Metabolism and neurogenesis
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The generation of neurons in the developing and adult mammalian brain by neural stem/progenitor cells (NSPCs) depends on a tight control of NSPC activity and neuronal differentiation that is regulated by a plethora of intrinsic and extrinsic molecular cues. Besides well-studied morphogenic signaling pathways and transcriptional codes that govern the distinct developmental steps from the dividing NSPC to a functional neuron, a critical role of cellular metabolism to determine the functional properties of NSPCs and newborn neurons has been recently identified. Here, we review advances in our understanding of how metabolism affects NSPC behavior and subsequent neuronal differentiation and suggest how metabolism may serve as a common signal integrator to ensure life-long addition of new neurons in the mammalian brain.

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Introduction
The brain is the most complex organ in mammals. The numbers of neural cells, their positioning within brain areas, the subtype specification of neurons, and the connectivity of individual neurons and subregions need to be tightly controlled to ensure the proper functioning of neural circuits [1,2]. During embryonic development dividing neural stem/progenitor cells (NSPCs) generate the vast majority of neurons that will populate the adult mammalian brain. The formation of the central nervous system has been extensively studied and key cellular and molecular principles have been identified that regulate the expansion of NSPCs, the induction of the generation of neurons, and subtype specification of neuronal as well as glial cells [1–3]. Notably, the process of generating neurons, called neurogenesis, does not stop with the end of embryonic and early postnatal development but continues throughout life in distinct regions, such as the hippocampal dentate gyrus (DG) and the subventricular zone (SVZ) [4]. Thus, identifying the mechanisms governing NSPC behavior is not only needed to understand the formation of the brain but is also required to understand the principles of neurogenesis that occurs throughout life in the mammalian brain. Given the complexity of the end product, it is not a surprise that each step during embryonic and adult neurogenesis is regulated by a variety of intrinsic mechanisms and cell-extrinsic cues, for example, regulated through defined transcriptional codes and key morphogenic signaling pathways [1,5,6].

Until recently a core component of each cell, its metabolism, has been largely neglected for the role it may play during neurogenesis. However, it seems quite obvious that cellular metabolism determining for example the cell’s energy status will be linked to NSPC activity and neuronal differentiation processes, as cell division and differentiation are associated with an increase in cell volume and biomass production and require substantial amounts of energy for DNA replication and organelle synthesis [7**]. Indeed, extensive analyses of the transcriptomes of distinct NSPC stages as well as transgenesis-based gain-of-function and loss-of-function studies indicated that distinct metabolic states play a critical role to govern developmental steps in the course of embryonic and adult neurogenesis (e.g., [6,8–11,12**,13*]). Here, we will concisely review recent evidence of how cellular metabolism affects NSPC activity and subsequent neuronal differentiation. Further, we will discuss how metabolism may serve as a molecular hub to integrate a variety of signaling pathways regulating neurogenesis during embryogenesis and in the adult mammalian brain.

Metabolic control of NSPC activity
To ease understanding, a simplified scheme of the major cellular metabolic pathways is shown in Figure 1.

Lipid metabolism and NSPC activity
Distinct lipid metabolic pathways have been known for many years as the ‘lipogenic phenotype’ in cancer, providing proliferation and survival advantages [14]. Interestingly, similar lipid metabolic pathways are important for adult neurogenesis [12**]. Proliferating adult NSPCs upregulate the production of lipids through fatty acid synthase (FASN)-dependent de novo lipogenesis, and pharmacological or genetic manipulation of this pathway is associated with a drastic reduction in proliferation and neurogenesis, suggesting a crucial role for newly formed lipids in NSPCs [12**]. Further, de novo lipogenesis in
A simplified scheme of the major cellular metabolic pathways. Glucose is taken up and metabolized into pyruvate in a process called glycolysis, with a relatively small amount of energy equivalents, adenosine triphosphate (ATP) and reduced nicotinamide adenine dinucleotide (NADH), generated. Pyruvate can be either fermented into lactate, which is subsequently secreted, or can be shuttled into the mitochondria and used in the tricarboxylic acid (TCA) cycle to generate NADH and reduced flavin adenine dinucleotide (FADH) for energy production. In the mitochondrial respiratory chain, the NADH and FADH generated during the TCA cycle are used in a complex process called oxidative phosphorylation (OXPHOS), requiring oxygen (O₂) and resulting in the generation of energy in the form of ATP. As a side product, reactive oxygen species (ROS) can be generated during OXPHOS. NADH and FADH are also generated in large amounts by the breakdown of fatty acids in a process termed fatty acid oxidation (FAO), occurring in mitochondria as well as in peroxisomes (not shown in the scheme). The resulting acetyl-CoA can be fuelled into the TCA cycle for further energy production and as a carbon source or can be exported from mitochondria via citrate for other use. For instance, acetyl-CoA is one of the building blocks for the generation of new lipids (lipogenesis) in a process involving fatty acid synthase (FASN), yielding palmitate, which can be subsequently used to generate more complex fatty acids. The reduced nicotinamide adenine dinucleotide phosphate (NADPH) required for lipogenesis can be generated during the pentose phosphate pathway (PPP), a metabolic pathway parallel to glycolysis.

