Neural stem cell metabolism revisited: a critical role for mitochondria

Valentina Scandella[1*], Francesco Petrelli[1*], Darcie L. Moore[2], Simon M.G. Braun[3] & Marlen Knobloch[1]

[1] Department of Biomedical Sciences, University of Lausanne, Lausanne, Switzerland.

[2] Department of Neuroscience, University of Wisconsin-Madison, United States of America.

[3] Department of Genetic medicine and development, University of Geneva, Geneva, Switzerland.

[*]equal contribution

Correspondence: marlen.knobloch@unil.ch

ORCID numbers:

V. Scandella: 0000-0002-3820-0687

F. Petrelli: 0000-0003-0696-7039

D.L. Moore: 0000-0002-0854-7655

S.MG. Braun: 0000-0003-2622-9914

M. Knobloch: 0000-0002-7490-0285

KEYWORDS:

neural stem cells, metabolism, mitochondria, quiescence

ABSTRACT:

Metabolism has emerged as a key regulator of stem cell behavior. Mitochondria are crucial metabolic organelles which are highly important for differentiated cells, yet considered less so for stem cells. However, recent studies have shown that mitochondria influence stem cell maintenance and fate decisions, inviting a revised look at this topic. In this review, we cover the current literature addressing the role of mitochondrial metabolism in mouse and human neural stem cells in the embryonic and adult brain. We summarize how mitochondria are implicated in fate regulation and how substrate oxidation affects NSC quiescence. We further explore scRNAseq data for metabolic signatures of adult NSCs, highlight emerging technologies reporting on metabolic signatures, and discuss mitochondrial metabolism in other stem cells.

The importance of neural stem cells in physiological and pathological conditions

The brain consists of billions of cells, which almost all originate from neural stem cells (NSCs). NSCs multiply and give rise to differentiated progeny that can generate neurons, astrocytes, and oligodendrocytes. A major wave of neurogenesis, the production of new neurons from NSCs, occurs in an orchestrated manner during embryonic brain development, creating the neurons that will be used throughout life. When NSCs and neurogenesis are not functioning properly at this stage, severe brain malformations or premature death can occur [1]. Indeed, molecular and genetic alterations, which lead to excessive proliferation of NSCs or premature differentiation into neurons cause several neurodevelopmental disorders characterized by both mental and/or motor disabilities [1]. Neurogenesis greatly decreases after embryonic development, yet does not cease completely. Both early postnatal and adult neurogenesis have been shown to occur in many mammalian species [2]. Newborn neurons generated postnatally, during adulthood, or even in old age, are able to integrate into the existing neuronal circuitry and can influence certain types of learning and memory, as well as contribute to mood regulation [3,4]. However, while the integration of new neurons does contribute to memory encoding, it can also promote memory loss by remodeling existing circuitries, especially during infancy, when the generation of new neurons is still high [5]. Thus, besides embryonic neurogenesis, postnatal and adult neurogenesis also need to be tightly regulated.

While early postnatal neurogenesis is still quite frequent, adult neurogenesis decreases dramatically during aging [6,7] and this decrease is more severe in the context of neurodegenerative disorders, including Alzheimer's disease [8,9]. This correlation has triggered hope that increasing neurogenesis might have beneficial effects in the context of Alzheimer's disease. Several studies in mice, using genetic enhancement of neurogenesis, suggest that this is indeed the case, opening up potential new therapeutic avenues [10,11]. Despite an ongoing debate about the importance of adult neurogenesis in humans [12,13], the findings that mammalian NSCs persist throughout life and that neurogenesis occurs beyond embryonic development have triggered hope that this might be exploited for brain repair [14].

NSC activity determines the production of new neurons

Understanding what drives neurogenesis is thus important for both embryonic development and adult brain function. The key regulatory step for the production of new neurons is

determined by the activity of the NSCs (**Fig.1**), which can fall into the following categories in the embryonic brain: 1) NSCs can either symmetrically self-renew, which expands the stem cell pool but does not lead to the production of newborn neurons ("symmetric proliferative"), 2) NSCs can divide asymmetrically, producing a daughter NSC and a more committed progenitor, which maintains the stem cell pool and leads via the committed progenitors to newborn neurons ("asymmetric neurogenic"), or 3) NSCs can terminally differentiate, which diminishes the stem cell pool, but can lead directly to newborn neurons ("symmetric self-consuming"). During embryonic brain development, these decisions occur in a regulated manner and change throughout development [15]. The symmetric proliferative embryonic NSCs are also called neuroepithelial cells (NECs), that transition into so-called radial glial cells (RGCs), which undergo asymmetric neurogenic and later symmetric self-consuming divisions (**Fig. 1**) [15]. For simplicity, we here use the term NSCs for all the different division modes. In the last period of embryonic brain development, NSCs switch from neurogenesis to gliogenesis, producing different glial precursors to generate astrocytes and oligodendrocytes [15,16]. This part of NSC regulation will not be discussed in this review.

In the adult mammalian brain, NSCs are mainly found in two neurogenic regions, the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the hippocampal dentate gyrus (DG). Nomenclature for NSCs of the two niches can vary (for instance Type B cells for SVZ and Type 1 cells for DG, [17]), but as NSCs still retain a radial process, they are also called radial glia-like cells (RGLs) [18]. RGLs can self-renew and give rise to intermediate progenitors, which further proliferate and differentiate into newborn neurons. Adult NSCs are primarily neurogenic but can also produce, to a lesser extent, local astroglial cells [19]. In both niches, NSCs that remain in adulthood originate from embryonic NSCs, which are set aside during embryonic and early postnatal development [20–22]. These adult NSCs are less active than their embryonic counterparts and are usually in a stage of shallow/resting quiescence, or deep/dormant quiescence, however, they can still self-renew and give rise to newborn neurons [23–28]. In this quiescent state, NSCs first need to be activated before they start to proliferate and to produce newborn neurons (**Fig. 1**). They can also undergo asymmetric neurogenic and symmetric self-consuming divisions, as has been recently shown by intravital live imaging [27,28]. Both intrinsic and extrinsic factors can directly influence adult NSCs and determine the net outcome of new neurons produced. The many studies addressing the regulation of NSCs have painted a complex picture influencing these decisions, such as physical activity, age, the niche, circulating

factors, neuronal activity, and genetic programs. These studies are covered in other recent reviews [29,30] and will not be discussed here.

Cellular metabolism: key for stem cell activity, but challenging to study

Cellular metabolism has emerged as a key regulator of stem cell activity (recently reviewed by Meacham and colleagues [31]). Several studies have shown that metabolic profiles change with cell state and that these can influence cell fate decisions and cell activity. In general, proliferating cells often use glycolysis as their main pathway to generate adenosine triphosphate (ATP) and the building blocks necessary for sustaining their growth [32]. With differentiation, cells shift to mitochondrial substrate oxidation, which results in the oxidative phosphorylation (OXPHOS) of ADP to ATP (**Box1**). This also seems to be the case for NSCs. However, recent publications suggest that the metabolic profile of NSCs is more complex than previously thought and that mitochondria play an important role in both embryonic and adult NSCs.

While it is clear that cellular metabolism is central for stem cell behavior, there are many challenges to study its specific role in different stem cell states, which also apply to the studies discussed in this review. *In vivo*, adult NSCs are rare cells that divide infrequently, thus cell numbers for metabolic profiling or metabolic measurements are very limited and most of such measurements are done with NSCs expanded *in vitro*. Furthermore, transitions between different cell stages occur within a continuum instead of clearly distinct states [33], making it challenging to identify and isolate specific populations of NSCs. Besides these NSC-specific limitations, studying cellular metabolism adds additional challenges as metabolic reactions are dynamic processes that depend on protein activity and substrate availability. Many studies that have defined the cellular metabolism of different NSC states have used single cell RNA sequencing (scRNA-Seq) data, which only reflects the mRNA levels of metabolic genes. While the upregulation of metabolic genes from the same pathway suggests that this pathway is used, transcriptomic data do not always reflect protein levels [34,35], thus these limitations need to be considered.

In this review, we cover recent literature addressing the role of mitochondrial substrate oxidation in mouse and human NSCs during embryonic development and in adulthood. We summarize evidence demonstrating how mitochondria are implicated in fate regulation and how substrate oxidation affects the quiescent versus activated state of adult NSCs. We

further explore scRNA-seq data for metabolic signatures of adult NSCs, highlight emerging technologies reporting on metabolic signatures, and discuss metabolic similarities of NSCs with other stem cell systems (**Box 2**). We briefly also cover key findings on metabolic regulation of Drosophila neurogenesis (**Box 3**), but refer the reader to a recent review by Petridi and colleagues [36] for further studies in Drosophila.

The changing view on the role of mitochondria in stem cells

Until recently, stem cells such as pluripotent stem cells (PSCs), embryonic stem cells (ESCs), and somatic stem cells (SSCs) have been considered primarily glycolytic [37,38]. Glycolysis regulates the fate and the function of stem cells not only through the synthesis of ATP and reductive equivalents, but also by enabling the production of lipids, hexosamines, ribose, and amino acids, which are required for proliferation [39–43]. Despite substantial evidence for this glycolytic profile, several studies have pointed out a fundamental role of mitochondria in controlling the behavior of PSCs, ESCs, and various types of SSCs [40,42–49]. Some of these studies directly showed that though mitochondrial pyruvate oxidation was significantly reduced, mitochondria in these stem cells were still active and maintained their ability to generate ATP by using other substrates, such as glutamine and fatty acids, for their metabolic needs [40,43,48,49].

