact, in the forebrain SVZ niche, transcriptional differences between quiescent and activated NSCs include the class of lipid metabolism genes (Codega et al. 2014).

The amount of *de novo* lipogenesis was demonstrated to influence quiescence behavior since the amount of new lipids produced is reduced in quiescent adult NSCs, whereas proliferating NSCs require the production of lipids to assure the maintenance of neurogenesis (Knobloch et al. 2013). Specifically, it was demonstrated that proliferating NSCs require fatty acid synthase (FASN)-dependent lipogenesis and that the thyroid

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hormone-inducible hepatic protein (THRSP; also known as SPOT14) is responsible to ensure quiescence of adult NSCs because it reduces the substrate availability of malonyl-CoA for FASN-dependent *de novo* lipogenesis (Knobloch et al. 2013). The importance of newly formed lipids in NSCs was evident when the pharmacological and genetic manipulation of this pathway was associated with a drastic reduction in proliferation and neurogenesis (Knobloch et al. 2013). Furthermore, the additional demonstration that FASN mRNA is upregulated in the hippocampus after running and that the pharmacological inhibition of FASN impaired exercise-mediated improvement in proliferation and spatial memory (Chorna et al. 2013) emphasizes the importance of *de novo* lipogenesis in the control of NSC activity. Additionally, it provides evidence of the dynamic regulation of this pathway upon a pro-neurogenic stimulus (Chorna et al. 2013) and that NSCs can alter lipid metabolism upon extrinsic signals (Knobloch et al. 2014). Even though it is not yet fully understood why proliferating NSCs are so dependent on the production of new lipids, it is likely that a considerable amount is used for new membrane production, which is required for cell growth and proliferation (Knobloch et al. 2013). However, NSCs were suggested to use lipids not only for membrane remodeling but also as an alternative energy source to glucose (Stoll et al. 2015), which is similar to what has been described for cancer cell survival (Carracedo et al. 2013). When glucose is limited, fatty acids are an extremely relevant energy source that can be incorporated from extracellular media or obtained from hydrolyzed triglycerides (Carracedo et al. 2013).

In contrast to mature cells within the adult brain, which depend on carbohydrates such as glucose for energy production, NSCs were reported to use fatty acids to support aerobic respiratory activity and cell division (Stoll et al. 2015). Specifically, fatty acid oxidation was found to be elevated in adult NSCs in the SVZ (Stoll et al. 2015) and to

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control the NSC pool during neocortical development by assuring proper self-renewing divisions (Xie et al. 2016). The importance of fatty acid oxidation was further demonstrated when its pharmacological inhibition resulted in decreased proliferation (Stoll et al. 2015), reduced the NSC pool due to increased differentiation (Xie et al. 2016), and ultimately, linked neuropsychiatric diseases (e.g., autism) to the deregulation of NSC activity during development (Xie et al. 2016).

Lipid metabolism also contributes to the interaction between the neurogenic niche and NSCs behavior. At least in *Drosophila*, the accumulation of lipids in lipid droplets occurs in response to oxidative stress to provide a protective environment that minimizes the damaging effects of polyunsaturated fatty acids peroxidation on NSCs and maintains the proliferation of neuroblasts (Bailey et al. 2015). Nevertheless, and even though they were proposed to have antioxidant roles in the stem cell niche of *Drosophila* (Bailey et al. 2015), lipid droplets, if in excess, can also negatively regulate adult NSC proliferation (Hamilton et al. 2015). Recently, associated with Alzheimer's disease pathology (Hamilton et al. 2015), lipid accumulation within ependymal cells lining the ventricular zone was correlated with the early onset of neurogenesis defects, both in animal models and in patients (Hamilton et al. 2015). In addition to the possible direct contribution to cognitive decline, the observed suppression of NSC activity due to lipid accumulation at the ependymal surface of the brain in Alzheimer's disease may explain why the brain's stem cell system does not mount a more robust protective or regenerative response in the context of the disease (Hamilton et al. 2015).

2.3 Metabolic patterns: shifting between glycolysis and oxidative phosphorylation

Glycolysis, in certain circumstances, is one of the major pathways that provides metabolic precursors for biosynthesis and energy production. The activities and

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metabolic flux of this pathway are precisely tuned to ensure optimal resource distribution in accordance with cellular function. Noticeably, quiescent NSCs have a predominantly glycolytic profile, as opposed to their more differentiated progeny that generate ATP mainly by moving to mitochondrial oxidative phosphorylation (Figure 2) (Rafalski and Brunet 2011). This metabolic shift between glycolysis and mitochondrial phosphorylation is essential for stem cell function and fate determination. Consistent with this view, and specifically for NSCs, rodents subjected to caloric restriction were described to display increased numbers of newly produced neural cells in the SGZ and in the higher expression of the brain-derived neurotrophic factor (BDNF) (Lee et al. 2000). In contrast, adult neurogenesis is impaired in several rodent models of diet-induced obesity and diabetes (Pani 2015). Nevertheless, and although glucose is the main substrate for brain metabolism, lactate can also be used as a metabolic substrate by NSCs (Alvarez et al. 2016).

The neurogenic niches where NSCs reside are characterized by a low oxygen tension (<1–6%) that creates a hypoxic environment that is favorable to NSC maintenance and proliferation, both *in vivo* and *in vitro* (Prozorovski et al. 2015). This is based on the promotion of glycolytic metabolism in NSCs by the hypoxic niche, over mitochondrial phosphorylation, which fulfills NSC biosynthetic needs (Kim et al. 2014). Indeed, NSCs have been described to be highly tolerant of hypoxia, but inhibition of glycolytic pathways, even in the presence of pyruvate and oxygen, significantly impairs their survival (Candelario et al. 2013). In addition to supporting the role of glycolytic pathways and oxygen availability for NSC behavior was the demonstration of the ingrowth of blood vessels into the developing cortex, which resulted in increased oxygen availability and promoted the well-known switch of NSC expansion to differentiation during brain development (Lange et al. 2016). The additional genetic perturbation of

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vessel formation resulting in hypoxia caused the NSC pool to expand, thereby reducing neurogenesis (Lange et al. 2016).

The adult NSC response to hypoxia towards a glycolytic metabolism is possible because they express glucose transporters 1 and 3 (Maurer et al. 2006). These transporters are regulated by hypoxic conditions to assure enhanced glycolytic flux that can supply the metabolic needs of NSCs necessary for neurogenesis (Kim et al. 2014). Also under hypoxia, the transcription of glycolytic genes is upregulated by the hypoxia-inducible factor (HIF), which is a class of transcription factors that are stabilized and activated under low oxygen availability. Even though it was demonstrated by some authors that NSC resistance to hypoxia is independent of HIF1 alpha signaling (Candelario et al. 2013), HIF1 deficiency in NSCs (Renault et al. 2009) determines the loss of quiescence and the premature depletion of the stem cell pool.

