

# Introduction to embryonic and adult neural stem cells: from the metabolic circuits of the niches to the metabolome

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## Proceedings

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*Stem cells: present and future*

Guest Editors: Gavino Faa, Vassilios Fanos, Antonio Giordano

## Abstract

Metabolomics has provided new insight into the biology that drives the phenotype of stem cells. During the recent years, metabolic circuits of embryonic and neural stem cells (NSCs) have been better elucidated. Many factors contribute to stem cell determination fate: metabolism, transcriptional signaling, epigenetics, extrinsic mechanisms such as short-range signals from the niche and humoral signals. The metabolism decides if a cell proliferates, differentiates or remains quiescent. Embryonic and adult NSCs share two features: they generate at least one daughter cell and can differentiate into specialized cells. NSCs use different pathways depending on their stage of differentiation: glycolysis is highest in proliferating stem cells and it is essential for stemness. Conversely, oxidative phosphorylation supports differentiated cells. Moreover, lipid metabolism maintains proliferation and neurogenesis; indeed, fatty acid oxidation and fatty acid synthesis represent a component of stem cell fate regulation.

## Keywords

Neural stem cells, metabolomics, metabolism, neurogenesis, mitochondria.

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## Stem cells

Stem cells are blank cells, unspecialized, capable of proliferating and regenerating, keeping at the same time an undifferentiated state. This process is called “asymmetric division” because it involves the production not only of new stem cells but also of cells that have the potential to differentiate into specialized cells. The third feature of stem cells is their paracrine function.

Thus, they act as a complex biopharmacy with different functions: tissue repair, regulation of cell differentiation and anti-inflammatory action [1, 2].

Stem cells can be divided into three groups, according to their different potential for development: totipotent cells, which constitute a zygote, capable of giving rise to a new individual; pluripotent cells (15 days after fertilization), capable of producing all types of tissue cells; and multipotent cells, more differentiated cells which maintain the self-renewal capacity but, in addition, can also differentiate into cells that belong to a specific tissue. Stem cells have been identified in many organs and tissues of human body. Neural stem cells (NSCs) are included in the multipotent group because they are tripotent cells, since they can proliferate and differentiate into neurons, oligodendrocytes and astrocytes [3].

## Neural stem cells: a little history

Up to 1960, scientists believed in the dualistic theory of His, according to which in the primitive neural tube there were two distinct types of

progenitor cells: the neuroblasts, producing germ cells and neurons, and the spongioblasts, the precursors of glial cells. It was thought that in the brain NSCs were absent and that neurons, once having developed in the brain, had no regenerative capacity. Scientists were convinced that stem cells could only belong to more plastic tissues like hematopoietic tissue [4].

These dogmas of neurobiology were revisited in 1961 when Smart and Leblond published the first article about evidence, in the mouse brain, of neuro-glial cells in the mitotic phase [5].

Those cells were not yet differentiated into neurons and therefore not yet migrated into the olfactory bulb. Later, Altman in 1962 and Altman and Das in 1965 published the results of their studies, according to which, in adult brains, the birth of new neurons occurred [6, 7]. Consequently, different authors described mitotic neurons in several species [8-14].

In the 2000s it became obvious that, in the adult brain, new neurons grow and specialize. However, the rate of neurogenesis was slower than in the fetal brain and the new neurons were capable of forming new synapses with pre-existing cells. It is estimated that the adult brain, at hippocampal level, forms about 700 new neurons a day [15, 16]. This active neurogenesis has an enormous importance for the concept of permanent structural plasticity of the brain and is not limited to specific periods of development. By 2013, the sites of the niche of NSCs in adult brain were also revealed: adult neurogenesis occurs in the subventricular zone (SVZ) of the anterolateral ventricle, in the hippocampal subgranular zone (SGZ), along the midline near the third and fourth ventricle, in the forebrain, the striatum, amygdala, hypothalamus, at the level of the substantia nigra and in the subcortical white matter [17-20].

## Embryonic neurogenesis

The embryonic plate is homogeneous in terms of cellular morphology because it is composed of neuro-epithelial cells that develop, after the formation of the neural tube, into radial glial cells. Radial glial cells are multipotent and become neurons during the first stage of gestation, while gliogenesis occurs in the last stage of gestation. Radial glial cells are bipolar, having an apical extension in contact with the lateral ventricles and a basal process that touches the pial surface. It seems that this feature is essential in determining

their function and behavior as they receive signals from several distinct regions of the brain [21, 22].

These cells have a high rate of replication, are able to repair themselves, form new radial glial cells and differentiate into neural progenitor cells and neurons through the process of asymmetric division. Near the birth, glial cells change their features and generate NSCs that will represent a useful pool for adult neurogenesis and astrocytes and oligodendrocytes [23, 24].

During intrauterine life, neural and pial proliferation is very active and localized in two zones: the ventricular and the subventricular. NSCs are localized in the ventricular zone. In the human embryo and fetus, distribution, morphology, growth and number of NSCs vary widely depending on the gestational age. They are variously distributed in the cortex, hippocampus, striatum, olfactory bulb, near the ventricles in the midbrain, cerebellum, spinal cord and retina [25]. During intrauterine brain development there is no evidence of quiescent NSCs, probably because of the high need of neural cells in the developing human brain [26].

### Adult neurogenesis

Currently, scientists believe that adult NSCs are the descendants of subventricular radial glial cells; consequently, they arise from embryonic neuroepithelial cells [27, 28].

The adult brain is less versatile than the embryonic one. The adult NSCs show several differences from radial cells: most of them are quiescent and, when duplicating, the cell cycle is longer than the embryonic one. They live in particular areas, complex and stable (the niches). Finally, they are not so multipotent as believed in the past. Thus, they can only turn into two specific neuronal types: periglomerular neurons in the SVZs and granule neurons in the dentate gyrus. In fact, adult neurogenesis is traditionally located in the SVZ of the anterolateral ventricle, characterized by cells that give rise to new neurons that migrate into the striatum in the adult human brain and olfactory bulb in the murine brain (through the rostral migratory olfactory route) [29-33].

In the SGZ is located another area of adult neurogenesis, which produces neurons for the dentate gyrus and astrocytes [34]. Recently, different authors have described new niches along the midline near the third and fourth ventricle [35,

36]. Further niches have been found also in the forebrain, the striatum, amygdala, at the level of the substantia nigra, the subcortical white matter and the hypothalamus [37, 38].

Since the morphology of adult NSCs is similar to that of embryonic radial glial cells, they are called radial glia-like cells. The adult subventricular region is very similar to SVZ of the embryo [33].