NSPCs is upregulated with running, a robust enhancer of adult NSPC proliferation, showing a direct influence of a pro-neurogenic stimulus on lipid metabolism in NSPCs [15]. The chronic pharmacological inhibition of FASN abolished the beneficial effects of exercise such as increased proliferation and cognitive enhancement [15], supporting the importance of lipid metabolism to control NSPC activity.

The amount of de novo lipogenesis also influences quiescence behavior as the production of new lipids is reduced in quiescent adult NSPCs through the action of a specifically expressed protein called Spot14 regulating the levels of Malonyl-CoA, one of the substrates for FASN [12**]. The dynamic response of Spot14-positive NSPCs to pro-neurogenic and anti-neurogenic stimuli further suggest that NSPCs can alter their lipid metabolism upon...
extrinsic signals [16], in line with increased FASN activity upon running [15]. However, how exactly these extrinsic signals translate into a metabolic change remains to be elucidated. Though it is not yet clear why proliferating adult NSPCs are so dependent on newly produced lipids, it is likely that a large amount is used for new membrane production required upon proliferation and differentiation, as suggested by radioactive tracing experiments [12*].

Interestingly, it has recently been suggested that NSPCs might not only use lipids as building blocks for membranes but also as an alternative energy source to glucose. Increased fatty acid oxidation (FAO), the breakdown of lipids, was found to be high in adult NSPCs in the SVZ and pharmacological inhibition of FAO resulted in reduced proliferation [17]. Furthermore, a recent report linked the clinical association of FAO deficits with neuropsychiatric diseases to a dysregulation of NSPC activity during development [18]. Inhibition of FAO resulted in a reduced NSPC pool, which was due to increased differentiation and reduced self-renewal of NSPCs, suggesting that FAO is indeed crucial for the maintenance of NSPCs [18]. How exactly FAO functions to maintain NSPCs remains to be determined.

Lipid metabolism also seems to be important for the interaction between the niche and NSPCs: two recent papers showed that the accumulation of lipids in so-called lipid droplets in niche cells might directly influence NSPC behavior [19,20]. In Drosophila, lipid droplets in niche cells protect both glia and neuroblasts by providing a ‘safe’ storage for polyunsaturated fatty acids during oxidative stress and thus limit damage inflicted by peroxidation reactions [19]. Whether similar mechanisms exist in the mammalian neurogenic niches is not known. However, an excess of lipid droplets in the ependymal cells lining the ventricular zone, part of the niche in the mammalian SVZ, have been recently associated with Alzheimer disease pathology [20]. In an Alzheimer mouse model, this lipid accumulation leads to decreased NSPC proliferation, which could be mimicked in wildtype mice by a local increase and subsequent accumulation of lipids, suggesting that perturbed lipid metabolism in disease might be directly influencing NSPC behavior [20]. Taken together, there is now strong evidence pointing towards an important regulatory role of lipid metabolism in NSPCs that might open new avenues for manipulating NSPC behavior.