As mentioned, during lineage progression, the activation of NSCs is accompanied by a downregulation of glycolysis and an upregulation of mitochondrial activity and oxidative phosphorylation (Figure 2). In fact, to support the growing energy demands of specialized progeny, several biochemical and structural alterations occur in order for mitochondria to utilize oxygen to generate ATP (Almeida and Vieira 2016). Importantly, mitochondrial metabolism also contributes to regulating NSC activity, since a certain level of aerobic metabolism might be necessary to prevent oncologic transformation of NSCs, as was recently suggested (Zhang et al. 2016).

The importance of mitochondrial metabolic pathways in the regulation of neuronal differentiation and maturation is evident when the inhibition of oxidative phosphorylation prevents cell cycle exit and terminal differentiation of NSCs by genetically targeting various components of the mitochondrial electron transport chain

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in *Drosophila* (Homem et al. 2014). In mammals, similar effects were described. It was recently demonstrated that the integrity of the mitochondrial electron transport chain and oxidative phosphorylation machinery are critical during the early phases of hippocampal neurogenesis since the proliferation and survival of intermediate progenitor cells, which are generated from activated NSCs, are compromised by pharmacological and genetic approaches (Beckervordersandforth et al. 2017). Moreover, impaired mitochondrial function was observed to contribute to the age-associated decline of hippocampal neurogenesis; the pharmacological enhancement of mitochondrial function promotes neurogenesis in the aging hippocampus (Beckervordersandforth et al. 2017). Together, this suggests the occurrence of a metabolic shift from usage of the glycolysis pathway to oxidative phosphorylation during NSC to intermediate progenitor cell transition, which is affected during aging (Beckervordersandforth et al. 2017).

3. Redox state of neural stem cells influences their fate

Closely related to the shift in cells metabolic profiles and concomitant with the use of mitochondrial metabolism during differentiation is the generation of reactive oxygen species (ROS). The oxidative phosphorylation that takes place in the mitochondria generates ROS as a byproduct (Madhavan 2015), and while ROS has long been valued for their damage-promoting detrimental effects, there is now a greater understanding of its role as a signaling molecule that is essential for both sustained NSC self-renewal capacity (Le Belle et al. 2011, Beckervordersandforth et al. 2017) and differentiation (Zheng et al. 2016). Furthermore, although quite controversial, there is evidence that the hypoxic microenvironment of neurogenic niches may exert effects on NSC stemness by generating free radicals, including ROS, in particular, subcellular compartments (Vieira et al. 2011).

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Intracellular ROS are formed by one-electron transfer from a redox donor to molecular oxygen (O_2). This initially generates a superoxide anion (O_2^-) that can be converted to hydrogen peroxide (H_2O_2) by superoxide dismutase enzymes. Other forms of ROS can also include hydroxyl radical (HO), nitric oxide radical (NO), and peroxynitrite ($ONOO^-$). Commonly associated with cause oxidative stress upon cellular accumulation, the negative effects of ROS are counteracted at the physiological levels by the action of specific antioxidant mechanisms. These include, among others, the action of antioxidants (e.g., ascorbic acid) and enzymes, such as superoxide dismutase (SOD), catalase and glutathione peroxidase (GPX) (Bigarella et al. 2014).

However, when ROS production outpaces its scavenging, this leads to its excessive accumulation, which shifts the intracellular redox environment into a more oxidized state and promotes oxidative reactions that lead to DNA damage and the oxidation of lipids and proteins. In fact, oxidative stress imposed by the cellular accumulation of ROS is the major contributor to disease and cell death (Dixon and Stockwell 2014) through the activation of multiple signaling pathways, including the p53 pathway (Martindale and Holbrook 2002).

With respect to stem cell physiology and behavior, ROS levels are critical in maintaining the self-renewal and differentiation of stem cells; therefore, ROS levels play major roles in the exhaustion of cycling stem cells. Throughout recent years, several reports have shown how oxidative stress promotes exit from the stem cell state and spontaneous cellular differentiation. For example, in the hematopoietic system, a low endogenous cellular ROS status contributes to the maintenance of their quiescent state, whereas a higher ROS state is associated with a greater proliferation that leads to a premature exhaustion of self-renewal (Jang and Sharkis 2007). Accordingly, FOXO-

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deficient hematopoietic stem cells accumulate oxidative damage due to a reduced expression of antioxidant enzymes (Tothova et al. 2007), while deregulated mTOR activation or accumulation of unrepaired DNA damage are associated with an increased generation of mitochondrial ROS and a loss of self-renewal capacity (Ito et al. 2004, Chen et al. 2008). Similar observations were obtained in the *Drosophila* testis germline stem cells (Tan et al. 2017) and in models of human embryonic stem cells (Hu et al. 2018), where increased levels of ROS induced a reduction in the number of stem cells by promoting their differentiation, while ROS reduction sustained stem cell self-renewal. Altogether, this leads to the hypothesis that keeping ROS at steady-levels within the stem cell niche is an important feature of stemness, which is directly related to the quiescent state of stem cells.

Certainly, this also holds true for neural progenitors, even though they are not as fully investigated as they are in other systems, such as the hematopoietic system, in which the involvement of ROS in the exhaustion of NSCs is also likely to occur. The formation of ROS is inhibited in hypoxic niches where quiescent NSCs reside (Ochocki and Simon 2013); however, in the context of reduced antioxidant defenses, the excess ROS production may induce NSCs to exit from quiescence or lead to mitochondrial damage in the long term (Cavallucci et al. 2016).

Nevertheless, it is surprising to observe that, contrary to the majority of the reports that describe the cellular toxicities of ROS, proliferative, self-renewing neural progenitors with phenotypic characteristics of NSCs were described to present increased ROS levels and to be highly responsive to ROS stimulation (Le Belle et al. 2011). In fact, pharmacological or genetic manipulations that interfered with cellular ROS levels were negatively impacted by NSC self-renewal and neurogenesis (Le Belle et al. 2011). In addition, oxygen species generated by membrane-bound NADPH-dependent oxidases

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(NOX) may also have a physiological role in NSC exit from quiescence (Le Belle et al. 2011).