Until few years ago it was thought that, differently from embryonic stem cells (ESCs), adult NSCs, did not enter in contact with the liquor. Since rodent SVZ possesses some similarities with humans and are better studied than human SVZ, they have helped us to understand the human cellular architecture of this area. In both the human and the rodent brain, the progenitor of adult SVZ are radial glia-like cells. However, differently from humans, in the murine SVZ it is possible to identify four types of cells:

1. *NSCs (or B cells)*, slowly dividing NSCs showing a non-mobile primary cilium that reaches the ventricular lumen. B cells have a long basal process ending near the blood vessels and another, apical, similar to the embryonic one dipped in the CSF. This process is surrounded by ependymal cells arranged to form a pinwheel feature [39]. It is thought that this organization is essential for neurogenesis and that the contact with the ventricle may have an important function in the physiology of the nervous system. B cells express Glial Fibrillary Acidic Protein (GFAP), Glutamate Aspartate Transporter (GLAST), Brain Lipid-Binding Protein (BLBP) and nestin [40-43]. However, up to now, it does not exist a specific marker to identify type B cells.
2. *Intermediate progenitor cells (C)*, rapidly proliferating cells that express the Epidermal Growth Factor (EGF) receptor [44].
3. *Type A cells*, neuroblasts expressing markers typical of newborn neurons: doublecortin and Polysialylated-Neural Cell Adhesion Molecule (PSA-NCAM).
4. *Type E or ependymal cells*, which are post-mitotic and multiciliated cells. They express S100B protein and CD24 [39, 45].

It is believed that B cells generate C cells, which in turn produce ependymal cells. The human SVZ, compared to the SVZ of rodents, displays a peculiar cell organization. It is composed of four layers: in the first there are multiciliated

ependymal cells; the second layer is formed by astrocytes and cell bodies interdigitated with expansions of ependymal cells; in the third there are B cells, oligodendrocytes-like precursors and ependymal cells; finally, the fourth layer is composed by neuronal bodies and myelin tracts [46-49].

The NSCs located in the hippocampus are capable of differentiating exclusively in neurons of the dentate gyrus. Their migration is limited to the areas near the site of origin. The radial glia-like cells (or cells I) generate granule neurons through a process that includes cells with an increasing degree of maturation variable from less mature cells (IIa, IIb, type II cells) up to adult neurons.

The “niches” contain, in addition to NSCs, neurons, astrocytes, axons, the extracellular matrix and a network of interconnected vessels. Since the capillaries in the niches are fenestrated, they are called “windows of the brain”. Thus, niches may receive, integrate and respond to messages that come from outside because they are adjacent to blood vessels (10-12  $\mu$ ) and in contact with other neurons and cells such as microglia and astrocytes. The NSCs of the SVZ area, in particular, are associated with projections of the vascular basal lamina called “fractones”, which are rich in extracellular matrix components and growth factors [50].

## Human metabolome

It seems that, in human body, there are about 500 different cell types showing a unique cellular metabolome. Human metabolome consists of a group of about 41,511 small molecules (metabolites) found by using mass spectrometry or Nuclear Magnetic Resonance (NMR) spectroscopy, in a given tissue or fluid, *in vitro* and 1H-Magnetic Resonance Spectroscopy (1H-MRS), which is the correlate of NMR, *in vivo*. The number of identified human metabolites is lower compared with transcriptomes (85,000) and proteomes (10,000,000) [50].

Metabolites in a biological system are the result of both genetic and environmental influences (epigenetics). Therefore, differently from genome and proteome, metabolomic profiles do not depend only on our genes. Endogenous metabolites are synthesized by the enzymes encoded by our genome or by our microfloral genomes and are also related to the environment (food, drinks, breathed substances,

gut microbioma). Metabolomics presents many advantages in comparison with transcriptomics and proteomics: it is cheaper, feasible in a large number of samples, it enables the identification of endogenous molecules belonging to many metabolic pathways and the whole genome is not required [52].

The level of each metabolite found in the final tissue phenotype results from what humans ingest, metabolize, catabolize or come into contact with. The profiles include a collection of peptides, lipids, amino acids, nucleic acids, carbohydrates, organic acids, vitamins, minerals, and exogenous compounds like food additives, drugs, toxins, pollutants with a small molecular weight (< 2,000 Da [53]).

Thus, the human metabolome consists of a mix of both endogenous and exogenous compounds. Metabolic profiling is generally applied to biofluids like urine, cerebrospinal fluid, plasma and saliva. Moreover, it is also possible to research metabolites in extracts of tissues and cell lysis. Thus, there are two kinds of stem cell sources, the “*in vitro*” ones (cellular) and the “*ex vitro*” ones (tissue), depending on the origin of the sample. The profile varies from the *metabolomic fingerprint*, corresponding to intracellular metabolites, to the *metabolomic footprinting*, which includes secreted metabolites mostly used in microbial metabolomics [53, 54].

Recently, scientists have discovered how to use both intracellular and secreted metabolites as possible biomarkers of specific cell types such as human pluripotent stem cells and neural progenitor cells [55].

Metabolites commonly detected by 1H-MRS as different peaks are N-acetylaspartate (NAA), creatine, choline, myo-inositol, alanine, lactate, glutamine, glutamate and some macromolecular proteins and lipids [56]. In the last few years, several studies have shown how stem cells use different metabolic circuits depending on their stage of differentiation [57].

Currently, the authors are investigating to determine whether very preterm infants have a low density of neural progenitor stem cells and if this low density could cause long-term cognitive deficits. Stem cells are very plastic and it is also possible that differentiated cells could revert back into stem cells. For this reason, these cells do not have a specific fixed metabolic profile, but a profile corresponding to every particular stage of differentiation.

Advances in metabolomics have underlined the role of specific pathways in the fate of stem cells. Thus, it is the metabolism that decides if a cell proliferates, differentiates or remains quiescent.

### Metabolic circuits in undifferentiated and differentiated stem cells

During the development of the brain, but also after birth and in adult life, the intracellular metabolic reactions have a very rapid kinetics. Metabolomics allows us to measure metabolite fluxes both within and outside cells in the different phases of differentiation. Neurogenesis is regulated by many extracellular cues like Epidermal, Fibroblast and Insulin-like Growth Factors. It is thought that, in NSCs, Insulin-like Growth Factor 1 (IGF1), EGF and Vascular Endothelial Growth Factor (VEGF) stimulate the P13K-Akt pathway, the main regulator of cellular metabolism. This pathway acts on the glucose, glutamine and lipid metabolism by using different effectors like Forkhead transcription factors (FOXO), mammalian Target of Rapamycin (mTOR), Hypoxia-Inducible Factor 1- $\alpha$  (HIF-1- $\alpha$ ), myelocytomatosis oncogene (Myc) and Tumor Suppressor Protein (p53). The mTOR and 5' AMP-activated Protein Kinase (AMPK) regulate the stem cells energy production by stimulating glycolysis and inhibiting oxidative phosphorylation (OXPHOS) [58]. FOXO factors can elicit the cell cycle arrest, resistance to oxidative stress and apoptosis [50, 60]. In particular FOXO3 is a member of a transcription factor family which regulates NSC pool. FOXO proteins regulate the stem cells cycle and the DNA repair [61]. Moreover, FOXO proteins activate genes to combat oxidative stress. Conversely, p53 is necessary for differentiation and neurite outgrowth.