Mitochondrial activity and NSPC behavior

Another emerging view on metabolic changes between stem cells and progeny suggests that mitochondrial mass/activity and oxidative phosphorylation increase with lineage progression whereas glycolytic activity is rather a stem cell feature [21]. This also seems to hold true for NSPCs and their differentiating neuronal progeny (see below), as recent publications suggest [22]. Low levels of oxygen, that is, hypoxia, has been known for a long time to promote NSPC maintenance and proliferation both in vitro and in vivo; however, the underlying mechanisms have remained poorly understood [23]. Two recent studies have now addressed how hypoxia influences NSPC metabolism. The first study addressed the reliance of embryonic and adult NSPCs on oxidative versus glycolytic metabolism, using pharmacological inhibition and alteration of substrate availability [24]. Indeed, NSPCs were highly tolerant to hypoxia, but inhibition of glycolytic pathways, even when another oxidizable substrate was provided, greatly impaired their survival [24]. Supporting a role of glycolytic versus oxidative metabolism for NSPC behavior, an elegant study connected the ingrowth of blood vessels into the developing cortex and the resulting increase in oxygen availability with the well-known switch of NSPC expansion to differentiation during brain development [25*]. Using a genetic perturbation of vessel formation, the authors showed that hypoxia due to the absence of ingrowing blood vessels caused the NSPC pool to expand whereas neurogenesis was dramatically reduced. Hyperoxygenation of the pregnant mothers restored tissue oxygen in the embryos despite the lack of blood vessels and rescued the phenotype. Hif1 was highly upregulated and functional studies using genetic knockout and overexpression approaches showed its regulatory role in this system, mainly by upregulating glycolysis [25*].

Although mitochondrial metabolism seems to be lower in NSPCs than in their neuronal progeny, mitochondria still contribute to NSPC activity regulation, for instance through FAO as outlined above. A certain level of oxidative metabolism might even be necessary to prevent oncologic transformation of NSPCs, as has been recently suggested: inhibition of mitochondrial metabolism in NSPCs led to a switch towards more glycolysis with higher proliferation and less inducible differentiation [26]. Genetic impairment of mitochondrial function increased tumor-forming capacity of NSPCs when transplanted into the brain of recipient mice, suggesting that a tight metabolic control might be crucial to prevent uncontrollable growth [26]. A reduction in mitochondria has also been linked to age related changes in NSPCs, accompanied by general alterations in metabolism [27]; however, the detailed mechanisms and consequences of lowered mitochondrial content require further studies, including in vivo analyses. Interestingly, a recent study showed that repletion of oxidized nicotinamide adenine dinucleotide (NAD+) appears to be efficient to improve mitochondrial function in aged somatic stem cells and extend lifespan [28].

Reactive oxygen species (ROS) production and defense affect NSPC activity

The generation of ROS is closely linked to mitochondrial metabolism. It is widely known that ROS can be toxic
side products of the electron transfer chain, causing damage to macromolecules such as proteins, lipids and DNA. Thus, antioxidant mechanisms antagonizing the negative effects of ROS are important for cellular health. Defects in these defense mechanisms have been associated with aging and disease, whereas successful reduction of ROS, for instance through caloric restriction, has been put forward as one underlying cause for an increase in longevity [29]. It is becoming evident, however, that a certain amount of ROS is important for signaling and may be involved in regulating stem cell behavior [30–33]. Thus, understanding the regulation of ROS and the maintenance of the cellular redox potential is also relevant for the field of stem cell biology where direct or indirect roles for ROS regulating NSPC activity have been described [30,34*]. Several key metabolic and lifespan regulators such as forking Box O (FoxO) transcription factors, sirtuins, as well as mammalian target of rapamycin (mTOR), have been linked to ROS production, ROS defense and redox potential and have been recently shown to be important for NSPCs. Ablation of FoxOs results in an initial increase in brain size followed by reduced neurogenesis and a reduction of the NSPC pool, suggesting an important role of FoxOs in controlling self-renewal [31,32]. ROS levels were increased upon FoxO ablation and gene expression signatures of FoxO knockout versus wildtype NSPCs point towards regulation of oxygen and glucose metabolism [31,32]. Indeed, a recent study focused on the metabolic alterations upon FoxO ablation and identified glycolysis and glutamine metabolism to be downregulated [35]. This downregulation resulted in increased oxidative stress and compromised the proliferative potential of NSPCs, most likely because both downregulated metabolic pathways normally contribute to the anti-oxidant defense program of a cell [35]. Although these studies show the importance of FoxOs for NSPCs and link it to metabolic changes, further studies are needed to better understand these effects.