The revised role of mitochondrial metabolism in embryonic NSCs

As with other stem cell types, embryonic NSCs also are considered primarily glycolytic and shift their metabolism towards OXPHOS during differentiation [50,51]. Using cultured NSCs derived from the embryonic cortex, Lange and colleagues showed that proliferative NSCs secrete a large amount of lactate. In contrast, during differentiation, NSCs drastically reduce their production of lactate, suggesting a redirection of pyruvate into mitochondria. Moreover, proliferative NSCs were enriched in hypoxia-inducible factor 1 (HIF-1)-dependent glycolytic genes such as hexokinase 2 (*Hk2)*, glyceraldehyde-3-phosphate dehydrogenase (*Gapdh)*, enolase 1 (*Eno1),* lactate dehydrogenase A *(Ldha)*, and the rate-limiting glycolytic enzyme phophofructokinase-1 (*Pfk1*). The downregulation of 6-Phosphofructo-2-Kinase/Fructose-2,6-Biphosphatase 3 *(Pfkfb3)*, a hypoxia-inducible activator of the rate-limiting glycolytic enzyme *Pfk1,* by *in utero* electroporation impaired the expansion and/or maintenance of NSCs during cortical development [50]. These findings highlight the importance of glycolysis and hypoxia for the proliferation of embryonic NSCs.

Despite this clear dependence on glycolysis, proliferating embryonic NSCs are also affected when mitochondrial proteins are manipulated. For example, in another study, the inhibition of mitochondrial complex I impaired ATP production and proliferation in embryonic NSCs [52]. Similarly, genetic deletion of mitochondrial *Aif*, an FAD-dependent NADH oxidase, caused mitochondrial dysfunction and altered NSC self-renewal, proliferation, as well as neuronal differentiation [53]. These data underline the importance of mitochondria in the regulation of embryonic NSCs. Interestingly, genetic disruption of complex II did not alter the generation or maintenance of embryonic NSCs, but led to severe brain malformations and early postnatal [54]. Why the effects of mitochondrial dysfunction in embryonic NSCs vary depending on the targeted proteins remains to be determined.

Interestingly, the importance of mitochondrial metabolism in embryonic NSCs seems to be related to their division mode (**Fig.1**). A recent study using C^{13} flux analysis reported that at embryonic day 10.5 (E10.5) of neocortical development, when NSCs (NECs) undergo symmetrical division to expand the stem cell pool, they use glucose-derived pyruvate to generate lactate, whereas at E13.5, asymmetrically dividing NSCs (RGCs) use glucose-derived pyruvate to feed the TCA cycle [51]. Furthermore, scRNA-seq at different stages of embryonic brain development showed that in contrast to the symmetrically proliferating NECs, which are characterized by a typical glycolytic profile, asymmetrically dividing neurogenic NSCs exhibit a distinct metabolic state characterized by high expression of genes involved in the citrate/TCA cycle and fatty acid beta-oxidation (FAO) pathway [51]. Interestingly, these metabolic changes in embryonic NSCs coincide with increased vascularization, suggesting that the availability of oxygen provided by ingrowing blood vessels plays an important role in embryonic NSC regulation [50].

Although glucose-derived pyruvate is considered the main fuel for mitochondria, FAO previously has been shown to be an important mitochondrial pathway regulating stem cell functions [49,55–57], including in embryonic NSCs [58]. Indeed, Xie and colleagues have shown that carnitine palmitoyl transferase 1 (CPT1), the rate-limiting enzyme of FAO that produces the long-chain acylcarnitines transported into mitochondria for oxidation (**Box 1**), is expressed in embryonic NSCs [58]. Inhibition of FAO by etomoxir, an inhibitor of CPT1a, impaired NSC expansion in E12.5 forebrain hemisphere cultures. In addition, *in utero* electroporation of *Cpt1a* short hairpin RNA (shRNA) into the embryonic neocortex at E12.5 reduced the pool of NSCs by potentiating NSC symmetric differentiation [58]. These data fit

well with the metabolic and gene expression profile of Dong and colleagues [51], and suggest that metabolic pathway activity can directly influence the division mode of embryonic NSCs. Even if it is not yet clear how exactly FAO impacts NSC function, reducing the mitochondrial oxidation of fatty acids impacts NSC self-renewal and dysregulates their differentiation [58]. Taken together, these findings highlight that embryonic NSCs require glycolysis for their expansion, and an active mitochondrial metabolism to support their asymmetric division as well as their differentiation.

Mitochondrial dynamics shape the state of embryonic NSCs

While metabolic measurements can give a clearer picture, it is not always possible to perform those, especially when working with *in vivo* systems. Nevertheless, mitochondrial morphology can be used as an indicator of a cell's metabolic state, as morphology is tightly coupled with mitochondrial activity. Mitochondria are not static organelles: they undergo dynamic morphological changes with fusion and fission events of the outer and inner mitochondrial membranes. Interestingly, cells containing elongated, fused mitochondria have higher OXPHOS levels than cells with fragmented mitochondria [59]. Mitochondrial fusion is orchestrated by mitofusins (MFN 1-2) and optic atrophy 1 (OPA1), whereas dynamin-related protein 1 (DRP1) regulates fission. Two recent studies have shown that mitochondrial dynamics are linked to embryonic NSC behavior [60,61]. Mitochondrial shape varies along with NSC division and stage progression during brain development. Specifically, embryonic uncommitted NSCs exhibit elongated mitochondrial morphology, while with neuronal commitment, mitochondria become fragmented in intermediate neural progenitors to finally elongate in postmitotic neurons [60,61]. Genetic ablation of *Mfn1/2* led to a severe mitochondrial fragmentation and a profound loss of NSC self-renewal capacity in embryonic mouse brains. More precisely, MFN1/2 disruption led to an increased number of dividing cells with a horizontal cleavage plane [60], a feature that has been associated with asymmetric division, leading to the generation of committed intermediate progenitors [62]. In contrast, induction of mitochondrial fusion by genetic deletion of *Drp1* triggered an increase in the number of uncommitted NSCs that divided symmetrically [60].

In addition, using a method to visualize mitochondria in NSCs during the entire process of neurogenesis, Iwata and colleagues discovered that mitochondrial dynamics influence the fate decisions of NSCs in a specific post-mitotic period. Moreover, by promoting mitochondrial fusion right after mitosis, they were able to affect the fate decisions of NSCs

and to increase their ability to self-renew. Interestingly, the post-mitotic control of cell fate through mitochondrial dynamics observed in mice was also valid in human NSCs. Although the time window of post-mitotic fate decisions is longer in humans, induced mitochondrial fusion in human cortical progenitors led to a decrease in committed progenitors and neurons [61]. Therefore, mitochondrial shape not only governs the division mode and maintenance of the NSC pool, but also redirects fate acquisition after mitosis (recently reviewed by [63]). Taken together, these studies show that embryonic NSCs, despite relying preferentially on glycolysis, contain active mitochondria which are needed for their maintenance and capacity to differentiate. Fused mitochondria lead to an increase in self-renewal capacities, whereas NSCs with fragmented mitochondria are destined towards neuronal commitment.

Do adult NSCs depend on mitochondria?

In the adult brain, NSCs are primarily quiescent, preventing stem cell exhaustion [64]. Similar to other SSCs (**Box 2**), quiescent NSCs (qNSCs) have been considered as glycolytic cells [33,65]. However, Khacho and colleagues showed that, comparable to their findings in embryonic NSCs, forced fragmentation of mitochondria in adult NSCs also reduced the number of uncommitted cells and progeny [60], suggesting a role for mitochondria in the regulation of adult NSCs.

A study by Beckervordersandforth and colleagues [65] addressed the role of mitochondria in the control of lineage progression. They found that with the transition from activated NSCs (aNSCs) to intermediate progenitors, genes involved in the TCA cycle and OXPHOS machinery were upregulated, supporting the metabolic shift towards oxidative metabolism in committed cells, with neurons being highly oxidative. Perturbation of mitochondrial function by ablation of the mitochondrial transcription factor A (*Tfam*) in NSCs led to a massive reduction in the number of intermediate progenitors and newborn neurons, supporting the importance of mitochondria on the way to differentiation.

Interestingly, while this study focused on the importance of mitochondria at a later stage in the lineage progression, the overall mitochondrial volume was similar between NSCs and intermediate progenitors, and there was no change in the mitochondrial membrane potential between aNSCs and qNSCs. Furthermore, proliferation and cell viability were substantially impaired in TFAM-depleted NSCs [65], suggesting that mitochondria are also important at earlier steps in the NSC lineage.

Quiescence to activation: Exploring the role of NSC metabolism using *in vivo* scRNA-seq data

Understanding the metabolic events that accompany the transition from quiescence to activation in NSCs is difficult, as this is a relatively rare event which occurs on a single cell level. Several studies have captured multiple *in vivo* NSC states in the SVZ and DG of adult mouse brains using scRNA-seq, ranging from qNSCs to aNSCs to intermediate progenitors and neuroblasts [24–26,28,33,66–68]. Interestingly, metabolic differences were always among the top terms in the gene enrichment analyses, despite very different strategies to isolate, label, and purify the NSC populations.