#### *The role of the oxygen in the neurodevelopment of the stem cells*

The partial pressure of oxygen in the brain ranges from 0.55 (4.1 mmHg) in the midbrain to 8% (60 mmHg) in the pia, so that the neurogenic stem cell niches are hypoxic. Probably stem cells have a selective survival advantage by maintaining an undifferentiated state with low oxidative stress. It is believed that low O<sub>2</sub> represses neuronal differentiation, regulates death and differentiation

of CNS precursor types. Hypoxia appears to increase neurogenesis *in vitro* activating the HIF, which, in turn, regulates the production of erythropoietin and VEGF. Oxygen is a source of Reactive Oxygen Species (ROS), which are a group of highly active, reduced forms of molecular oxygen such as superoxide and hydroxyl radical. ROS are produced by mitochondrial OXPHOS and are normally neutralized by antioxidants. It seems that endogenous ROS increases self-renewal and neurogenesis while FOXO are involved in proliferation, differentiation and cellular survival. Under stress conditions ROS can increase and damage the DNA [62]. In the brain ROS can also be generated following a systemic inflammation. Excessive levels of ROS can cause mitochondrial dysfunction, which consequently inhibits the Tricarboxylic Acid cycle (TCA cycle) and the electron transport chain. FOXO protect against ROS by stimulating antioxidants and DNA repair enzymes. Interestingly, mitochondrial dysfunction and impaired bioenergetics seem to be a common denominator of neurological diseases like autism, depression, bipolar disorder, chronic fatigue syndrome, schizophrenia and Parkinson's disease [63]. In addition, in post-mortem brain tissue obtained from patients affected by Parkinson's disease, a reduction of the number of SVZ and SGZ progenitors has been found [64].

However, ROS are not only harmful because they play an important role in cell fate decision.

### Metabolic and spectroscopic profiling

Metabolic and spectroscopic profiling of stem cells encompasses the following compounds: mobile lipids visible on proton NMR (NMR mobile lipids), the choline-containing compounds, the energy metabolism (glycolysis vs. OXPHOS). Maturation markers are absent. NMR mobile lipids are associated with apoptosis and necrosis. They are not stem cell-specific and have been associated with cellular stress. It is believed that peak of the the fatty acid methylene at 1.28 pp is a marker of neural progenitor cells [65].

*Choline-containing compounds* are related to membrane lipid synthesis and cellular proliferation. They decrease with cell differentiation and maturation of the postnatal rat brain and human fetal brain [66-68].

*Amino acids* play a key role in the discrimination of differentiated cells. Alanine, glycine, and glutamate are elevated in mesenchymal stem cells.

Glutamine is quantitatively the most significant amino acid in plasma. In the brain, glutamine is converted to glutamate by a glutaminase (GLS). Adult neural stem progenitor cells express GLS and glutaminolysis is very important for redox balance and glutathione (GSH) biosynthesis. Transcription factors such as FOXO, Myc and p53 regulate this pathway through target gene activation.

Glutamine can be used as a carbon source after having been converted to glutamate and then alpha-ketoglutarate, a key component of TCA. Zhang et al. in 2014 demonstrated that ESCs rely on glutamine to fuel their proliferative state [69]. Serine and glycine are synthesized in stem cells from the glycolysis. These amino acids are involved in biosynthesis, redox and methylation, being precursors of purines, GSH and lipids. Therefore, they can regulate ROS levels through GSH. There is a high concentration of taurine in the developing brain of rats, while it decreases during maturation. Taurine stimulates NSC proliferation and threonine is important for murine ESC propagation.

Threonine is an essential amino acid whose levels increase during differentiation in association with an increase in one-carbon metabolism for purine biosynthesis [68, 70, 71]. Threonine contributes to the synthesis of glycine; consequently, it is involved in epigenetic regulation.

The RNA and DNA content is high in ESCs. During the development of the brain, the amount of RNA decreases, while the concentration of lipid and protein rises. NAA is a metabolite found in mature neurons whose function has not yet been clarified. It is used as a marker of mature neurons together with myo-inositol. Markers of mature cells are absent in neural progenitor cells and increase during the development of the fetal brain [72-76]. However, in 2013, Chung et al., using a high-resolution <sup>1</sup>H-MRS to study undifferentiated and differentiated human striatal NSCs, demonstrated that the absolute levels of NAA, the traditional indicator of mature neurons, were not significantly different between differentiated and undifferentiated cells [77]. Moreover, differently from previous evidence, the study by Chung and colleagues revealed that the concentration of myo-inositol was almost 50-fold lower with differentiation. In addition, threonine, glutamate and alanine concentrations decreased between three- and five-fold in dif-