Currently assumed to be important for signaling in NSC behavior, and critically affecting the appropriate balance between self-renewal and differentiation (Le Belle et al. 2011, Prozorovski et al. 2015), ROS plays roles as second messengers and in the activation of normal cellular processes. In fact, there is current evidence that oxidative conditions can also influence the fate of a progenitor cell. Within the hippocampus, during the course of differentiation, subtle alterations of the redox state of neural progenitors into a more oxidized environment favors differentiation towards an astroglial lineage, whereas a more reduced environment favors neuronal differentiation (Le Belle et al. 2011, Prozorovski et al. 2015). Noticeably, this observed oxidation-mediated increase in glial cells was shown to be Sirt-1-mediated, rather than to be linked to an enhanced proliferation of astrocyte precursors or to the restriction of the multipotentiality of neural precursors (Prozorovski et al. 2008). In this case, and under oxidative conditions, Sirt-1 was shown to be upregulated in neural progenitors and bind to the transcription factor *Hes1*, which subsequently inhibited pro-neuronal *Mash1*. In addition, the shRNA-mediated knockdown of *Sirt1* in neural progenitors prevented oxidation-mediated suppression of neurogenesis, thus inducing a shift in the fate of neural progenitors (Prozorovski et al. 2008).

Noticeably, when ascorbic acid, which is a well-known low-molecular-weight antioxidant, was applied to neural precursor cells, it potentiated a neuronal fate commitment by increasing ROS metabolism in a Wnt/β-catenin/ROS signaling-dependent manner. Conversely, treatment with the ROS scavengers *N*-acetyl-*L*-cysteine (NAC) and ruthenium exerted antioxidant activities and inhibited neurogenesis (Rharass et al. 2017).

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Interestingly, the neurogenic process itself generates localized ROS (Walton et al. 2012). As a high-energy consumption process, adult neurogenesis transiently generates localized oxidative stress, thus contributing to ROS-mediated effects within the neurogenic niche (Walton et al. 2012). In accordance, an increased expression of oxidized markers was observed in the SGZ; further, *in vitro* approaches revealed that induction of NSC differentiation results in an immediate increase in overall ROS production (Walton et al. 2012). In contrast, neurogenesis ablation reduces oxidative stress within the SGZ, therefore highlighting that oxidative stress is generated in the normal course of neurogenesis (Walton et al. 2012).

Noticeably, within the cell, other numerous oxidant sources contribute to ROS generation. In addition to organelles (including mitochondria), various enzymes generate ROS as part of their enzymatic reaction cycles. Furthermore, the presence of transition metals required for the function of ROS-producing enzymes, such as iron, leads hydrogen peroxide to generate damaging hydroxyl radicals. Although not directly linked to cell metabolism and differentiation, this type of cellular signaling mechanism cannot be excluded because of its putative importance in contributing to cell physiology and, consequently, in overall brain function.

3.1 Iron contribution to reactive oxygen species production and cellular function

Iron is one of the most abundant essential elements in living organisms. Physiological processes such as DNA synthesis and cell division, as well as oxygen transport and storage, require iron as a cofactor (Mladenka et al. 2006). Additionally, H_2O_2-induced signaling effects are also dependent on iron availability (Galaris and Pantopoulos 2008). Iron, incorporated within heme or in iron-sulfur [Fe-S] clusters, is required for the action of ROS-producing enzymes, including NADPH oxidases, which are also present at the active sites of the H_2O_2-destroying enzyme catalase (Dixon and Stockwell 2014).

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However, the existence of pools of labile, redox-active iron ions in the cytosol, the mitochondrial matrix and the lysosomes (Dixon and Stockwell 2014), which are capable of directly catalyzing free radicals via the Fenton reaction, result in the formation of iron-mediated ROS that ultimately leads to cellular oxidative stress and cell death. Notably, a form of iron-dependent, oxidative cell death designated ferroptosis was recently described in mammalian cells (Dixon et al. 2012). Ferroptosis is morphologically, biochemically and genetically distinct from apoptosis, autophagy and reported forms of necrosis and is characterized by the overwhelming iron-dependent accumulation of lethal lipid ROS (Dixon et al. 2012). Several modulators have been described in the process. Glutathione peroxidase 4 is an essential regulator of ferroptotic cell death (Yang et al. 2014), functioning as a central target of ferroptosis inducers. Of relevance, ferroptosis can be prevented by lipophilic antioxidants, such as vitamin E, and by the iron chelator deferoxamine (Dixon et al. 2012), but not by apoptosis or necrosis inhibitors. Of interest, ferroptosis and lipid peroxidation processes were recently identified as metabolic checkpoints required for direct neuronal reprogramming (Gascon et al. 2016). Specifically, ferroptosis inhibitors improved the generation of inducible neurons from a range of somatic cells and *in vivo* after brain injury (Gascon et al. 2016). Together, this suggests a fine balance at the cellular and systemic levels in order to maintain iron concentration that is precisely controlled.

3.2 Iron homeostasis and neurogenesis

A fine balance exists at the cellular and systemic levels in order to meticulously control and maintain the iron concentration. While there is no biological mechanism to excrete iron from the body, the balance of iron levels is tightly controlled at the absorption level.

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At the cellular level, most mammalian cells acquire iron via receptor-mediated endocytosis of iron-loaded transferrin (Garrick and Garrick 2009).

Specifically, in the central nervous system (CNS), the brain is unique among the organs with regard to iron metabolism. The existence of the blood-brain barrier limits the entrance of plasma iron but has transport mechanisms that allow iron to move from the luminal surface of the endothelial cells into the brain (Zecca et al. 2004). Moreover, the concentration of iron in the different brain regions varies significantly. For instance, brain regions that are more associated with motor functions tend to have more iron than nonmotor-related ones, which can, at least in part, explain why movement disorders are commonly associated with iron imbalance (Zecca et al. 2004).

Specifically, oligodendrocytes are commonly enriched in iron, even though neurons and microglia also present ferritin, thus indicating that all neural cell types have the capacity to store iron (Zecca et al. 2004). This is intimately related to the importance of iron in physiological brain functions that include neural respiration, myelin synthesis, neurotransmission and synaptic plasticity (Weinreb et al. 2013).

Particularly related to the mechanisms pertained to iron signaling and NSC physiology, several reports have highlighted how iron levels are important for the proper maintenance of NSCs. For instance, the increase in SVZ NSC proliferation was described to be mediated by transferrin incorporation into cells (Silvestroff et al. 2012, Silvestroff et al. 2013), and it also controls the progression of NSCs into mature oligodendrocytes by significantly affecting NSC metabolic activity (Silvestroff et al. 2013).