To get a better picture of whether mitochondrial substrate oxidation does play a role in adult NSCs, we selected two DG and two SVZ studies for further analysis [25,26,33,67] and specifically checked the expression of key genes [69] in the glycolysis pathway, in the TCA cycle, in FAO, in glutamine metabolism, and in OXPHOS (**Fig. 2A**). As all studies analysed different types of qNSCs and aNSCs with different sequencing protocols and analysis pipelines, we focused on the provided lists of differentially expressed genes (DEGs), and applied the same cut-off criteria for all studies (adjusted p-value < 0.1 or top 1000 DEGs). Interestingly, this analysis revealed a mixed pattern of DEGs, with some genes in a metabolic pathway higher expressed in aNSCs and other genes in the same pathway higher expressed in qNSCs. FAO provided the clearest picture, with almost all key players consistently higher in qNSCs, suggesting that the oxidation of fatty acids plays an important role in the quiescent state (**Fig. 2A**). Furthermore, metabolic genes in DG and SVZ NSCs were also not always changed in the same direction between qNSCs and aNSCs. Whether this is due to a true difference in their metabolic profile or whether this is influenced by the different technical procedures or the selected markers and resulting selected NSC populations, remains to be determined. Finally, the higher expression of several key genes involved in mitochondrial substrate oxidation, such as genes of the TCA cycle, FAO, and OXPHOS in qNSCs supports that mitochondria are indeed important for adult qNSCs.

The expression pattern of metabolic genes does not always reflect the metabolic state

While gene expression data can reveal differential expression of metabolic genes, metabolic pathways are not only regulated at the gene expression level, but are heavily influenced by protein levels, enzymatic activities, and substrate availability. scRNA-seq data thus should be taken with a certain level of precaution when studying metabolism. To obtain a more

rounded view, RNA-seq data can be combined with proteomic and metabolomic analyses. Indeed, in a recent study, Wani and colleagues observed discrepancies between transcriptome and protein levels for cytosolic and mitochondrial proteins in an *in vitro* model of NSC quiescence [70]. They found that mitochondrial TCA, FAO, and OXPHOS-related mRNA levels did not necessarily reflect protein levels (**Fig. 2B**), whereas cytosolic proteins correlated well. These findings suggest that scRNA-seq might not always mirror the true metabolic status. Wani and colleagues further compared proteomic changes of aNSCs and qNSCs with a focus on energy metabolism-related proteins. Enrichment analyses revealed that qNSCs upregulated proteins involved in OXPHOS and FAO [70], as was previously shown [56].

The revised role of mitochondrial metabolism in adult NSCs

Following up on the changes in the proteome between qNSCs and aNSCs, Wani and colleagues also revealed a large change in the mitochondrial proteome. They found that among the different mitochondrial proteases, the activity of the *i*-AAA peptidase YME1L was significantly increased in qNSCs compared to aNSCs [70]. YME1L controls the levels of diverse mitochondrial proteins and its genetic deletion in qNSCs caused an impairment of self-renewal and led to premature differentiation. The loss of YME1L activity was accompanied by a massive downregulation of proteins involved in the FAO pathway as well as other mitochondrial proteins. However, whether the loss of the FAO machinery is a cause or a consequence of dysregulated NSCs remains to be determined. Nevertheless, these findings demonstrate that the transition between quiescence and activation is accompanied by a change in mitochondrial proteases, which in turn alter mitochondrial proteins and their function [70].

Two recent studies, discussed in more detail below, have further highlighted the importance of mitochondrial metabolism as a direct regulator of quiescence and fate decisions in adult NSCs. Glucose-derived pyruvate and FAO, two major mitochondrial pathways that can fuel the TCA cycle (**Box 1**), are necessary to maintain and regulate the quiescent state in NSCs [56,71]. Lipid metabolism has been shown to play a pivotal role in the regulation of adult NSCs, and the shift between the build-up and breakdown of lipids influenced the balance between qNSCs and aNSCs [56,72]. qNSCs express high levels of proteins involved in FAO, including CPT1a (**Box 1**). Importantly, pharmacological and/or genetic inhibition of *Cpt1a* impaired NSC function and significantly decreased neurogenesis [56,57]. Shifting from FAO to *de novo* lipogenesis by manipulating malonyl-CoA levels was sufficient to activate qNSCs,

showing that FAO is required for NSC quiescence [56]. However, why exactly mitochondrial FAO is so important for the quiescent state in adult NSCs remains to be determined.

In a recent study, Petrelli and colleagues add further proof that the metabolic state of qNSCs is not a “low metabolic state”, but an active metabolic state, which requires a functional mitochondrial metabolism [71]. They showed that qNSCs have a complex mitochondrial network formed largely by elongated and active mitochondria, whereas proliferating NSCs had more fragmented mitochondria. These findings fit well with the data from Wani and colleagues [70] discussed above. Furthermore, blocking the transport of glucose-derived pyruvate into mitochondria (**Box 1**) by both pharmacological inhibition and genetic deletion of MPC1, triggered the activation of qNSCs, and resulted in an increased number of newborn neurons. This activation of qNSCs appears to be mediated by an increase in intracellular aspartate levels [71]. Aspartate *per se* has been shown to support the proliferation of cells by promoting DNA, RNA, and protein synthesis even in the context of ETC impairment [73]. Intracellular aspartate levels are primarily derived from glutamine and/or glutamate oxidation into mitochondria, which is directly linked to ETC function by promoting the generation of oxaloacetate in the TCA cycle [73,74]. Whether the increased aspartate comes from an increased glutamine and/or glutamate oxidation in *Mpc1* conditional knockout NSCs remains to be investigated. Surprisingly, *Mpc1* deletion in NSCs did not alter their ability to generate newborn neurons, suggesting that NSCs and their progeny might be metabolically more flexible than previously thought [71]. In addition, *Mpc1* deletion in NSCs was also effective to activate qNSCs in aged mice, leading to increased neurogenesis in this context [71].

Taken together, these findings highlight the relevance of mitochondrial metabolism in the maintenance of the quiescent state in NSCs by the oxidation of fatty acids and glucose-derived pyruvate. Whether quiescent NSCs need oxidation of these two substrates for energy purposes and/or to modulate the availability of TCA intermediates and their ability to regulate gene expression still remains to be determined.

Novel technologies to explore the metabolic differences between qNSCs and aNSCs

The development of novel technologies has broadened the possibilities to study cellular metabolism. These include genetically encoded probes and dyes that monitor and visualize metabolites in different compartments [75], a subset of which are listed in **Table 1**. Genetically encoded reporters, such as fluorescent proteins and biosensors can be expressed in cells and allow for real-time monitoring of metabolite dynamics within targeted organelles. Indeed, using a genetically encoded calcium sensor, Gengatharan and colleagues could visualize fluctuations of intracellular levels of calcium in qNSCs and aNSCs [76]. Additionally, various fluorescent dyes and probes have been developed to specifically stain and detect metabolites in different cellular compartments without the need of genetic manipulation. In the context of NSCs, the use of reactive oxygen species indicators has allowed for the discrimination of quiescent and proliferating adult NSCs [77]. Moreover, recent advancements in spatial metabolomics and proteomics allow for high-resolution mapping of a huge variety of metabolites and proteins in fixed tissue sections and cells, even at the single cell level [78]. A newly developed mass spectrometry-based method furthermore allows the study of the interaction between metabolites and proteins [79]. Combining these novel technologies with the aforementioned genetically encoded probes and dyes will provide a more comprehensive understanding of both the spatial and dynamic aspects of cellular metabolism.

In addition to probes and dyes, there are non-invasive methods to report on the dynamic metabolic changes occurring within cells. One such strategy is to analyze the intrinsic fluorescence of specific metabolic cofactors to identify unique metabolic signatures in live, single cells. Many molecules in cells are autofluorescent, including metabolic coenzymes. These molecules can be visualized using distinct excitation and emission spectra to provide information on their intensity [80]. In addition, dynamic changes of these molecules and their fluorescence can be measured using fluorescent lifetime imaging microscopy (FLIM), where one excites fluorophores using specific wavelengths of light pulses, and measures the time until the fluorophores emit photons. For the metabolic cofactors NADH, NADPH, and FAD, the time to emission is different if these molecules are in a free or bound state, and thus reflects protein-binding activity [80]. Many studies have used the autofluorescent properties of these molecules to identify defined shifts in metabolic processes, yet it is difficult to fully understand the underlying metabolic pathway changes. Instead, these measures are useful in defining unique signatures underlying distinct cell states or cell fates.

As discussed above, as qNSCs are metabolically different from aNSCs, using metabolism as a readout of cell function may be a better indicator and predictor of these subtle differences in cell state than transcriptional signatures. In adult NSCs, it was recently shown that a combination of the signals detected using the excitation/emission parameters for NAD(P)H and FAD was highly predictive of whether NSCs were quiescent or activated both *in vitro* and *in vivo* [81]. Morrow and colleagues found that the bright signal detected using the FAD parameters in NSCs was not FAD, but co-localized within a subset of lysosomes, and was highly enriched in qNSCs [81]. Lysosomes previously have been shown to be increased in qNSCs [66,82], and are able to contribute to lipid metabolism through the lysosomal acid lipase (LAL), which can generate free fatty acids [83]. Thus, the combination of NAD(P)H and a lysosomal-based fluorescence (termed punctate autofluorescence (PAF)) may report both on mitochondrial and lipid metabolism [81]. Interestingly, the authors found that PAF intensity alone was highly predictive of cell state and highest in qNSCs, and correlated well with transcriptional changes previously described between qNSCs and aNSCs [24,81]. Furthermore, the highest PAF intensity associated with the most dormant quiescent population, and graded changes in gene expression of specific markers correlated with changing PAF intensity levels [81]. Thus, the technique of using intensity or FLIM-based imaging of autofluorescent molecules involved in metabolism is a non-destructive, label-free, long-term imaging approach that can be used with traditional methods such as fluorescent activated cell sorting (FACS). This allows for the identification of unique fingerprints reporting on the underlying metabolic dynamics, and can be used to separate and predict cell state or differentiation status.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

In this review, we highlight the emerging evidence that mitochondrial metabolism plays an active role in the regulation of embryonic and adult neurogenesis. The major findings are summarized in **Figure 3**. Moreover, we have revisited the role of mitochondria and substrate oxidation for the cellular state of quiescence, which has been considered for many years as a “low metabolic state”. The evidence that mitochondrial metabolic pathways, such as pyruvate oxidation and FAO directly influence this state demonstrate that the traditional view of cellular metabolism in quiescent NSCs needs reconsideration.