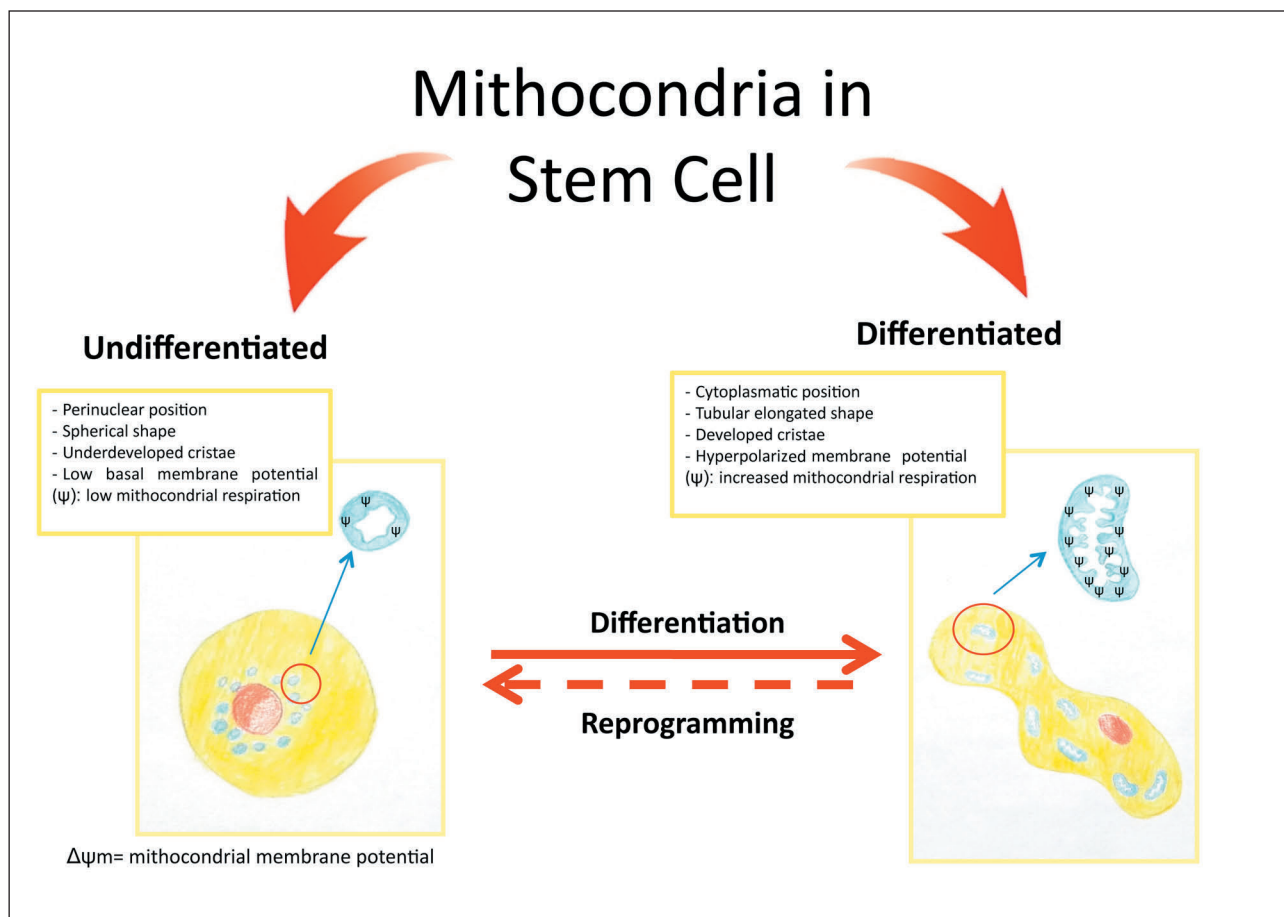
ferentiated cells. Although many metabolites decreased, lipid concentration increased upon differentiation.

Takubo et al. in 2013, using metabolome analysis in mice, found a biomarker of HSCs, the fructose 1,6-bisphosphate, which increases pyruvate levels by activating pyruvate kinase (PK) [78].

## Energy metabolism

Mitochondria are specialized organelles of bacterial origin that are involved in energy production, cell signaling, apoptosis and calcium homeostasis [79].

These semiautonomous organelles are the site of the pathways of the intermediate metabolism, amino acid biosynthesis, Fatty Acid Oxidation (FAO) and steroid metabolism. In addition, they are the most important source of endogenous ROS produced through OXPHOS. ROS lead to mutation and alter protein folding and DNA structure. Recent studies have demonstrated that ROS have not only deleterious effects for the cells but also a physiological role [80]. In the past, mitochondria were considered the “energy powerhouse” of cells [81]. It is known that mitochondria play an important role in pluripotency. Recently, scientists have discovered that these organelles are relatively inactive in stem cells, while they become very hardworking in differentiated cells. Pluripotent stem cells show immature mitochondria with a globular shape, cristae-poor morphology, decreased OXPHOS (with high lactate production) and perinuclear localization. All these features indicate a less active mitochondrial state. Conversely, mitochondria of differentiated cells are elongated, cristae-rich and have an activated TCA cycle [82, 83] (**Fig. 1**). Probably the anaerobic metabolism typical of pluripotent stem cells is promoted by mitochondrial Uncoupling Protein 2 (UCP2) together with the high levels of hexokinase II and the low levels of pyruvate dehydrogenase [84, 85]. UCP2 can also suppress ROS production [83]. However, the precise indicator of stem cell differentiation is not the metabolic shift toward OXPHOS, but rather the ratio of glycolysis/mitochondrial glucose oxidation [69]. Thus, not only can mitochondria mediate catabolic and anabolic processes, but they also intervene in the regulation and differentiation of stem cells. These organelles have their own genome, the circular



**Figure 1.** Mitochondrial differentiation and reprogramming involves structural and functional shift.

mitochondrial DNA, encoding for 13 essential protein subunits of I, III, IV and V complexes of the respiratory chain [86, 87]. Low mitochondrial DNA is associated with more undifferentiated cells and anaerobic metabolism, whereas the increase of mitochondrial number and mass is related to the differentiation and aerobic metabolism [88-90].