Changes in NSC proliferation, differentiation and survival due to either an excess or deficiency of iron have also been described but are not necessarily limited to iron-mediated oxidative stress, as we described previously. The *Fbxl5* KO mice (a member

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of the F-box family of protein that senses iron and promotes the degradation of iron regulatory proteins) presents increased levels of iron as a result of the accumulation of iron regulatory proteins (IRPs) and unregulated iron uptake (Moroishi et al. 2011). When specifically ablated in nestin-expressing neural stem progenitor cells, FBXL5 deficiency results in the aberrant proliferation of embryonic NSCs and the consequent defects in neurogenesis due to a progressive increase in the number of neural stem progenitor cells and astrogliogenesis. These effects were shown to be the result of the activation of the PI3K-Akt-mTOR signaling since the subsequent pharmacological inhibition of mTOR signaling with rapamycin attenuated the difference in the cellularity of proliferative regions and led to the normalization of neural stem progenitor cells that were positive for nestin and Ki67 (Yamauchi et al. 2017).

On the other hand, iron deficiency during perinatal development impairs neuronal differentiation in the rat hippocampus by reducing the activity of BDNF (Tran et al. 2008). Similarly, iron deficiency during the pre- and/or postnatal period reduced hippocampal DG in rat pups, which persisted until adulthood (Ranade et al. 2013). Noticeably, these effects promoted by iron deficiency were associated with altered corticosterone levels and glucocorticoid receptor expression (Ranade et al. 2013). Ultimately, these observations provided an explanation for the molecular basis for behavioral deficits related to perinatal iron deficiency (Lozoff et al. 2006).

Other putative mechanisms, whereby neurogenesis can be potentially affected by iron homeostasis, rely on neurotransmission. Studies have shown that the serotonin transporter expression is decreased by iron deficiency (Burhans et al. 2005), which, in turn, could affect neurogenesis, since the role of serotonin in adult hippocampal neurogenesis is well-documented (Alenina and Klempin 2015). Nevertheless, no clear evidence of this association exists.

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Furthermore, a tight connection between iron deprivation and hypoxic signaling is not surprising and is known to have similar consequences at the molecular level. Under hypoxia, the expression of major iron homeostasis genes, including transferrin, is activated by HIF to provide increased iron availability as an attempt to enhance oxygen uptake and delivery to hypoxic cells (Chepelev and Willmore 2011). In particular, the depletion of iron by its chelation with deferoxamine, with the aim to stabilize HIF1 alpha in neural progenitors, mimics hypoxia states that ultimately causes cell death (Milosevic et al. 2009).

Even though it is well established that mammalian cells acquire iron via transferrin-mediated endocytosis, the fact that deletion of the transferrin pathways does not block organogenesis suggests the presence of alternative methods to deliver iron. In fact, in recent years, lipocalin-2 (LCN2) has emerged as an alternative pathway for physiological iron delivery and uptake (Yang et al. 2002). Known for its role in immune responses to limit iron availability for bacterial growth (Flo et al. 2004), LCN2 also plays a role in cell physiology (Yang et al. 2002) and in the pathophysiology of many diseases (Ferreira et al. 2015), including kidney injury (Schmidt-Ott et al. 2007) and neurodegenerative processes (Choi et al. 2011, Marques et al. 2012, Naude et al. 2013). These functions have been attributed to the ability of LCN2 to regulate physiological intracellular iron homeostasis, independently of transferrin and through its specific cellular membrane receptor 24p3R (Yang et al. 2002, Devireddy et al. 2005). However, the description of a mechanism of iron cellular content regulation, mediated by LCN2 and with an impact on NSC physiology and brain function, was only very recently described (Ferreira et al. 2018).

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4. Lipocalin-2 in neurogenesis: dawn of a new era of metabolic regulation?

Lipocalin-2 (LCN2) is an acute-phase protein that, by binding to iron-loaded siderophores, acts as a potent bacteriostatic agent in the iron-depletion strategy of the immune system to control pathogens (Flo et al. 2004). The recent identification of a mammalian endogenous siderophore to which LCN2 can bind (Bao et al. 2010, Devireddy et al. 2010), along with the existence of a specific cell surface receptor for LCN2, the 24p3R (Devireddy et al. 2005) suggests a physiological role for LCN2 in iron trafficking and homeostasis via a transferrin-independent mechanism (Yang et al. 2002). Irrespectively of the cell type, the modulation of iron cellular content by LCN2 is largely dependent on the state of the ligand: iron-containing (holo-) and iron-free (apo-) LCN2 (Devireddy et al. 2005). The internalization of holo-LCN2 allows for the release of iron from the complex and an increase in the intracellular iron concentration (Devireddy et al. 2005). Contrastingly, apo-LCN2 internalization chelates intracellular iron and transfers it to the extracellular medium, reducing its intracellular levels (Devireddy et al. 2005). Importantly, this modulation of cell iron content was shown to impact cell proliferation and apoptosis (Devireddy et al. 2005) and was described to be relevant in kidney development (Yang et al. 2002) during acute anemia (Miharada et al. 2005), in iron delivery to spermatozoa (Elangovan et al. 2004), and in the attenuation of iron-related oxidative stress (Yamada et al. 2016).

In the CNS, less is known about the processes involving LCN2, namely, by which cells it is produced/secreted, and its impact on overall brain functioning [see (Ferreira et al. 2015)]. Nevertheless, in this context, the existence of a fine-tuned mediation of intracellular iron by LCN2 was shown to be important in the regulation of hippocampal neuronal dendritic spine density and morphology, with a further impact on structural plasticity and function (Mucha et al. 2011). The observed effects are assumed to be

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dependent on 24p3R-mediated endocytosis, since the LCN2 receptor is specifically expressed by neuronal cells (Ip et al. 2011) and, in cultured hippocampal neurons, was shown to bind and internalize LCN2 when both apo- and holo-forms are applied (Chia et al. 2015). The importance of these LCN2-mediated mechanisms in neural plasticity was observed when *Lcn2* deletion translated into increased anxiety, depressive-like behavior, spatial reference memory impairments and decreased long-term potentiation (Ferreira et al. 2013). Additionally, LCN2 has also been attributed to important roles in the context of neurodegeneration, including multiple sclerosis (Marques et al. 2012), Alzheimer's disease (Mesquita et al. 2014) and Parkinson's disease (Kim et al. 2016).

In this same line of evidence, and relying on its known roles as an iron-transport protein, it was recently shown that adult neurogenesis is markedly declined in mice lacking the expression of LCN2 (Ferreira et al. 2018).