Despite the clear evidence that mitochondria are important for NSCs, it remains to be determined why they use mitochondrial substrate oxidation (see **Outstanding Questions**).

In addition to being important for the production of ATP, it is becoming clear that metabolites produced in the TCA cycle via anaplerotic reactions can directly influence cell fate decision by modulating the activity of chromatin-modifying enzymes and subsequently gene expression [84]. Two new studies highlight the relevance of a specific epigenetic modification, namely histone acetylation, in the control of embryonic and adult NSCs. In the first study, Iwata and colleagues showed that specific NAD-dependent deacetylases are activated in response to an active ETC and an increased NAD/NADH ratio, resulting in a decrease in specific acetylated histone modifications and increased neurogenesis. Promoting these enzymes in cells with fused mitochondria was sufficient to increase the number of neuronal progeny, suggesting a crosstalk between oxidation state, mitochondrial shape, and cell fate decisions [61,63]. In addition, Liu and colleagues showed that the accumulation of D-2-hydroxyglutarate (D-2-HG) impairs the activation of qNSCs by reducing the levels of acetyl-CoA and histone acetylation. Interestingly, restoring the levels of acetyl-CoA and histone acetylation by acetate administration was sufficient to rescue NSC activation and neurogenesis [85]. This emerging research field will almost certainly provide new answers to how mitochondrial metabolites beyond acetyl-CoA affect the epigenome of NSCs to regulate gene expression and cell fate.

Technological advances, as discussed above, will further allow researchers to address metabolic questions looking at the protein and metabolite level, rather than relying primarily on gene expression data. Further, non-destructive, label-free methods utilizing intrinsically autofluorescent metabolic cofactors can not only define metabolic signatures associated with cell states, but can also be used to prospectively predict cell state in live cells for long-term imaging [81]. Such technologies will further our understanding of how mitochondrial metabolism influences the fate decisions and the functions of embryonic and adult NSCs.

FIGURES AND TEXT BOXES:

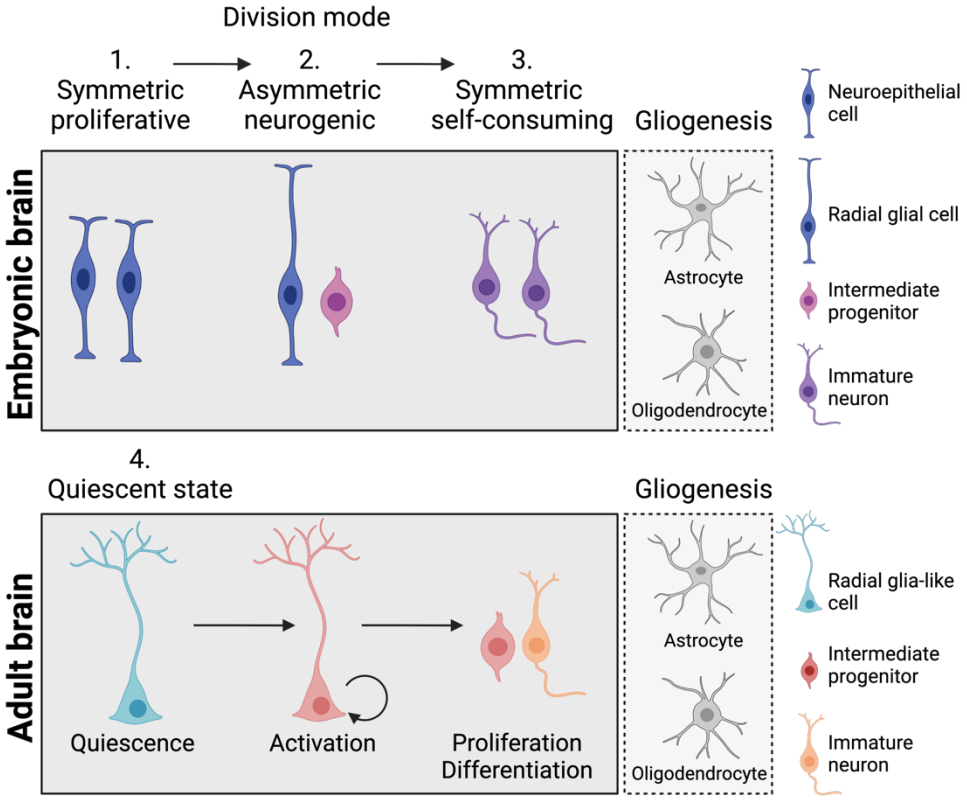


Figure 1: Division modes of embryonic and adult NSCs

In the embryonic brain, the earliest NSCs (also called neuroepithelial cells) divide symmetrically to self-renew and increase the stem cell pool (1). They develop into radial glial cells (RGCs) and start dividing asymmetrically to sustain the RGC pool and to produce progenitors that eventually differentiate into neurons (2). Towards the end of embryonic brain development, the RGCs divide again symmetrically in a self-consuming way to give rise to neurons (3). During brain development, some RGCs are set aside and become radial glia-like cells (RGL), which are the NSCs in adulthood (4). Adult NSCs are mainly in a quiescent state and first need to be activated to proliferate and differentiate into neurons. Embryonic and adult NSCs can also switch to gliogenesis to give rise to astrocytes and oligodendrocytes (dotted boxes). This part is not discussed in this review, but shown for completeness.

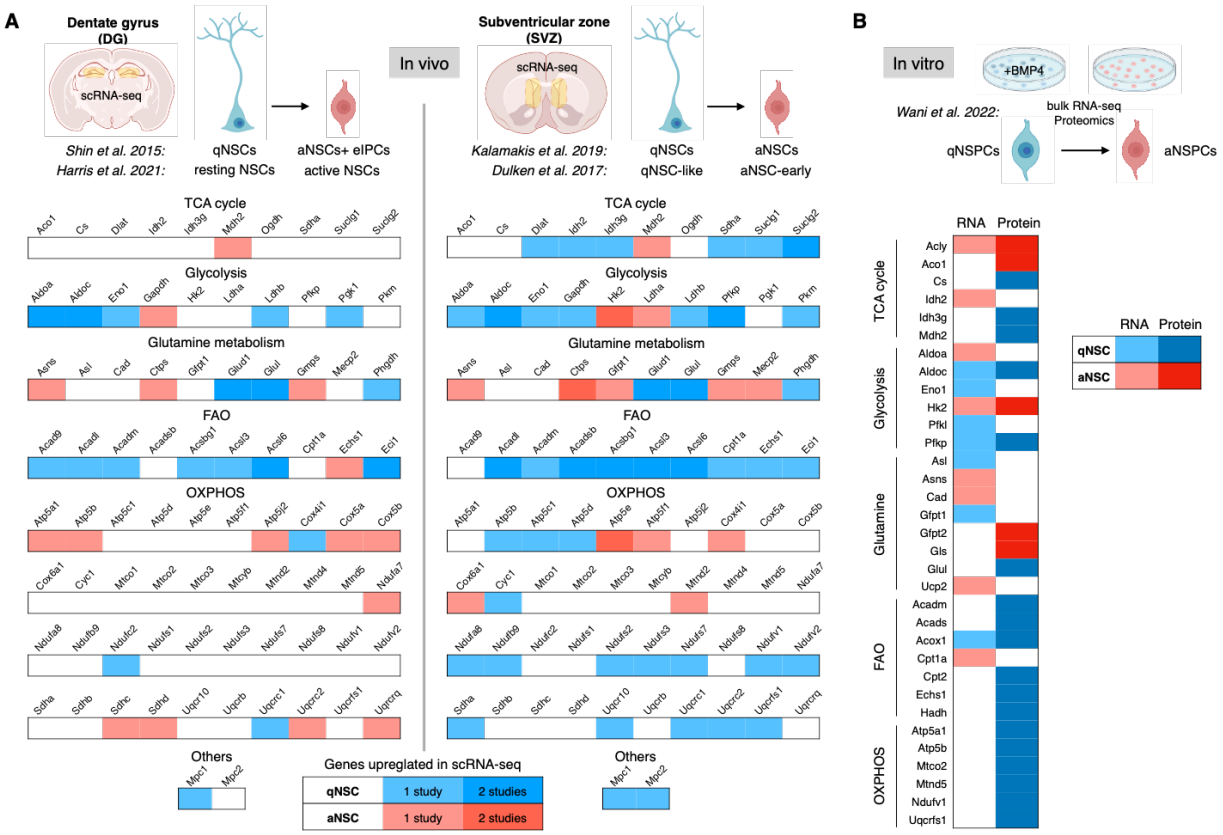
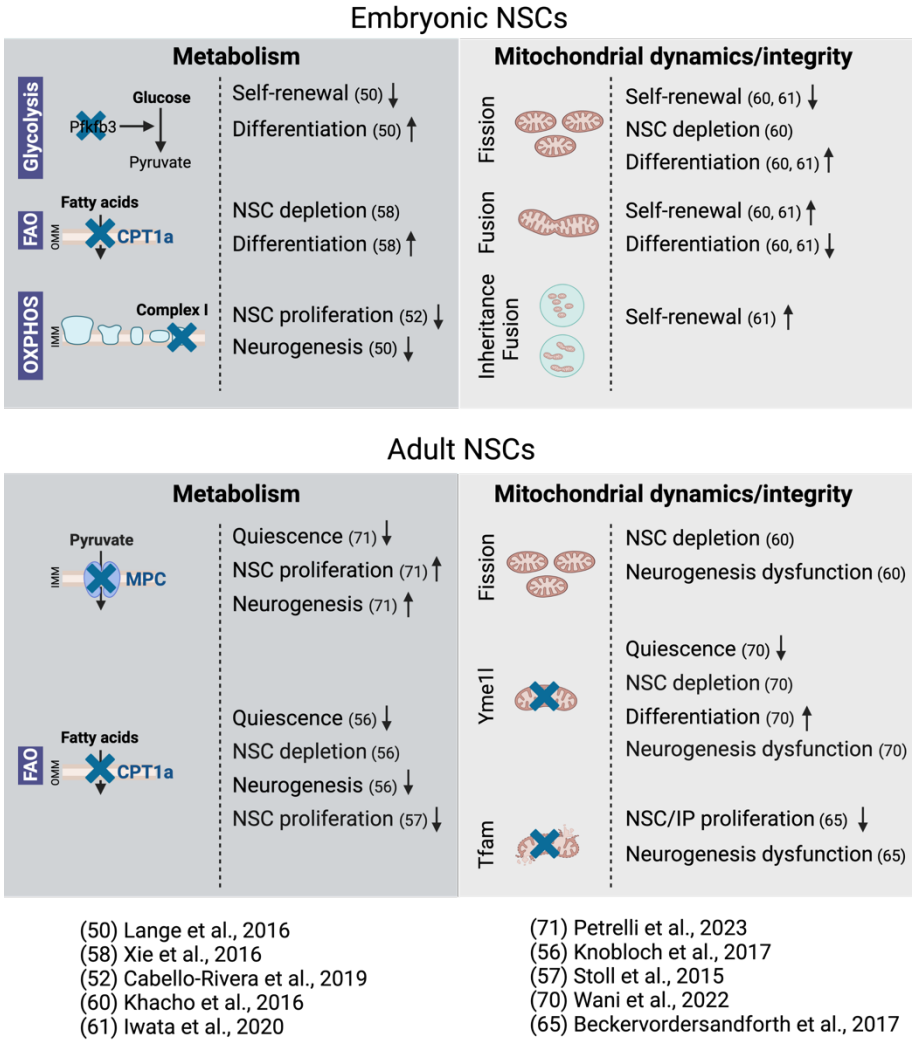