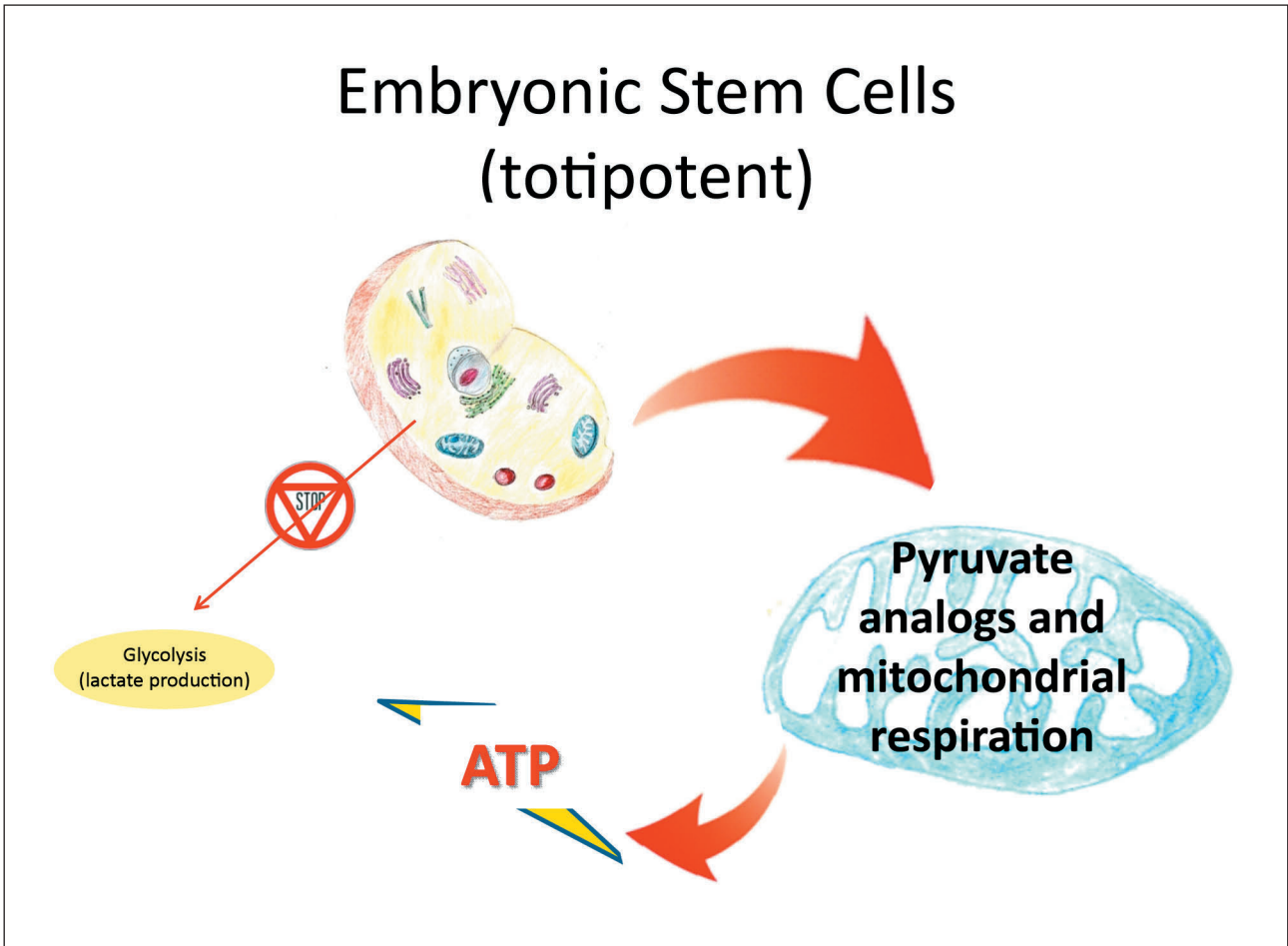
### Glucose metabolism

For each molecule of glucose, 38 ATPs can be generated, providing energy for protein synthesis, growth and proliferation [91]. Under hypoxia, ATP can be produced at a rapid rate because glycolysis can create a larger percentage of ATP than OXPHOS [92, 93].

$O_2$  concentrations can regulate the differentiation of NSCs. Hypoxia reduces proliferation maintaining cell quiescence. Since HIF-1- $\alpha$  is essential to maintain anaerobic glycolysis, it could reprogram cells to go back to a glycolytic phenotype typical of undifferentiated cells [94, 95].

In totipotent (embryonic) stem cells, glycolysis is impaired because two different rate-limiting enzymes (hexokinase and phosphofructokinase-1) have low activity and ATP synthesis depends only on pyruvate analogs and mitochondrial phosphorylation (**Fig. 2, Tab. 1**). Mitochondria, at this stage, are structurally immature. Consequently, in these unspecialized cells, the net oxygen consumption is low. It is believed that differentiation from ESCs into neural progenitors is stimulated by both eicosanoid pathway and fatty acid metabolism. Thus, the inhibition of the eicosanoid pathway increases the unsaturated fatty acids concentration and preserves pluripotency. Unsaturated fatty acids contain a high number of carbon-carbon double and triple bonds, which makes them susceptible to oxidative reactions [96].

Pluripotent and multipotent stem cells like NSCs rely on glycolysis regardless of the oxygen concentration; for this reason, this process is called aerobic glycolysis (**Fig. 3, Tab. 1**). This switch from mitochondrial phosphorylation to a



**Figure 2.** Metabolism of embryonic stem cells (ESCs) depends on pyruvate analogs and mitochondrial phosphorylation while glycolysis is impaired.

**Table 1.** Finding on undifferentiated and differentiated stem cell metabolism.

Type of cell	Glycolysis	Mitochondrial respiration	Lipolysis (fatty acid oxidation)
Totipotent	+	++	++++
Pluripotent	+++++	+	+++
Multipotent	+++++	++	++
Differentiated	+++	+++++	+

high rate of glycolysis is followed by hyperlactate production. Although glycolysis produces only two ATPs, in NSCs this pathway is very fast. Consequently, it produces a higher number of ATP than mitochondrial OXPHOS.

During this state, the cells increase growth and metabolic activity; the glucose uptake and flux into the cells arise because of the upregulation of the expression of the Glucose Transporters

GLUT1 and GLUT3 [97]. From the glucose catabolism, different biosynthetic pathways start, involving glycosaminoglycans, proteins and nucleotides. Indeed, amino acid and nucleotide synthesis increases due to the increase of glycolytic intermediates. Nucleotide synthesis is very important in order to maintain pluripotency and proliferation. Similarly, NSCs show a high glycolytic rate that declines during differentiation [84].

The metabolism in the quiescent NSCs is oriented to avoid cellular damage from ROS. NSCs live in hypoxic niches and their metabolism depends on glycolysis (Fig. 4, Tab. 2). Since ROS causes apoptosis, it seems that quiescent NSCs prefer a slow-cycling state to avoid being damaged because glycolysis is associated with a reduction in ROS production. It is believed that GSH is the main cellular antioxidant and the enzymes controlling its synthesis, Glutamate-Cysteine Ligase (GCLM) and GSH Synthetase