Shown to be highly present in the serum (Ferreira et al. 2018), but not in the physiological brain, the regulation of adult neurogenesis by LCN2 is suggested to occur extrinsically to the brain, rather than as an intrinsic CNS-derived cue. Once produced at the periphery, e.g., upon synthesis at the periphery by neutrophils (Kjeldsen et al. 1993), osteoblasts (Mosialou et al. 2017) or adipose tissue (Mosialou et al. 2017), and hypothetically delivered to the brain by the endothelial cells of the blood vessels that surround the neurogenic niches and the neural progenitors (Figure 3a), LCN2 controls adult neurogenesis by selectively binding to 24p3R-expressing NSCs (Sox2- and Nestin-positive cells) for the control of intracellular iron (Ferreira et al. 2018). In the absence of LCN2, an increase in the numbers of NSCs was observed and was attributed to an impaired cell cycle transition from type-1 quiescent cells to type-2 proliferative cells (Ferreira et al. 2018). This compromised maintenance of stemness and cell proliferation

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was due to an iron-mediated aberrant ROS formation [(Ferreira et al. 2018); Figure 3b]. Due to a lack of iron efflux in NSCs as result of LCN2 absence, together with impaired antioxidant regulation, the accumulation of iron drives an iron-dependent increase in ROS levels that induce cell-cycle arrest and, ultimately, cell death (Ferreira et al. 2018). While some suggest that increased ROS levels promote NSC proliferation and differentiation, which we also reviewed here (Figure 2), others suggested that increased ROS levels in NSCs can, in fact, result in cell death (Chuikov et al. 2010), similar to that which occurs in the LCN2-null mice model (Ferreira et al. 2018). It is, therefore, plausible to assume that ROS levels in the brain of LCN2-null mice are within the range of those that promote cell cycle arrest and death. Moreover, the absence of an efficient defense mechanism against oxidative stress in LCN2-null NSCs was evident when treatment with the well-known antioxidant NAC was sufficient to overcome some of the observed impairments.

Although it is clear that the absence of LCN2 likely induces an increase in the levels of iron, which in turn result in oxidative stress, and ultimately cell death, the precise levels of iron in the brains of LCN2-null mice still need to be clarified. Furthermore, how exactly such levels translate the impact on cell proliferation is not understood, since in other models of iron overload (Yamauchi et al. 2017), the unregulated iron uptake results in the aberrant proliferation of embryonic NSCs and defective neurogenesis (Yamauchi et al. 2017).

In addition, the precise signaling pathways underlying the control of iron-mediated oxidative stress by LCN2 still need to be clarified. For instance, a hypothesis can be drawn on the possibility that ROS accumulation can induce cell death through the induction of proapoptotic proteins and its translocation into the mitochondria (Figure 3b). On the other hand, accumulative intracellular ROS could induce a sustained

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expression of inflammatory mediators (e.g., iNOS, TGFβ1), which are known to contribute to cell death (Figure 3b). Furthermore, we can consider that, being LCN2 an immune mediator, the absence of LCN2 can directly induce alterations in the inflammatory status of the NSCs *per se*, which may have a direct impact on cell survival. In addition, an assumed inexistence of the nuclear factor erythroid 2-related factor 2 (Nrf2) system (a transcription regulator of the expression of antioxidant proteins that protect against oxidative damage) in LCN2-null NSCs could also explain the lack of an appropriate antioxidant transcription that, in turn, affects overall neurogenesis (Figure 3b).

4.1 Lipocalin-2 in neurogenesis: beyond iron

Certainly, the potential role of the cells at the periphery that produce LCN2 in the regulation of brain cell genesis warrants further investigation. Nevertheless, the demonstration of this type of regulation by LCN2 also turned out to be a good example of how the regulation of adult neurogenesis can occur extrinsically to the brain. Whether this type of regulation by LCN2 can also affect mitochondria biogenesis, energy production or even other types of NSC metabolism is still unknown.

In addition to acting on the intrinsic functions of NSCs, LCN2 may also contribute to regulating the neurogenic niches environment. For instance, increased levels of adrenal corticosteroids have been widely described to inhibit neurogenic activity (Cameron and Gould 1994). Of notice, LCN2-null mice were described to present a sustained hyperactivation of the hypothalamic-pituitary-adrenal axis that resulted in the increased production of corticosterone (Ferreira et al. 2013). The absence of LCN2 could, in turn, negatively modify the neurogenic niche environment through corticosteroids, thus affecting the neurogenesis process.

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Moreover, the regulation of adult neurogenesis by LCN2 could also be attributed to its described roles in cell metabolism. In fact, the concept that LCN2 can regulate cell metabolism is not new, and several studies have already demonstrated LCN2 as a critical regulator of energy metabolism in glucose and lipid homeostasis (Asimakopoulou et al. 2014) and even in insulin resistance (Kamble et al. 2016, Mosialou et al. 2017). As a multifaceted protein, the role of LCN2 in cell physiology can also go beyond iron homeostasis. Known to be broadly expressed in several tissues and in diverse conditions with a modulatory induction by specific factors, LCN2 has also been described to regulate cell metabolism through the control of inflammatory responses. Its production in adipose tissue and in the liver in response to metabolic stress and to cytokines points to the involvement of LCN2 in adipocyte metabolism and in inflammation (Zhang et al. 2014).

Noticeably, also in the brain, LCN2 has been extensively described to be expressed in response to injury and inflammation, so it can engage in critical roles in the progression and establishment of neuroinflammation [as reviewed in (Ferreira et al. 2015)]. In particular, reports have shown LCN2 secretion by astrocytes to target and modulate microglia activation and polarization into the M1 phenotype and to target astrocytes in an autocrine fashion manner for pro-inflammatory activation [as reviewed in (Ferreira et al. 2015)]. Interestingly, astroglial cells can sharply affect neurogenesis by regulating progenitor proliferation and differentiation and the survival of new adult-born neurons through the release of inflammatory cytokines (Belarbi and Rosi 2013). Noticeably, LCN2 could also enter through this route and, in the case of neuroinflammation, have an impact on neurogenesis by regulating the brain inflammatory status.