Figure 2: Exploring the role of NSC metabolism using *in vivo* scRNA-seq data

(**A**) Schematic overview of scRNA-seq studies used for meta-analysis and differentially expressed genes (DEGs) related to metabolic pathways in adult qNSCs (blue) and aNSCs (red) from the mouse DG and SVZ. For each neurogenic niche, we selected two studies: Shin *et al* [33] performed scRNA-seq analysis of DG NSCs from Nes-CFP^{nuc} mice and provided DEGs between qNSC and aNSCs/early intermediate progenitor cells (eIPCs) (top 1000 up- and down-regulated genes). Harris *et al* [25] performed scRNA-seq analysis of DG NSCs from Ki67$^{TD\text{-}NES}$ mice and provided DEGs between resting NSCs and aNSCs (padj < 0.1, 138 up- and 618 down-regulated genes). Kalamakis *et al* [26] performed scRNA-seq analysis of SVZ NSCs isolated with defined markers by FACS and provided genes specifically expressed in two qNSC clusters and three aNSC clusters (merged qNSC and aNSC clusters, 1199 up- and 893 down-regulated genes). Dulken *et al* [86] performed scRNA-seq analysis of SVZ NSCs isolated with defined markers by FACS and provided DEGs between qNSC-like and aNSC-early populations (top 1000 up- and down-regulated genes). The 82 genes selected for this analysis represent key genes from each major metabolic pathway: TCA cycle, Glycolysis, Glutamine metabolism, FAO and OXPHOS. Color code indicates the following: light blue: higher in qNSCs (1 study); dark blue: higher in qNSCs (2 studies); light red: higher in aNSCs (1 study); dark red: higher in aNSCs (2 studies).

(**B**) Wani *et al* [70] performed bulk RNA-seq and proteomics analysis of cultured DG qNSCs (+BMP4) and aNSCs (padj < 0.1 and |FC| > 1.5, 2832 up- and 2523 down-regulated genes). Comparison of transcriptomic and proteomic datasets from cultured qNSCs (blue) and aNSCs (red) from Wani *et al* [70]. Heatmap of 33 metabolic genes reveals that differences in gene expression do not always reflect similar differences in protein

abundance between qNSCs and aNSCs. Color code indicates the following: light blue: higher in qNSCs (RNA); dark blue: higher in qNSCs (protein); light red: higher in aNSCs (RNA); dark red: higher in aNSCs (protein).



(50) Lange et al., 2016
(58) Xie et al., 2016
(52) Cabello-Rivera et al., 2019
(60) Khacho et al., 2016
(61) Iwata et al., 2020

(71) Petrelli et al., 2023
(56) Knobloch et al., 2017
(57) Stoll et al., 2015
(70) Wani et al., 2022
(65) Beckervordersandforth et al., 2017

Figure 3: Summary of the main manipulations of metabolism and mitochondrial dynamics reported in this review. Shown are the manipulations and their effects on NSC behavior during embryonic development and adulthood. Numbers refer to the studies that reported the effects.

Box 1: Mitochondrial substrate oxidation

Glycolysis and mitochondrial substrate oxidation (also referred to as mitochondrial "respiration") are the two main metabolic pathways for a cell to generate energy in the form of adenosine triphosphate (ATP). Mitochondria are considered the "powerhouse" of a cell since their capacity to produce ATP is much higher than through glycolysis. Glucose-derived pyruvate, glutamine-derived alpha-ketoglutarate (aKG), and fatty acid-derived acetyl-CoA are among the main substrates used in mitochondria.

Mitochondrial ATP is generated by oxidation of substrates through a process involving several steps: Through the tricarboxylic acid cycle (TCA), reducing equivalents Nicotinamide adenine dinucleotide (NADH) and Flavin adenine dinucleotide ($FADH_2$) are produced and used to drive electrons into the electron transport chain (ETC), with oxygen (O_2) as the terminal electron acceptor. The ETC is coupled to the generation of the mitochondrial membrane potential, which is used by the F_1F_0-ATP synthase to produce ATP via oxidative phosphorylation (OXPHOS) [87].

Glucose: Glucose is first metabolized into pyruvate through glycolysis. In the cytoplasm, pyruvate can be converted into lactate or can be imported into mitochondria through the mitochondrial pyruvate carrier (MPC). Once in the mitochondria, pyruvate is converted to acetyl-CoA, which enters the TCA cycle, or can be directly carboxylated to form oxaloacetate.

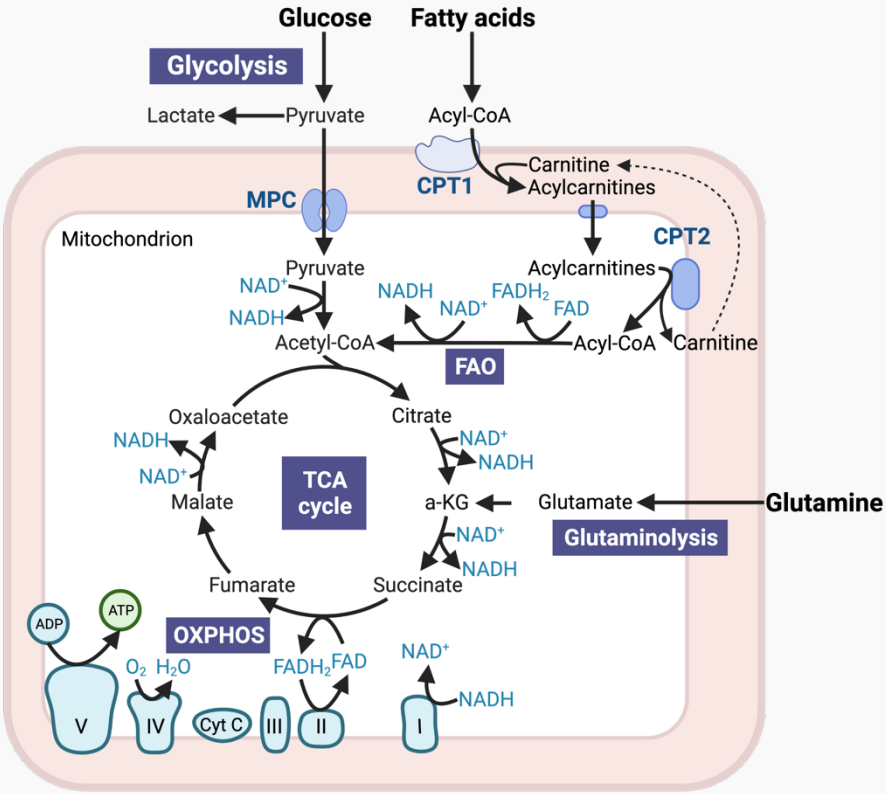
Fatty acids (FAs): Short-chain FAs and medium-chain FAs can passively cross the mitochondrial membrane, while long-chain FAs are transported into the mitochondria through the activity of carnitine-palmitoyltransferase 1 (CPT1). The oxidation of FAs through fatty acid β-oxidation (FAO) leads to the production of acetyl-CoAs that enter the TCA cycle, and $FADH_2$ and NADH that feed directly into the ETC.

Glutamine: Glutamine, a non-essential amino acid is transformed into glutamate by the action of glutaminase. In the mitochondria, glutamate is subsequently converted into aKG, which can either enter the TCA cycle to produce energy and reducing equivalents, or can be converted into citrate by reductive carboxylation to sustain fatty acid synthesis.