## Pluripotent and Multipotent Stem Cells

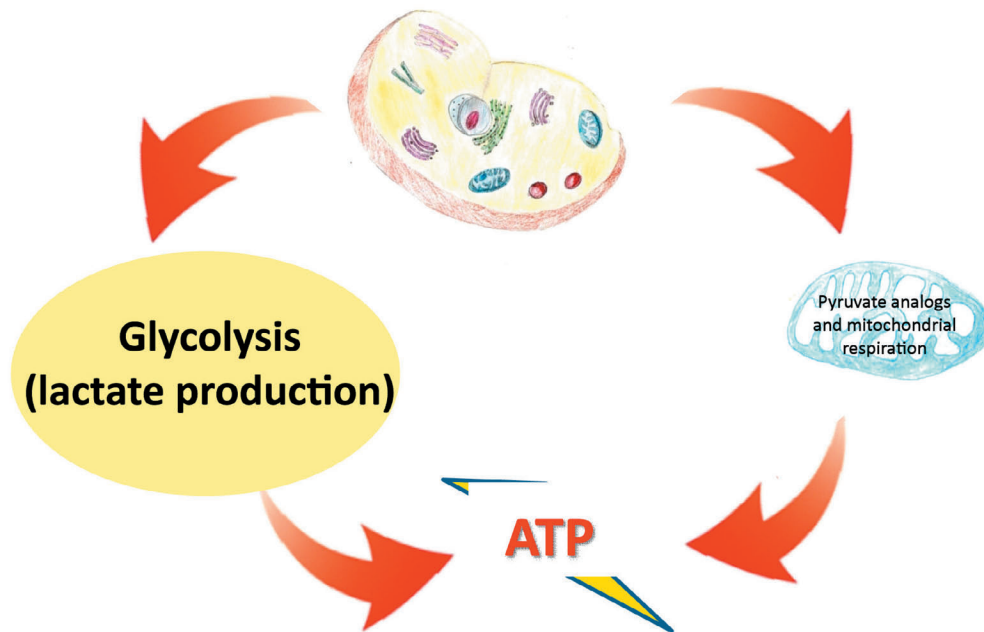


Figure 3. Metabolism of pluripotent and multipotent stem cells depends on glycolysis with hyperlactate production.

## Quiescent Neural Stem Cells

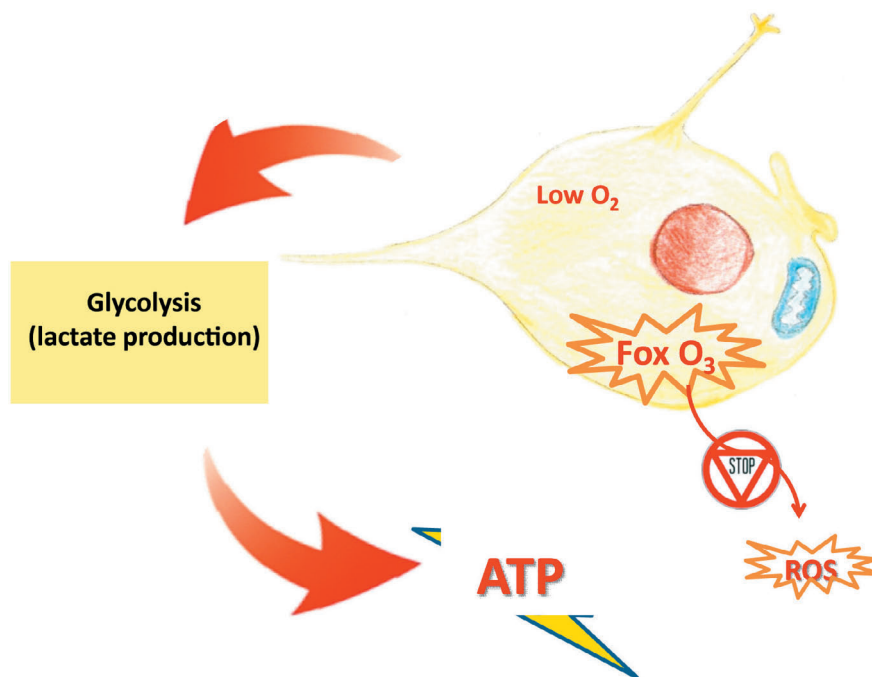


Figure 4. Metabolism in quiescent neural stem cells (NSCs) depends on glycolysis, which is associated with a low production of Reactive Oxygen Species (ROS).

**Table 2.** Finding on quiescent neural stem cells (NSCs) and neural progenitors metabolism.

	Glycolysis	Mitochondrial respiration	ROS	FOXO3	Lipogenesis (fatty acid synthase)
qNSCs	+++++	+	+	+++++	+
Neural progenitors	+++	+++++	++++	+	+++

qNSCs: quiescent neural stem cells; ROS: Reactive Oxygen Species; FOXO3: a member of Forkhead transcription factor family.

(GSS), are elevated in stem cells in order to prevent oxidative stress. Once neural progenitors start to differentiate, the glycolysis rate decreases and becomes more coupled to the Krebs cycle. This process is confirmed by a decreased lactate production.

Together with the development of mitochondria, the metabolism of more differentiated cells depends on mitochondrial oxidation for proliferation (**Fig. 5**). This preferred metabolic circuit is confirmed by metabolomic studies: many of the metabolites identified in differentiated cells were pyrimidine and purine nucleotides, and tricarboxylic acids involved in the Krebs cycle.

Neural progenitors, which live in a condition of normoxia, upregulate both glycolysis and OXPHOS (**Fig. 6**). It is believed that both fatty acid synthesis and the increase of ROS resulting from FOXO3 suppression act as primer of neural proliferation. In 2009, Renault and coworkers showed that, in mice, FOXO3 regulates the NSC pool and that its activity is higher in self-renewing NSCs than in differentiated progeny [61].

### Analogies between embryonic development and stem cell fate

The study of the developmental embryogenesis has led to the concept of “metabolic centric control” of stem cell differentiation. The oocyte has a high number of mitochondria, which, despite being spherical, small and having few and truncated cristae, can easily generate oxidative ATP to support metabolic requirement [98] (conversely, women in their forties show senescent mitochondria with abnormal morphology and declined function). Since the rate of glycolysis is inhibited due to the reduced function of certain enzymes, after fecundation, the single cell embryo relies much more on oxidative metabolism [99]. During morula formation, the mitochondrial content per cell declines, while the number of glucose transporters increases; consequently, gly-

colytic rates increase at this stage. Anaerobic glycolysis is also necessary for the cells, which have to live in the hypoxic environment of the uterine wall. Following implantation, blood flow begins in the embryo and glycolysis declines, while oxidative metabolism restarts. Similarly to their tissue of origin, pluripotent and multipotent stem cells have immature mitochondria with a perinuclear position, reduced oxidative capacity and are dependent on glycolysis. The metabolism of differentiated cells, similarly to the implanted embryo cells, rely on oxidative metabolism [100].

### Lipid metabolism

As glycolysis in neural stem progenitors cells regulates development, proliferation and differentiation, lipid metabolism maintains proliferation and neurogenesis. It is believed that unsaturated fatty acid preserve pluripotency. Yanes et al., in 2010, discovered that ESCs have a high level of unsaturated lipids and fatty acids while their levels decrease during differentiation [96].

Indeed, omega 6 and omega 3 fatty acids (arachidonic, docosahexanoic and linoleic acid) are typical substrates of pluripotent state.