Furthermore, given that LCN2 is highly expressed during both intestinal and metabolic inflammation observed in obesity (Moschen et al. 2017), some authors also proposed

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LCN2 as an adipokine (Yan et al. 2007, Yoo et al. 2014), with the potential to be used as a biomarker in inflammatory and metabolic diseases (Yan et al. 2007). Noticeably, LCN2 was also recently identified as a bone-derived hormone with the capacity to regulate metabolism in the brain (Mosialou et al. 2017). By specifically targeting the melanocortin-4 receptor (MC4R) in the hypothalamus, LCN2 can suppress appetite (Mosialou et al. 2017). In addition, LCN2 also regulates insulin secretion and increases insulin sensitivity and glucose tolerance, as well as plays a direct functional role in hepatic lipid metabolism by regulating the expression of lipid droplet proteins (Asimakopoulou et al. 2014). Furthermore, LCN2 was also found to directly bind membrane phosphatidylethanolamine to reinforce lipid raft reorganization via the protein kinase A-dependent mechanism and to promote sperm fertility by facilitating cholesterol efflux (Watanabe et al. 2014).

Altogether, this highlights the pleiotropism associated with the impact of LCN2 on metabolism, which suggests that the regulation of adult neurogenesis could occur beyond its capacity to traffic iron within the cells in a cellular non-autonomous manner.

The regulation of neurogenesis by LCN2 was revealed to be crucial for proper brain functioning during young adulthood. The absence of LCN2, in addition to promoting impairments in hippocampal long-term potentiation (Ferreira et al. 2013), promotes anxious and depressive-like behaviors in mice, as well as cognitive impairment in spatial learning tasks (Ferreira et al. 2013) and in contextual discrimination (Figure 3c) (Ferreira et al. 2018). These processes can, at least in part, be attributed to LCN2 capacity to control iron-mediated processes for cell genesis, ultimately regulating the hippocampal function, which became evident when the treatment of LCN2-null mice with the iron chelator deferoxamine rescued cell differentiation and contextual discrimination

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(Ferreira et al. 2018). Ultimately, the identification of these events and their regulation by LCN2 certainly increased our knowledge of the etiology of the processes that underlie brain physiological function and are even more important when considering the number of reports that, in recent years, demonstrated the nature of secreted LCN2 in neurodegenerative disorders (Choi et al. 2011, Marques et al. 2012, Kim et al. 2016).

As the brain ages, iron is known to accumulate in regions that are typically affected by Alzheimer's and Parkinson's diseases (Zecca et al. 2004). Consequently, high concentrations of reactive iron can contribute to increased oxidative stress (Dixon and Stockwell 2014), thus inducing neuronal vulnerability that can translate into neuronal dysfunction. Therefore, knowledge of how to modulate LCN2, for instance, the control of iron homeostasis and oxidative stress, at younger stages could help in the prevention of such phenomena later in life.

From a clinical point of view, LCN2 could be therapeutically targeted to prevent iron-mediated oxidative stress, envisaging improvements in cognition or cell genesis. Although up until now no specific modulator for LCN2 has been identified, certainly the identification of one that is based on iron chelation, antioxidant strategies and/or inflammatory inhibition would prove to be effective.

5. Concluding remarks

The targeting of metabolic pathways may hold a potential for novel therapeutic approaches to treat diseases that are associated with declining adult neurogenesis, such as depression or age-related cognitive decline. On the other hand, the identification of novel signaling mechanisms governing adult neurogenesis is pivotal to ensure life-long neurogenesis in the mammalian brain. Even though some breakthroughs have been achieved in recent years, future studies are still needed to further dissect and characterize

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the precise underlying cellular mechanisms, for instance, iron-mediated oxidation in NSCs, as well as during which conditions it is required for proper proliferation.

The results showing that adult NSCs require controlled levels of iron trafficking for proper neurogenesis reinforces the view that oxidation levels need to be maintained at controlled levels for NSC self-renewal and proliferation. Whether iron-mediated oxidative stress can be considered a novel metabolic checkpoint in the neurogenic process is still an open question. Nevertheless, LCN2 seems to be a crucial a molecular bridge in iron-dependent oxidation, potentially by integrating other signals, to ensure NSC self-renewal and proliferation. Of importance are also the signaling cascades at the periphery, which can overlap and cooperate within neurogenic niches.

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Figure 1: Neurogenic niches of the adult mammalian brain. Two niches are consensually accepted to exist in the adult mammalian brain: the hippocampal dentate gyrus (DG) and the subventricular zone (SVZ) - olfactory bulb (OB) system. Within the hippocampal DG, resident neural stem cells (type-1 radial glia-like cells) in the subgranular zone (SGZ) give rise to new progenitor cells (type-2 and type-3 cells) that migrate towards the granular cell layer (GCL), where they fully differentiate into mature granule neurons. At the lateral ventricles, type-B1 radial glia-like stem cells give rise to fast proliferating type-C cells that, in turn, originate type-A cells (neuroblasts). These cells then migrate through the RMS towards the OB, where they differentiate into granule neurons and integrate into the local circuitries.

BBB, blood-brain barrier; BV, blood vessel; DG, dentate gyrus; EPL, external plexiform layer; GCL, granule cell layer; GL, glomerular layer; LV, lateral ventricle; ML, molecular layer; OB, olfactory bulb; RMS, rostral migratory stream; SGZ, subgranular zone; SVZ, subventricular zone.

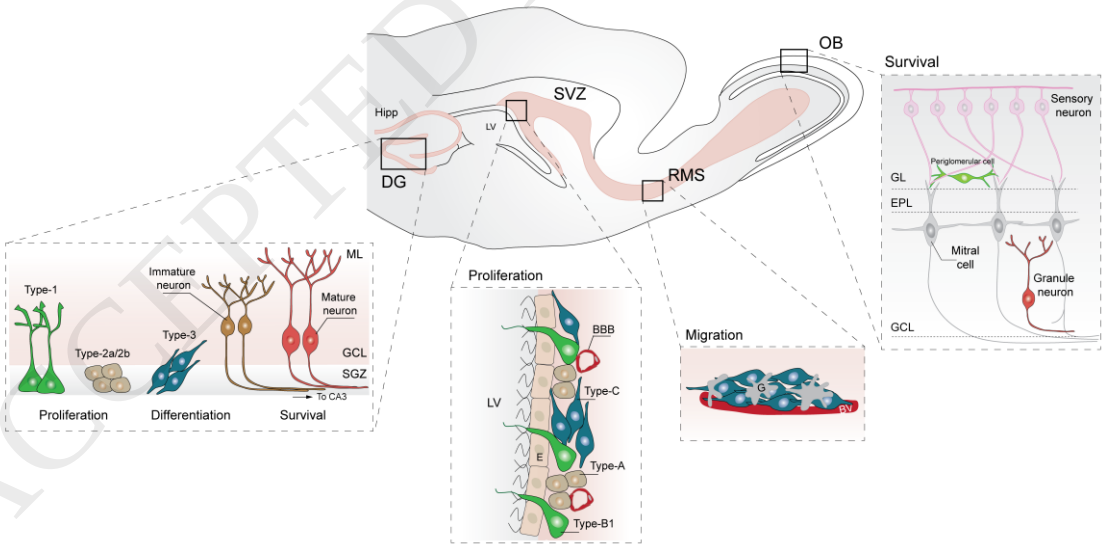

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Figure 2: Representative scheme of metabolic regulation in NSCs and their neuronal progeny. Quiescence of NSCs relies on their glycolytic status, while activated NSCs require *de novo* lipogenesis for proper proliferation and integration. A more oxidized environment regulates neuronal differentiation and maturation. OXPHOS, oxidative phosphorylation; ROS, reactive oxygen species.