Similarly, sirtuins have been shown to be critical for neurogenesis. Sirtuins are protein deacetylases with manifold actions and implications in various processes such as life span, inflammation and cancer [36]. Their deacetylating activity on histones has direct transcriptional consequences and their dependence on NAD+ as a co-enzyme makes them perfect sensors of the redox state of a cell. Thus far, most of the publications studying sirtuins in the context of neurogenesis have shown effects on differentiation (see below). However, two recent publications have provided evidence that sirtuins might also regulate NSPC self-renewal and proliferation [37,38]. Extracellular glucose levels regulated NSPC proliferation via a coordinated mechanism of Hes-1 expression, repressed by sirtuin 1 and activated by CREB, directly linking nutrient availability to NSPC behavior [38]. Remarkably, and despite the fact that many laboratories traditionally use high glucose medium to culture NSPCs, low glucose had beneficial effects on the self-renewal of NSPCs [38], indicating that the classic culture conditions may need to be adapted with increasing knowledge of metabolic requirements of NSPCs.

Nutrient availability also activates mTOR signaling [39]. Both inhibition and hyperactivation of mTOR have recently been shown to affect NSPC quiescence, proliferation and differentiation, suggesting a tight balance of optimal mTOR activity [40–42]. Interestingly, mTOR activity diminishes with age in NSPCs and might be relevant for the age-associated decline in neurogenesis, supported by the finding that mTOR inhibition in NSPCs in vitro resulted in a reversible quiescence-like phenotype [41]. Indeed, it was shown that stimulation of mTOR signaling in aged mice increases NSPC proliferation [43]. In summary there is clear evidence that metabolism indeed regulates NSPC activity; however, detailed and comprehensive metabolic analyses will be required to fully understand how the different key players affect the metabolic state of NSPCs.

**Metabolic mechanisms regulating neuronal differentiation and maturation**

Many metabolic pathways that alter NSPC behavior ultimately also affect the generation of newborn neurons. Thus, it is difficult to identify specific pathways that only regulate neuronal differentiation and maturation but do not affect NSPCs per se. As outlined above, an increase in mitochondrial metabolism is emerging as a key feature associated with stem cell differentiation [21,44]. In an elegant study in Drosophila, a steroid-hormone-mediated metabolic switch from glycolysis to oxidative phosphorylation was shown to trigger cell cycle exit and terminal differentiation of NSPCs during metamorphosis [45**]. Inhibition of oxidative phosphorylation by genetically targeting various components of the mitochondrial electron transport chain prevented this differentiation and extended the life span of the neuroblasts [45**], suggesting a direct regulation of NSPC differentiation in Drosophila via oxidative phosphorylation. In the mammalian system, an extensive increase of mitochondrial mass in adult newborn neurons compared to NSPCs has been described [8]. Upon exercise, the increase in mitochondria was even more profound, and virus-mediated genetic manipulation of mitochondrial mass either inhibited neurogenesis (when mitochondria generation was knocked down) or further enhanced neurogenesis (when mitochondria generation was augmented), providing a functional link between mitochondrial metabolism and neuronal maturation [8]. Similarly, genetic mitochondrial damage in NSPCs had a detrimental effect on the generation of neurons and oligodendrocytes during development, with only minor effects on the NSPC pool [46]. Interestingly, a large amount of adult newborn neurons die during maturation, a well-described but poorly understood
Schematic representation of the major metabolic pathways in NSPCs and their neuronal progeny. Shown are the major changes in metabolic pathways occurring in quiescent and proliferating NSPCs as well as in immature neurons in the adult dentate gyrus and the developing forebrain. Please refer to the text for details.
phenomenon [47]. Given the large increase in mitochondria during the differentiation process, a concomitant increase in ROS production may contribute to increased cell death. Indeed, increased oxidative stress was reported to occur as a side effect of NSPC proliferation/differentiation [48], although the direct consequences were not elaborated. A recent publication in the field of cellular reprogramming showed that direct conversion of fibroblasts or astrocytes into neurons was accompanied by a significant increase in oxidative stress, which led to massive cell death and inefficient generation of neurons [49]. Anti-oxidative treatment significantly lowered cell death and resulted in a better yield of reprogrammed neurons. Strikingly, activating the vitamin D pathway, which is known for its anti-oxidative action, more than doubled direct reprogramming in vivo [49], emphasizing the important role of oxidative phosphorylation and the redox state for differentiation.