The metabolic intermediates of the TCA cycle are also used for biosynthetic processes, such as the production of fatty acids and non-essential amino acids. Thus, beside its primary role

for energy production, mitochondrial substrate oxidation is also important to balance the concentrations of TCA cycle intermediates [88]

In addition, by acting as co-factors or substrates for epigenetic enzymes, intermediates of mitochondrial substrate oxidation have also been shown to modulate histone modifications and therefore gene expression [89,90].



Scheme of the major mitochondrial substrate oxidation pathways

Box 2: The metabolic profile of quiescent adult somatic stem cells

Unlike ESCs, adult SSCs are primarily in a quiescent state, typically defined as being in the reversible G0 stage of the cell cycle. They are nested in specific niches of different organs, including brain, muscle, bone marrow, and skin. Quiescent adult stem cells can be stimulated to activate and enter the cell cycle, maintaining organ homeostasis during the life of the organism [91]. Until recently, the quiescent state has been defined as a "low metabolic state" [92]. As discussed above, highly proliferative cells primarily use glycolysis to produce ATP and to synthesize biomolecules to increase their mass, whereas more differentiated cells shift to OXPHOS [32,93]. Once entering quiescence, SSCs are no longer dividing. So why would they use only glycolysis to feed their metabolic needs and not mitochondrial metabolism? The notion that quiescent SSCs are primarily glycolytic originates from various studies in adult hematopoietic stem cells (HSCs), which reported that quiescent HSCs rely primarily on glycolysis for ATP production, and glucose-derived pyruvate is converted to lactate rather than oxidized in the mitochondria [94,95]. Furthermore, it had been shown that HSCs have low mitochondrial activity, supporting a glycolytic profile (recently reviewed by [96,97]. However, two recent studies have demonstrated that quiescent HSCs take up less glucose than proliferative HSCs, which also express higher levels of glycolytic enzymes [98,99]. These findings suggest that in direct comparison to proliferative HSCs, quiescent HSCs are less glycolytic, and that glycolysis is activated when HSCs enter the cell cycle rather than when they are in a quiescent state. A similar metabolic regulation occurs in muscle stem cells, in which glycolysis increases after their activation [100,101], which is also the case for other adult stem cells [44,102]. Since these new findings support the concept that the quiescent state is less glycolytic than the activated or proliferative state, the main question is: what is the role of mitochondria in quiescence? Several recent studies demonstrated that mitochondrial oxidation of fatty acids, glutamine, and glucose-derived pyruvate is necessary to control the quiescent state in different stem cell types [55,56,71,101,103,104]. Taken together, these findings support the idea that the quiescent state of SSCs is not just a "low metabolic state," but rather an active metabolic state characterized by a functional mitochondrial metabolism.

Box 3: Metabolic lessons from *Drosophila*

For many years, *Drosophila* has been used as a model system to study the metabolic requirements of NSCs, also called neuroblasts (NBs). These NBs maintain their ability to self-renew and differentiate during embryonic and larval stages, while at the end of the pupal stage, NBs terminate their proliferation program and undergo apoptosis or symmetric neural differentiation [105]. Several studies have suggested that OXPHOS is dispensable for proliferating NBs and that they rely mainly on glycolysis. Larval proliferative NBs have smaller mitochondria in comparison to their differentiated progeny [106]. Moreover, a mutation in the *Drosophila* gene *qless*, coding for an important enzyme involved in the synthesis of the ETC component coenzyme Q, does not interfere with NB proliferation [107]. Furthermore, Homem and colleagues showed that mitochondrial OXPHOS is indeed necessary to terminate NB proliferation [108]. The inhibition of several OXPHOS subunits by RNAi-mediated knockdown in NBs resulted in sustained proliferation at the end of the pupal stage and in the adult fly brain. Additionally, these findings demonstrated that this metabolic shift is not just a consequence of differentiation per se but a necessary metabolic process for neural fate decisions [108].

However, other studies have reported the presence of mitochondrial OXPHOS activity in *Drosophila* NBs, suggesting a more complex picture. For instance, it has been shown that larval NBs maintain functional ETC activity under hypoxia conditions [109]. Moreover, RNAi-mediated knockdown of OXPHOS genes reduced NB proliferation by increasing cell cycle length [110]. These data suggest that mitochondrial OXPHOS activity is also important for NB proliferation during *Drosophila* larval brain development. These discrepancies might be partially explained by differences in the types of NBs analysed in these studies. As the behavior of *Drosophila* NBs is also finely-regulated by nutrients, oxygen levels, and systemic and local signals, differences in their microenvironment likely influence their metabolic profile. We refer the reader to a recent review by Petridi and colleagues, who discuss the role of mitochondrial metabolism in *Drosophila* NSCs in more detail [36].

626 **Table 1: Novel techniques to study metabolic properties**
627

Nature of the probe or sensor	Metabolic target	Technique	Cell types	References
Autofluorescence	NADH/NADPH FAD	FLIM / PAF	**NSCs**	[81]
Dye/ small molecule fluorescent probe	Mitochondrial ROS	MitoSox	**NSCs**	[77]
	Cellular ROS	Superoxide indicator dihydroethidium (DHE)		
Dye/ small molecule fluorescent probe	Mitochondrial membrane potential	TMRM, Mitotracker Green	Neurons, **NSCs**	[111–113]
Dye/ small molecule fluorescent probe	Lipids	Bodipy C12	Neurons, astrocytes	[114]
Genetically-encoded fluorescent sensor	Calcium	GCaMPs	**NSCs**	[76]
Genetically-encoded fluorescent sensor	Glutathione redox potential	Redox biosensor (Grx1 fused to roGFP2)	Neurons	[115]
Genetically-encoded fluorescent sensor	Lactate / pyruvate	Laconic / Pyronic	Neurons, astrocytes	[116]
Genetically-encoded fluorescent sensor	NAD/NADH	SoNar	Epithelial cells	[117]
Genetically-encoded fluorescent sensor	ATP	iATPSnFRs	Neurons, astrocytes	[118]
Mass spectrometry	Proteins	Expansion Proteomics	Whole Brain	[119]
Mass spectrometry	Proteins	MALDI- imaging mass spectrometry	Whole Brain	[120]

628

629

ACKNOWLEDGEMENTS :

Figures were created using BioRender. The authors would like to thank Melissa Skala from UW-Madison for comments on the “novel technologies” section.

REFERENCES:

1. Klingler, E. *et al.* (2021) Mapping the molecular and cellular complexity of cortical malformations. *Science* 371, eaba4517

2. Denoth-Lippuner, A. and Jessberger, S. (2021) Formation and integration of new neurons in the adult hippocampus. *Nat Rev Neurosci* 22, 223–236

3. Toda, T. *et al.* (2019) The role of adult hippocampal neurogenesis in brain health and disease. *Mol Psychiatr* 24, 67–87

4. Tartt, A.N. *et al.* (2022) Dysregulation of adult hippocampal neuroplasticity in major depression: pathogenesis and therapeutic implications. *Mol Psychiatr* 27, 2689–2699

5. Akers, K.G. *et al.* (2014) Hippocampal Neurogenesis Regulates Forgetting During Adulthood and Infancy. *Science* 344, 598–602

6. Kuhn, H.G. *et al.* (1996) Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. *The Journal of Neuroscience* 16, 2027–2033

7. Ben Abdallah, N.M.B. *et al.* (2010) Early age-related changes in adult hippocampal neurogenesis in C57 mice. *Neurobiology of Aging* 31, 151–161

8. Moreno-Jiménez, E.P. *et al.* (2019) Adult hippocampal neurogenesis is abundant in neurologically healthy subjects and drops sharply in patients with Alzheimer’s disease. *Nature Medicine*

9. Terreros-Roncal, J. *et al.* (2021) Impact of neurodegenerative diseases on human adult hippocampal neurogenesis. *Science* 374, 1106–1113

10. Mishra, R. *et al.* (2022) Augmenting neurogenesis rescues memory impairments in Alzheimer’s disease by restoring the memory-storing neurons. *J Exp Med* 219, e20220391

11. Li, Y.-D. *et al.* (2023) Activation of hypothalamic-enhanced adult-born neurons restores cognitive and affective function in Alzheimer’s disease. *Cell Stem Cell* 30, 415-432.e6

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

13. Lucassen, P.J. *et al.* (2020) Adult neurogenesis, human after all (again): Classic, optimized, and future approaches. *Behav Brain Res* 381, 112458

14. Quadrato, G. *et al.* (2014) Adult neurogenesis in brain repair: cellular plasticity vs. cellular replacement. *Front Neurosci* 8, 17

15. Kriegstein, A. and Alvarez-Buylla, A. (2009) The Glial Nature of Embryonic and Adult Neural Stem Cells. *Annual Review of Neuroscience* 32, 149–184

16. Clavreul, S. *et al.* (2022) Astrocyte development in the cerebral cortex: Complexity of their origin, genesis, and maturation. *Front Neurosci-switz* 16, 916055

17. Martínez-Cerdeño, V. and Noctor, S.C. (2018) Neural Progenitor Cell Terminology. *Front Neuroanat* 12, 104

18. Miranda-Negrón, Y. and García-Arrarás, J.E. (2022) Radial glia and radial glia-like cells: Their role in neurogenesis and regeneration. *Front Neurosci* 16, 1006037

19. Bonaguidi, M.A. *et al.* (2011) In Vivo Clonal Analysis Reveals Self-Renewing and Multipotent Adult Neural Stem Cell Characteristics. *Cell* 145, 1142–1155