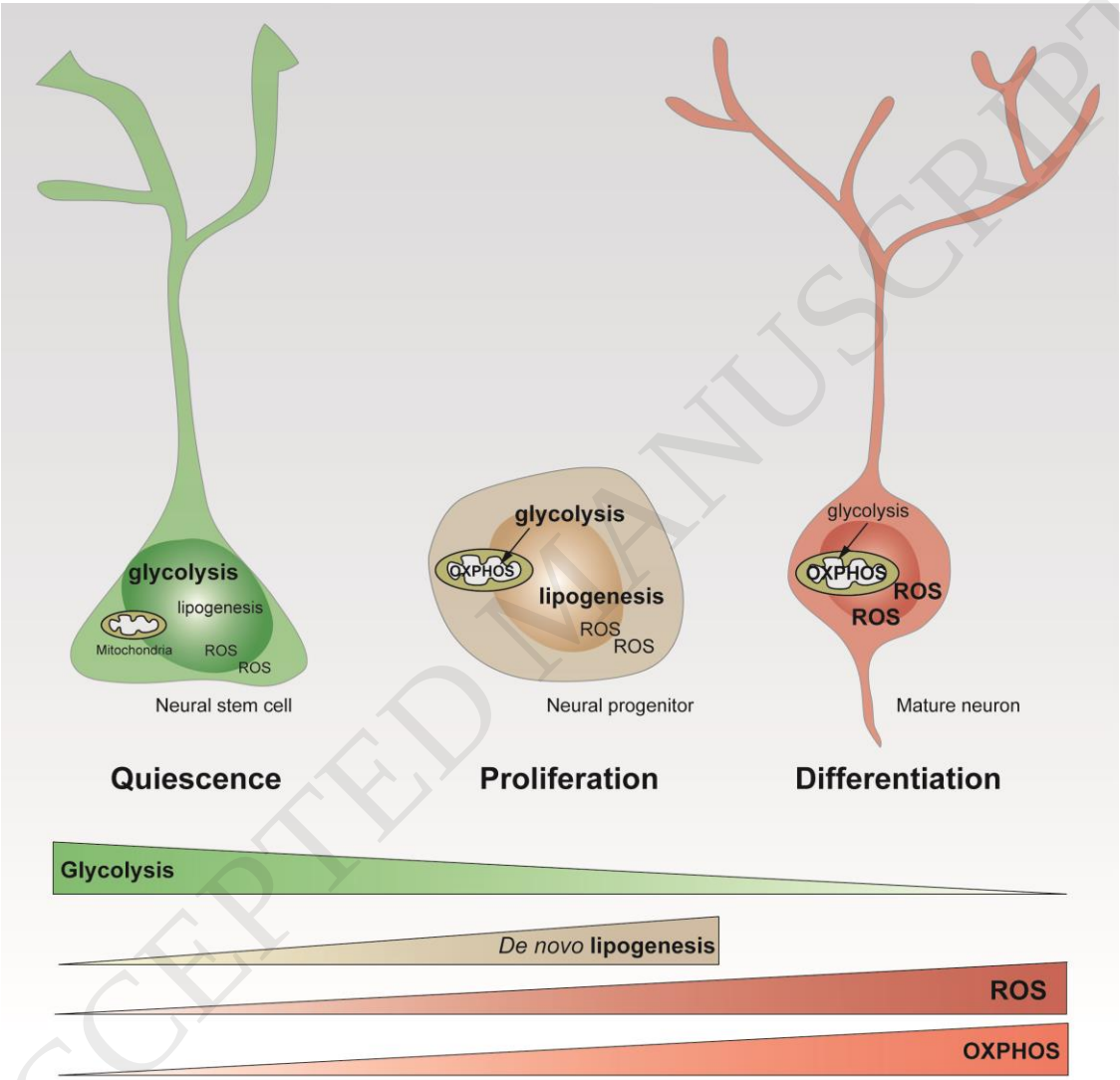

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Figure 3: Putative mechanism of LCN2-mediated control of hippocampal neurogenesis. Lipocalin-2 produced at the periphery circulates in the bloodstream and impacts the hippocampal neurogenic niche to regulate NSC proliferation and self-renewal. (**a**) Possibly delivered by the endothelial cells of the blood vessels that surround the neurogenic niche, LCN2 binds to 24p3R-expressing NSCs to regulate intracellular iron. (**b**) When LCN2 is absent, an intracellular iron accumulation in NSCs occurs, thus leading to iron-related oxidative stress that imposes cell death, impaired neurogenesis and (**c**) deficient contextual discriminative behaviors. Putative signaling mechanisms underlying the effects observed in NSCs of LCN2-null mice are also depicted.