Later, Ito et al. in 2012, identified that fatty acid breakdown pathway (FAO) improved the undifferentiated stem cell maintenance [101]. Conversely, in adult stem cells lipogenesis seems to be very active (**Tab. 1** and **Tab. 2**). Indeed, Knobloch et al. in 2013 demonstrated that adult neural stem progenitor cells express high levels of Fatty acid synthase (Fasn), the rate-limiting enzyme for lipogenesis. Furthermore, the inhibition of the eicosanoid pathway promotes pluripotency and FAO is undetectable in more differentiated cells [102, 103]. Adult stem cell turnover is regulated by epigenetic modifications like DNA methylation [104]. The S-adenosyl methionine cycle is very important in the differentiation from ESCs and pluripotent stem cells (PSCs). The metabolites

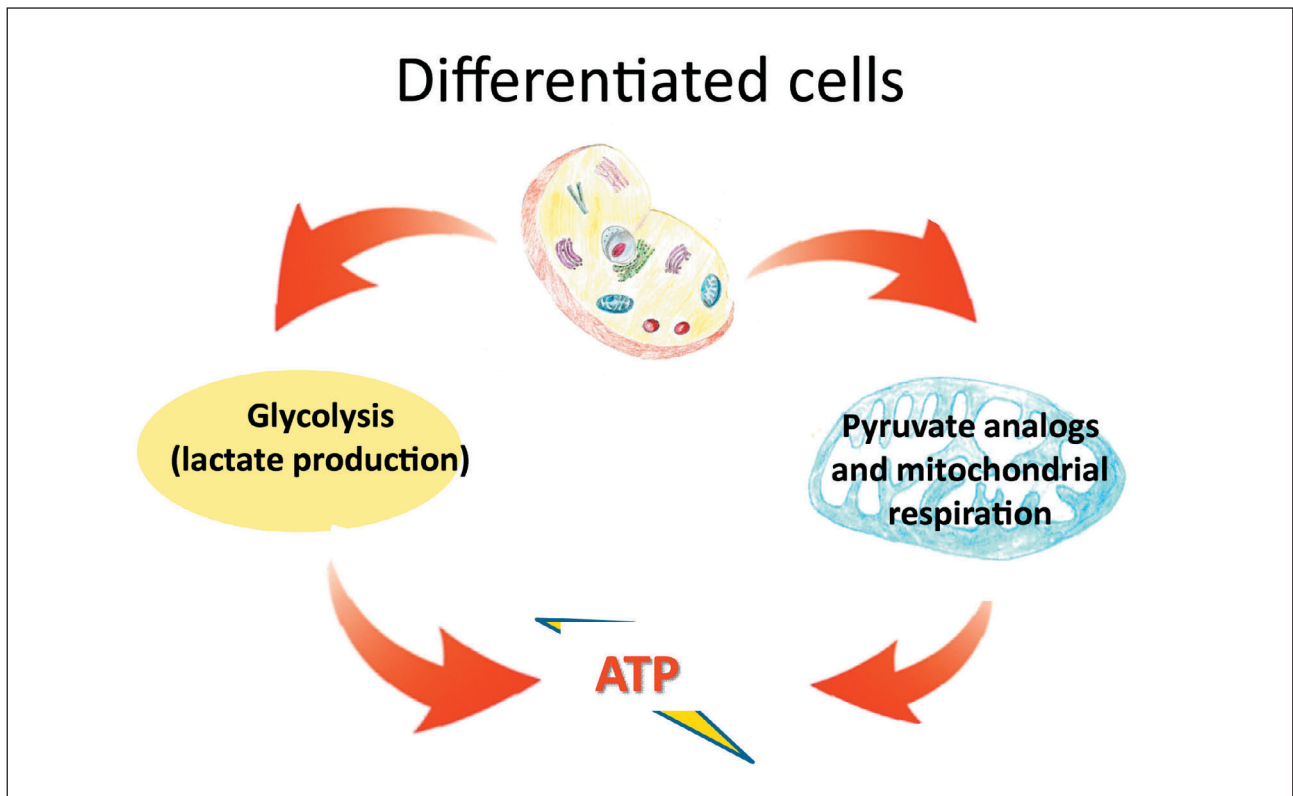


Figure 5. Metabolism in differentiated cells depends on mitochondrial oxidation.