20. Fuentealba, L.C. *et al.* (2015) Embryonic Origin of Postnatal Neural Stem Cells. *Cell* 161, 1644–1655

21. Furutachi, S. *et al.* (2015) Slowly dividing neural progenitors are an embryonic origin of adult neural stem cells. *Nat Neurosci* 18, 657–665

22. Berg, D.A. *et al.* (2019) A Common Embryonic Origin of Stem Cells Drives Developmental and Adult Neurogenesis. *Cell* 177, 654-668.e15

23. Urbán, N. *et al.* (2016) Return to quiescence of mouse neural stem cells by degradation of a proactivation protein. *Science* 353, 292–295

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

25. Harris, L. *et al.* (2021) Coordinated changes in cellular behavior ensure the lifelong maintenance of the hippocampal stem cell population. *Cell Stem Cell* 28, 863-876.e6

26. Kalamakis, G. *et al.* (2019) Quiescence Modulates Stem Cell Maintenance and Regenerative Capacity in the Aging Brain. *Cell* 176, 1407-1419.e14

27. Pilz, G.-A. *et al.* (2018) Live imaging of neurogenesis in the adult mouse hippocampus. *Science* 359, 658–662

28. Bottes, S. *et al.* (2021) Long-term self-renewing stem cells in the adult mouse hippocampus identified by intravital imaging. *Nat Neurosci* 24, 225–233

29. Negredo, P.N. *et al.* (2020) Aging and Rejuvenation of Neural Stem Cells and Their Niches. *Cell Stem Cell* 27, 202–223

30. Matsubara, S. *et al.* (2021) Regulation of Adult Mammalian Neural Stem Cells and Neurogenesis by Cell Extrinsic and Intrinsic Factors. *Cells* 10, 1145

699 31. Meacham, C.E. *et al.* (2022) Metabolic regulation of somatic stem cells in vivo. *Nat Rev*
700 *Mol Cell Bio* 23, 428–443

701 32. Metallo, C.M. and Vander Heiden, M.G. (2013) Understanding Metabolic Regulation and
702 Its Influence on Cell Physiology. *Mol Cell* 49, 388–398

703 33. Shin, J. *et al.* (2015) Single-Cell RNA-Seq with Waterfall Reveals Molecular Cascades
704 underlying Adult Neurogenesis. *Cell Stem Cell* 17(3), 360–72.

705 34. Maier, T. *et al.* (2009) Correlation of mRNA and protein in complex biological samples.
706 *Febs Lett* 583, 3966–3973

707 35. Koussounadis, A. *et al.* (2015) Relationship between differentially expressed mRNA and
708 mRNA-protein correlations in a xenograft model system. *Sci Rep* 5, 10775

709 36. Petridi, S. *et al.* (2022) Mitochondrial respiration and dynamics of in vivo neural stem
710 cells. *Development* 149

711 37. Rafalski, V.A. *et al.* (2013) Energy metabolism and energy-sensing pathways in
712 mammalian embryonic and adult stem cell fate. *J. Cell Sci.* 125, 5597–5608

713 38. Folmes, C.D.L. and Terzic, A. (2016) Energy metabolism in the acquisition and
714 maintenance of stemness. *Semin. Cell Dev. Biol.* 52, 68–75

715 39. Prigione, A. *et al.* (2011) Human Induced Pluripotent Stem Cells Harbor Homoplasmic
716 and Heteroplasmic Mitochondrial DNA Mutations While Maintaining Human Embryonic
717 Stem Cell–like Metabolic Reprogramming. *Stem Cells* 29, 1338–1348

718 40. Zhang, J. *et al.* (2012) Metabolic Regulation in Pluripotent Stem Cells during
719 Reprogramming and Self-Renewal. *Cell Stem Cell* 11, 589–595

720 41. Folmes, C.D.L. *et al.* (2011) Somatic Oxidative Bioenergetics Transitions into
721 Pluripotency-Dependent Glycolysis to Facilitate Nuclear Reprogramming. *Cell Metab.* 14,
722 264–271

723 42. Takubo, K. *et al.* (2013) Regulation of Glycolysis by Pdk Functions as a Metabolic
724 Checkpoint for Cell Cycle Quiescence in Hematopoietic Stem Cells. *Cell Stem Cell* 12, 49–
725 61

726 43. Schell, J.C. *et al.* (2017) Control of intestinal stem cell function and proliferation by
727 mitochondrial pyruvate metabolism. *Nat. Cell Biol.* 19, 1027–1036

728 44. Flores, A. *et al.* (2017) Lactate dehydrogenase activity drives hair follicle stem cell
729 activation. *Nat. Cell Biol.* 19, 1017–1026

730 45. Maryanovich, M. *et al.* (2015) An MTCH2 pathway repressing mitochondria metabolism
731 regulates haematopoietic stem cell fate. *Nat Commun* 6, 7901

732 46. Rodríguez-Colman, M.J. *et al.* (2017) Interplay between metabolic identities in the
733 intestinal crypt supports stem cell function. *Nature* 543, 424–427

47. Varum, S. *et al.* (2011) Energy Metabolism in Human Pluripotent Stem Cells and Their Differentiated Counterparts. *Plos One* 6, e20914

48. Tohyama, S. *et al.* (2016) Glutamine Oxidation Is Indispensable for Survival of Human Pluripotent Stem Cells. *Cell Metab.* 23, 663–674

49. Mihaylova, M.M. *et al.* (2018) Fasting Activates Fatty Acid Oxidation to Enhance Intestinal Stem Cell Function during Homeostasis and Aging. *Cell Stem Cell* 22, 769–778

50. Lange, C. *et al.* (2016) Relief of hypoxia by angiogenesis promotes neural stem cell differentiation by targeting glycolysis. *The EMBO Journal* 35, 924–941

51. Dong, X. *et al.* (2022) Metabolic lactate production coordinates vasculature development and progenitor behavior in the developing mouse neocortex. *Nat Neurosci* 25, 865–875

52. Cabello-Rivera, D. *et al.* (2019) Mitochondrial Complex I Function Is Essential for Neural Stem/Progenitor Cells Proliferation and Differentiation. *Front Neurosci* 13, 664

53. Khacho, M. *et al.* (2017) Mitochondrial dysfunction underlies cognitive defects as a result of neural stem cell depletion and impaired neurogenesis. *Hum Mol Genet* 26, 3327–3341

54. Díaz-Castro, B. *et al.* (2015) Resistance of glia-like central and peripheral neural stem cells to genetically induced mitochondrial dysfunction—differential effects on neurogenesis. *Embo Rep* 16, 1511–1519

55. Ito, K. *et al.* (2012) A PML-PPAR-delta pathway for fatty acid oxidation regulates hematopoietic stem cell maintenance. *Nat. Med.* 18, 1350–1358

56. Knobloch, M. *et al.* (2017) A Fatty Acid Oxidation-Dependent Metabolic Shift Regulates Adult Neural Stem Cell Activity. *Cell Reports* 20, 2144–2155

57. Stoll, E.A. *et al.* (2015) Neural Stem Cells in the Adult Subventricular Zone Oxidize Fatty Acids to Produce Energy and Support Neurogenic Activity. *Stem Cells* 33, 2306–2319

58. Xie, Z. *et al.* (2016) Inborn Errors of Long-Chain Fatty Acid beta-Oxidation Link Neural Stem Cell Self-Renewal to Autism. *Cell Reports* 142, 991–999

59. Giacomello, M. *et al.* (2020) The cell biology of mitochondrial membrane dynamics. *Nat Rev Mol Cell Bio* 21, 204–224

60. Khacho, M. *et al.* (2016) Mitochondrial Dynamics Impacts Stem Cell Identity and Fate Decisions by Regulating a Nuclear Transcriptional Program. *Cell Stem Cell* 19, 232–247

61. Iwata, R. *et al.* (2020) Mitochondrial dynamics in postmitotic cells regulate neurogenesis. *Science* 369, 858–862

62. Matsuzaki, F. and Shitamukai, A. (2015) Cell Division Modes and Cleavage Planes of Neural Progenitors during Mammalian Cortical Development. *Csh Perspect Biol* 7, a015719

63. Iwata, R. and Vanderhaeghen, P. (2021) Regulatory roles of mitochondria and metabolism in neurogenesis. *Curr Opin Neurobiol* 69, 231–240

769 64. Urbán, N. *et al.* (2019) Quiescence of Adult Mammalian Neural Stem Cells: A Highly
770 Regulated Rest. *Neuron* 104, 834–848

771 65. Beckervordersandforth, R. *et al.* (2017) Role of Mitochondrial Metabolism in the Control
772 of Early Lineage Progression and Aging Phenotypes in Adult Hippocampal Neurogenesis.
773 *Neuron* 93, 560-573.e6

774 66. Leeman, D.S. *et al.* (2018) Lysosome activation clears aggregates and enhances
775 quiescent neural stem cell activation during aging. *Science* 359, 1277–1283

776 67. Dulken, B.W. *et al.* (2019) Single-cell analysis reveals T cell infiltration in old neurogenic
777 niches. *Nature* 571, 205–210

778 68. Basak, O. *et al.* (2018) Troy+ brain stem cells cycle through quiescence and regulate
779 their number by sensing niche occupancy. *Proc National Acad Sci* 115, E610–E619

780 69. Düking, T. *et al.* (2022) Ketogenic diet uncovers differential metabolic plasticity of brain
781 cells. *Sci Adv* 8, eabo7639

782 70. Wani, G.A. *et al.* (2022) Metabolic control of adult neural stem cell self-renewal by the
783 mitochondrial protease YME1L. *Cell Reports* 38, 110370