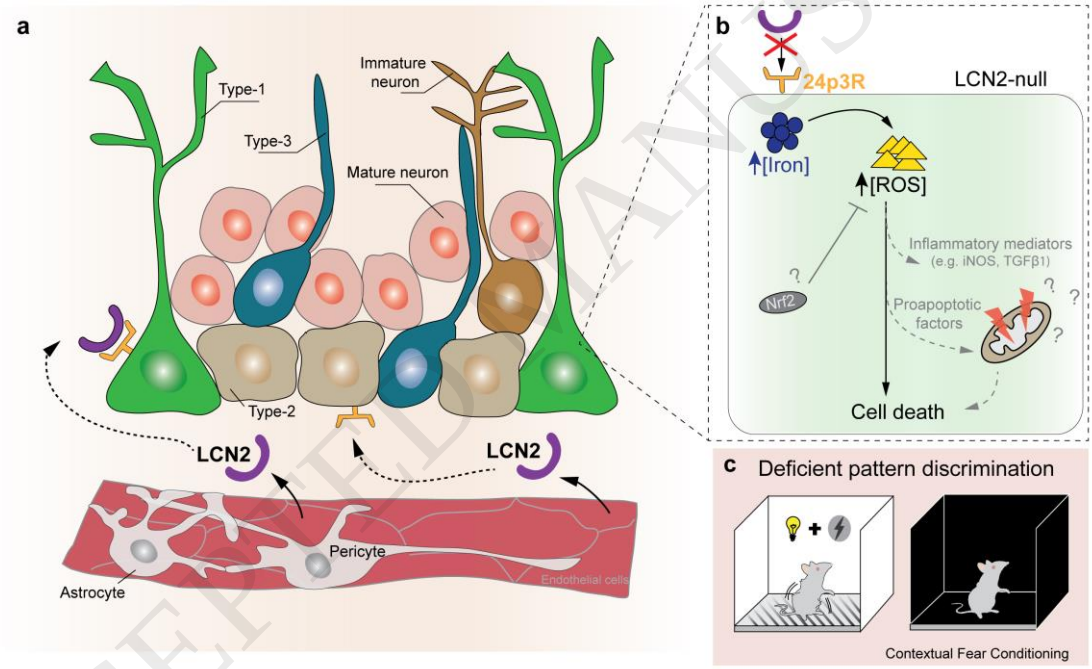

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Table 1: Regulation of adult neurogenesis in the subgranular zone (SGZ) and subventricular zone (SVZ)

Regulators	Proliferation		Survival		Neuronal differentiation		Putative mechanism	References
	SVZ	SGZ	SVZ	SGZ	SVZ	SGZ		
Transcription factors								
Sox2	+	+					Shh signaling	(Favaro et al. 2009)
Pax6		+			+			(Maekawa et al. 2005)
Tbr2		+			+	+		(Hodge et al. 2012)
Prox1						+	Wnt signaling	(Lavado et al. 2010)
NeuroD1			+	+	+	+		(Gao et al. 2009)
Signaling molecules								
BMP		+						(Bond et al. 2014)
Shh	+	+						(Lai et al. 2003)
Wnt	+				+	+		(Lie et al. 2005)
Notch	+	+			-			(Ables et al. 2010)
Neurotrophic/growth factors								
FGF-2	+	+						(Kang and Hebert 2015)
EGF	+	=						(Kuhn et al. 1997)
IGF	+	+		+		+		(Aberg et al. 2000, Hsieh et al. 2004)
VEGF		+						(Jin et al. 2002)
BDNF		+	+	+	+	+		(Choi et al. 2009)
Neurotransmitters								
Dopamine	-	-			-			(Hoglinger et al. 2004)
Serotonin	+	+			+			(Brezun and Daszuta 1999)
Glutamate		-		=			mGluR, NMDAR	(Tashiro et al. 2006)
GABA	-				-			(Tozuka et al. 2005)
Hormones								
Corticosterone		-						(Cameron and Gould 1994)
Estrogen		+		=		=		(Tanapat et al. 1999)
Drugs								
Antidepressant		+					BDNF	(Malberg et al. 2000)
Lithium		+					Wnt signaling	(Wexler et al. 2008)
Behavior								
Enrichment	=	+	=	+	=	+	VEGF	(Kempermann et al. 1997)
Running	=	+	=	+			VEGF	(van Praag et al. 1999)
Learning		=		+		=		(Dobrossy et al. 2003)
Pathology								
Ischemia	+	-		+	+	+	NMDAR	(Liu et al. 1998)
Inflammation	-	-		-		-	IL-6, TNF-α	(Ekdahl et al. 2003)
AD, PD, HD	+	+						(Jin et al. 2004)
Stress		-		=			Glucocorticoids	(Gould et al. 1998)
Aging	-	-		=		-	Corticosteroids	(Kuhn et al. 1996)
Diabetes		-						(Stranahan et al. 2008)

The table is based on results from key publications on adult neurogenesis and provides a general overview of the diverse regulatory factors of adult neurogenesis. "+": increase; "–" decrease; "=": no change; unmarked indicates "not examined/unknown." Abbreviations: AD, Alzheimer's disease; BDNF, brain-derived neurotrophic factor; BMP, bone morphogenetic protein; EGF, epidermal growth factor; FGF-2, fibroblast growth factor; GABA, γ-aminobutyric acid; HD, Huntington's disease; IGF, insulin growth factor; IL,

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interleukin; NeuroD1, neuronal differentiation 1; NMDAR, N-methyl-D-aspartate receptor; Pax6, paired box protein-6; PD, Parkinson's disease; Prox1, prospero homeobox-1; Shh, Sonic hedgehog; Tbr2, T-box brain protein 2; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

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Table 2: The role of the energy-sensing molecular mechanism in neural stem cell maintenance

Signaling pathway	Effect on neural stem cells and neurogenesis		Mechanism of action	Reference
Insulin/ IGF-1	Induces hippocampal NSCs proliferation *in vivo* and *in vitro*		Dependent on EGF and FGF presence	(Aberg et al. 2000, Reynolds and Rietze 2005)
	Promotes NSCs exit from quiescence		-	(Renault et al. 2009)
	IGF-1 ablation	Delays age-related decline of olfactory bulb neurogenesis	-	(Chaker et al. 2015)
		Enhances hypothalamic α-tanycyte self-renewal	-	(Chaker et al. 2016)
FoxO	Promotes cellular quiescence, oxidative stress resistance, inhibition of premature oligodendrocyte differentiation		Transcriptional regulation of cell cycle genes (e.g., *Cyclin D1)*	(Renault et al. 2009, Rafalski and Brunet 2011)
	FoxO3 acts to maintain NSC quiescence and prevent premature exhaustion			
mTOR	Activated in response to insulin to promote neuronal differentiation		-	(Hay and Sonenberg 2004, Han et al. 2008)
AMPK	Regulation of proliferation and survival of embryonic NSCs		mTOR, p53 and FoxO3 expression	(Dasgupta and Milbrandt 2009)
Sirtuins	Sirt-1 promotes embryonic NSC differentiation to either a neurogenic or a glial fate, in response to oxidative stress		FoxO transcription factors; Wnt signaling pathway	(Hisahara et al. 2008, Prozorovski et al. 2008)
	Sirt-1 mediates the NSC response to glucose availability		CREB transcription factor	(Fusco et al. 2016)

Abbreviations: CREB, cAMP response element binding protein; EGF, epidermal growth factor; FGF, fibroblast growth factor; FoxO, forkhead Box O; IGF-1, insulin growth factor-1; NSCs, neural stem cells; Sirt-1, sirtuin-1.

ACCEPTED MANUSCRIPT