Support for the dependence of proper differentiation on an optimal redox state also comes from several recent publications studying sirtuins in the context of neurogenesis. As described above, sirtuins are good redox state sensors, as they depend on NAD+, the abundance of which is directly reflecting the oxidative state of a cell. Activation of sirtuin 1 repressed neuronal differentiation whereas knockdown led to an increase in neurogenesis in vitro and in vivo [50,51], proposing that oxidative challenges that often accompany aging and disease might directly suppress neurogenesis via activation of sirtuin 1. However, two recent studies rather suggest that such regulation may be more complex: while one study show that inducible genetic activation of sirtuin 1 in adult mice increases oligodendrocyte progenitors rather than neurons and enhances proliferation of NSPCs [53], others found that the ablation of the NAD+ producing enzyme Nampt as well as knockout of sirtuins led to reduced oligodendrocyte production and reduced NSPC proliferation [52]. These divergent results are most likely caused by the complex and manifold actions of sirtuins. Future detailed characterization of the metabolic changes upon manipulation of sirtuins might shed light onto these findings.

In addition, certain lipid metabolic pathways might also directly influence neuronal maturation and differentiation. As outlined above, several recent studies have uncovered the importance of lipid metabolism for NSPC quiescence and proliferation and also showed altered neurogenesis, yet most likely through alteration of NSPC behavior rather than through a direct influence on differentiation. Cholesterol metabolism however might be a pathway of specific importance for developing neurons [54**]. The specific ablation of endogenous cholesterol production in NSPCs led to massive apoptosis of newborn neurons during development, resulting in death at birth, whereas NSPCs were able to compensate the lack of cholesterol by increased uptake from the circulation [54**]. The reason for this difference in coping with a lack of cholesterol remains to be elucidated.

Conclusions

Substantial progress has been made in the last decades to decipher the mechanisms underlying the formation of the brain and the life-long addition of neurons by NSPCs. In addition to key transcriptional regulators and signaling pathways it has been recently discovered that cell metabolism plays a role in controlling NSPC activity and subsequent neuronal differentiation (summarized in Figure 2). Future studies will have to determine the exact metabolic switches (or shifts) occurring with distinct cellular states of NSPCs (e.g., quiescence versus activation). Furthermore, it will be of interest to determine how fast metabolic adaptations occur and if for example metabolic differences may already occur for each prospective daughter cell during asymmetric cell division of NSPCs.

Given its central role in determining the cellular state, cell metabolism may serve as a signal integrator that ‘translates’ a variety of signals into an integrated metabolic response that may affect cellular physiology and behavior on multiple levels (e.g., by changing energy state, fuel source, biomass production, and epigenetics). Thus, studying the interplay between transcriptional programs, morphogenic signaling, and its down-stream or up-stream regulation of the metabolic state may substantially improve our understanding of how NSPCs orchestrate the construction of the brain.

Conflict of interest statement

Nothing declared.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest


A thorough review covering the metabolic regulation of stem cell activity.


This study showed that adult neural stem cells depend on a specialized lipid metabolism for proper proliferation.


This study elegantly links angiogenesis to neural stem cell proliferation in the developing forebrain with levels of glycolysis.


This study shows how shifts in mitochondrial metabolism affect stem cell identity and differentiation in the developing forebrain.


This study represents one of the pioneering studies linking metabolism to neuronal stem cell activity.


This study identified a crucial step of metabolic adaptations in the context of cellular reprogramming.


52. Stein LR, Imai S: Specific ablation of Nampt in adult neural stem cells recapitulates their functional defects during aging. EMBO J 2014, 33:1321-1340.


This study was one of the first studies to link metabolic processes (i.e., cholesterol biosynthesis) to neural stem cell behavior in the developing forebrain.