784 71. Petrelli, F. *et al.* (2023) Mitochondrial pyruvate metabolism regulates the activation of
785 quiescent adult neural stem cells. *Sci Adv* 9, eadd5220

786 72. Knobloch, M. *et al.* (2013) Metabolic control of adult neural stem cell activity by Fasn-
787 dependent lipogenesis. *Nature* 493, 226–230

788 73. Sullivan, L.B. *et al.* (2015) Supporting Aspartate Biosynthesis Is an Essential Function
789 of Respiration in Proliferating Cells. *Cell* 162, 552–563

790 74. Birsoy, K. *et al.* (2015) An Essential Role of the Mitochondrial Electron Transport Chain
791 in Cell Proliferation Is to Enable Aspartate Synthesis. *Cell* 162, 540–551

792 75. Chandris, P. *et al.* (2022) Imaging Approaches for the Study of Metabolism in Real Time
793 Using Genetically Encoded Reporters. *Frontiers Cell Dev Biology* 9, 725114

794 76. Gengatharan, A. *et al.* (2021) Adult neural stem cell activation in mice is regulated by
795 the day/night cycle and intracellular calcium dynamics. *Cell* 184, 709-722.e13

796 77. Adusumilli, V.S. *et al.* (2020) ROS Dynamics Delineate Functional States of
797 Hippocampal Neural Stem Cells and Link to Their Activity-Dependent Exit from Quiescence.
798 *Cell Stem Cell* 28, 300–314

799 78. Taylor, M.J. *et al.* (2021) Spatially Resolved Mass Spectrometry at the Single Cell:
800 Recent Innovations in Proteomics and Metabolomics. *J Am Soc Mass Spectr* 32, 872–894

801 79. Hicks, K.G. *et al.* (2023) Protein-metabolite interactomics of carbohydrate metabolism
802 reveal regulation of lactate dehydrogenase. *Science* 379, 996–1003

80. Datta, R. *et al.* (2020) Fluorescence lifetime imaging microscopy: fundamentals and advances in instrumentation, analysis, and applications. *J Biomed Opt* 25, 071203–071203

81. Morrow, C.S. *et al.* (2022) Autofluorescence is a biomarker of neural stem cell activation state. *Biorxiv* DOI: 10.1101/2022.12.14.520430

82. Kobayashi, T. *et al.* (2019) Enhanced lysosomal degradation maintains the quiescent state of neural stem cells. *Nat Commun* 10, 5446

83. Zhang, H. (2018) Lysosomal acid lipase and lipid metabolism: new mechanisms, new questions, and new therapies. *Curr Opin Lipidol* 29, 218–223

84. Reid, M.A. *et al.* (2017) The impact of cellular metabolism on chromatin dynamics and epigenetics. *Nat Cell Biol* 19, 1298–1306

85. Liu, Y. *et al.* (2023) D-2-hydroxyglutarate dehydrogenase governs adult neural stem cell activation and promotes histone acetylation via ATP-citrate lyase. *Cell Reports* 42, 112067

86. Dulken, B.W. *et al.* (2017) Single-Cell Transcriptomic Analysis Defines Heterogeneity and Transcriptional Dynamics in the Adult Neural Stem Cell Lineage. *Cell Reports* 18, 777–790

87. Salway, J.G. (2004) *Metabolism at a Glance*, (3rd edition.), Blackwell Publishing

88. Owen, O.E. *et al.* (2002) The Key Role of Anaplerosis and Cataplerosis for Citric Acid Cycle Function. *J Biol Chem* 277, 30409–30412

89. Fan, J. *et al.* (2015) Metabolic Regulation of Histone Post-Translational Modifications. *Acs Chem Biol* 10, 95–108

90. Ryall, J.G. *et al.* (2015) Metabolic Reprogramming of Stem Cell Epigenetics. *Cell Stem Cell* 17, 651–662

91. Brunet, A. *et al.* (2023) Ageing and rejuvenation of tissue stem cells and their niches. *Nat Rev Mol Cell Bio* 24, 45–62

92. Cheung, T.H. and Rando, T.A. (2013) Molecular regulation of stem cell quiescence. *Nat Rev Mol Cell Bio* 14, 329–340

93. Lunt, S.Y. and Vander Heiden, M.G. (2011) Aerobic Glycolysis: Meeting the Metabolic Requirements of Cell Proliferation. *Annu Rev Cell Dev Bi* 27, 441–464

94. Chandel, N.S. *et al.* (2016) Metabolic regulation of stem cell function in tissue homeostasis and organismal ageing. *Nat. Cell Biol.* 18, 823–832

95. Coller, H.A. (2019) The paradox of metabolism in quiescent stem cells. *Febs Lett* 593, 2817–2839

96. Nakamura-Ishizu, A. *et al.* (2020) Hematopoietic Stem Cell Metabolism during Development and Aging. *Dev Cell* 54, 239–255

837 97. Filippi, M.-D. and Ghaffari, S. (2019) Mitochondria in the maintenance of hematopoietic
838 stem cells: new perspectives and opportunities. *Blood* 133, 1943–1952

839 98. Jun, S. *et al.* (2021) The requirement for pyruvate dehydrogenase in leukemogenesis
840 depends on cell lineage. *Cell Metab.* 33, 1777-1792.e8

841 99. Liang, R. *et al.* (2020) Restraining Lysosomal Activity Preserves Hematopoietic Stem
842 Cell Quiescence and Potency. *Cell Stem Cell* 26, 359-376.e7

843 100. Yucel, N. *et al.* (2019) Glucose Metabolism Drives Histone Acetylation Landscape
844 Transitions that Dictate Muscle Stem Cell Function. *Cell Reports* 27, 3939-3955.e6

845 101. Ryall, J.G. *et al.* (2015) The NAD+-Dependent SIRT1 Deacetylase Translates a
846 Metabolic Switch into Regulatory Epigenetics in Skeletal Muscle Stem Cells. *Cell Stem Cell*
847 16, 171–183

848 102. Zheng, X. *et al.* (2016) Metabolic reprogramming during neuronal differentiation from
849 aerobic glycolysis to neuronal oxidative phosphorylation. *Elife* 5, e13374

850 103. Yu, Y. *et al.* (2019) Glutamine Metabolism Regulates Proliferation and Lineage
851 Allocation in Skeletal Stem Cells. *Cell Metab.* 29, 966-978.e4

852 104. Kim, C.S. *et al.* (2020) Glutamine Metabolism Controls Stem Cell Fate Reversibility and
853 Long-Term Maintenance in the Hair Follicle. *Cell Metab.* 32, 629-642.e8

854 105. Homem, C.C.F. and Knoblich, J.A. (2012) Drosophila neuroblasts: a model for stem
855 cell biology. *Development* 139, 4297–4310

856 106. Sen, A. *et al.* (2013) Drosophila Clueless Is Highly Expressed in Larval Neuroblasts,
857 Affects Mitochondrial Localization and Suppresses Mitochondrial Oxidative Damage. *Plos*
858 *One* 8, e54283

859 107. Grant, J. *et al.* (2010) A Drosophila model for primary coenzyme Q deficiency and
860 dietary rescue in the developing nervous system. *Dis Model Mech* 3, 799–806

861 108. Homem, C.C.F. *et al.* (2014) Ecdysone and Mediator Change Energy Metabolism to
862 Terminate Proliferation in Drosophila Neural Stem Cells. *Cell* 158, 874–888

863 109. Bailey, A.P. *et al.* (2015) Antioxidant Role for Lipid Droplets in a Stem Cell Niche of
864 Drosophila. *Cell* 163, 340–353

865 110. van den Ameele, J. and Brand, A.H. (2019) Neural stem cell temporal patterning and
866 brain tumour growth rely on oxidative phosphorylation. *Elife* 8, e47887

867 111. Motori, E. *et al.* (2020) Neuronal metabolic rewiring promotes resilience to
868 neurodegeneration caused by mitochondrial dysfunction. *Sci Adv* 6, eaba8271

869 112. Kim, D.K. *et al.* (2022) Aβ-induced mitochondrial dysfunction in neural progenitors
870 controls KDM5A to influence neuronal differentiation. *Exp Mol Medicine* 54, 1461–1471

871 113. Baranov, S.V. *et al.* (2019) Mitochondria modulate programmed neuritic retraction. *Proc*
872 *National Acad Sci* 116, 650–659

873 114. Ioannou, M.S. *et al.* (2019) Neuron-Astrocyte Metabolic Coupling Protects against
874 Activity-Induced Fatty Acid Toxicity. *Cell* 177, 1522-1535.e14

875 115. Breckwoldt, M.O. *et al.* (2014) Multiparametric optical analysis of mitochondrial redox
876 signals during neuronal physiology and pathology in vivo. *Nat Med* 20, 555–560

877 116. Mächler, P. *et al.* (2016) In Vivo Evidence for a Lactate Gradient from Astrocytes to
878 Neurons. *Cell Metab* 23, 94–102

879 117. Zhao, Y. *et al.* (2016) In vivo monitoring of cellular energy metabolism using SoNar, a
880 highly responsive sensor for NAD+/NADH redox state. *Nat Protoc* 11, 1345–1359

881 118. Lobas, M.A. *et al.* (2019) A genetically encoded single-wavelength sensor for imaging
882 cytosolic and cell surface ATP. *Nat Commun* 10, 711

883 119. Li, L. *et al.* (2022) Spatially resolved proteomics via tissue expansion. *Nat Commun* 13,
884 7242

885 120. Delcourt, V. *et al.* (2018) Spatially-Resolved Top-down Proteomics Bridged to MALDI
886 MS Imaging Reveals the Molecular Physiome of Brain Regions. *Mol Cell Proteom Mcp* 17,
887 357–372

888