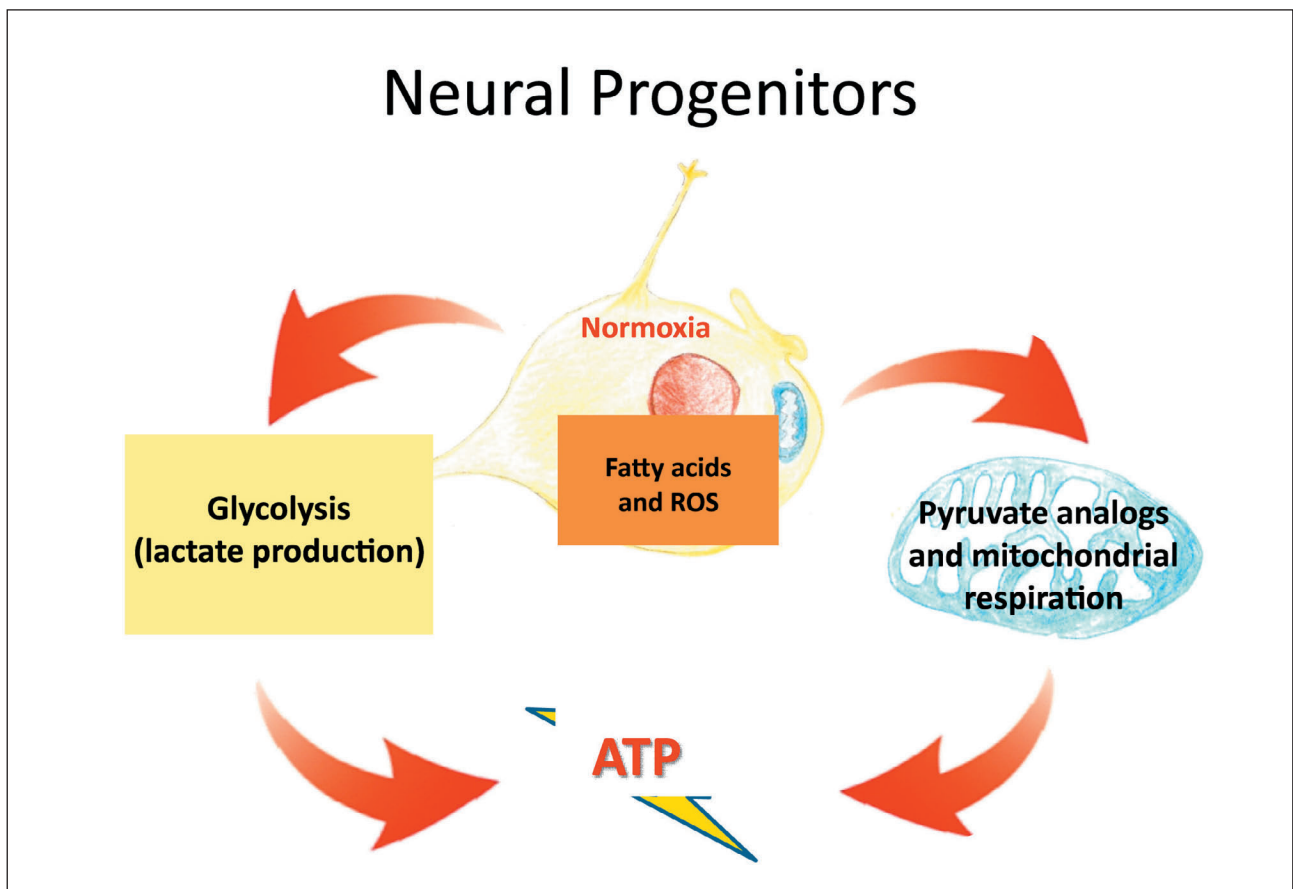


Figure 6. Metabolism in neural progenitors depends on both glycolysis and oxidative phosphorylation (OXPHOS).

belonging to this cycle are more elevated in PSCs than ESCs. Methionine deprivation causes cell apoptosis [105, 106].

### Systemic energy metabolism

NSCs are influenced by systemic energy metabolism. Indeed, while dietary restriction (without malnutrition) and exercise promote the birth of new neurons, on the contrary stress, neuroinflammation, diabetes and aging reduce neurogenesis (Fig. 7) [107-110].

Recently Rando and Simmons published a review article about the evidence that an adverse intrauterine environment (pregnancies complicated by intrauterine growth restriction, obesity, diabetes and parental diet) could influence the metabolic phenotype in offspring. Thus, different insults affecting cells during specific developmental windows, corresponding to rapid cell proliferation, can impair mitochondrial function leading to long-lasting effects in the descendants [111].

### Conclusions

Current efforts are focused on determining how metabolomics may be applied to improve the knowledge of the mechanisms that occur in the neurogenesis and of those that cause a specific metabolic dysfunction.

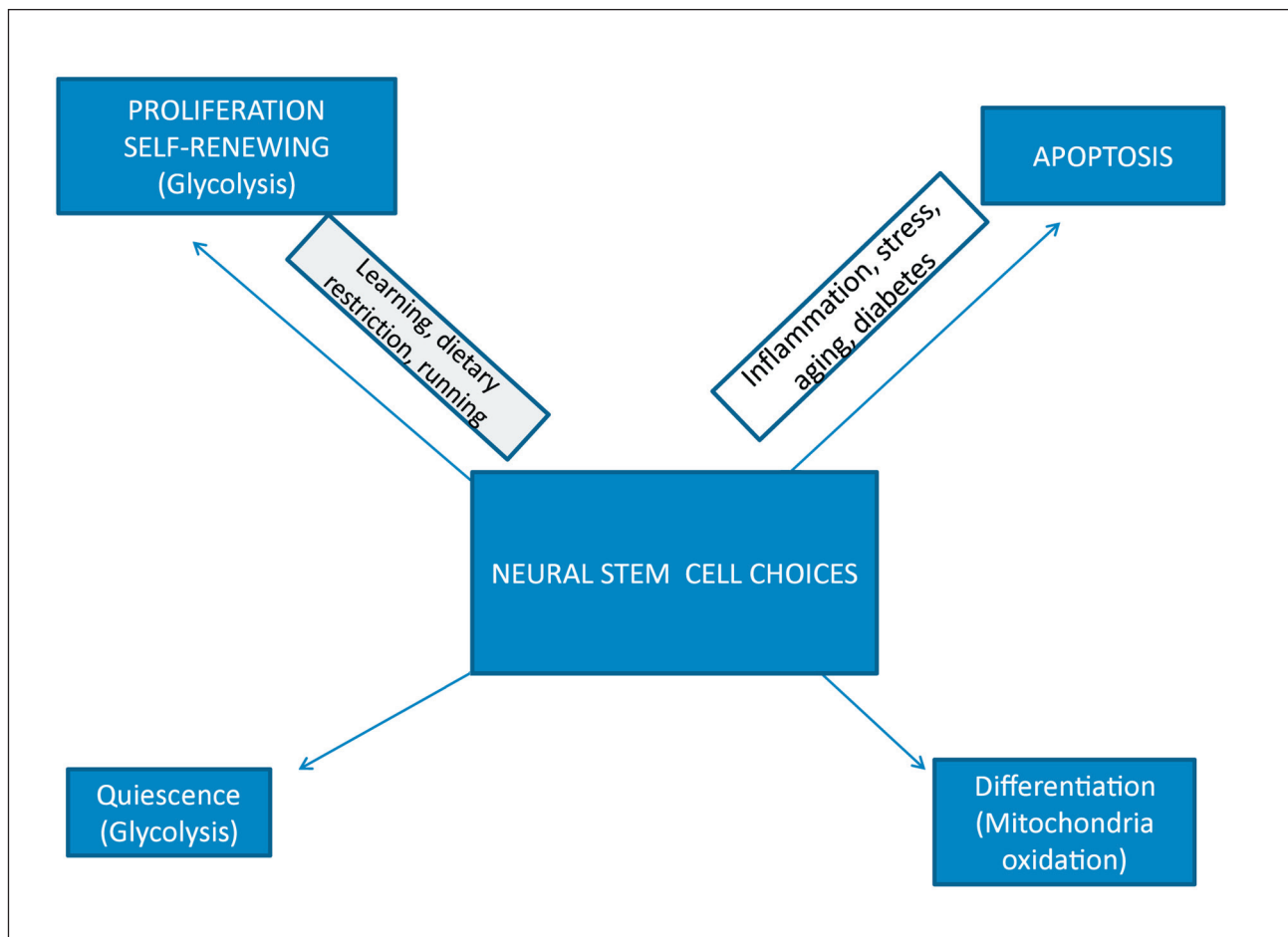
Moreover, the changes in metabolites may be useful in clarifying the mechanism that increases the production and survival of neurons and in developing new therapies against neurodegenerative diseases.

Additional studies are required to delve deeper into the stem cell metabolism.

The growing interest in metabolomics will give us a more complete understanding of both biology and pathology of stem cells with potential therapeutic applications in the next few years.

### Declaration of interest

The Authors declare that there is no conflict of interest.



**Figure 7.** Scheme depicting the choices of the neural stem cell.

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