Adult neural stem cell fate is determined by thyroid hormone activation of mitochondrial metabolism

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ABSTRACT

Objective: In the adult brain, neural stem cells (NSCs) located in the subventricular zone (SVZ) produce both neuronal and glial cells. Thyroid hormones (THs) regulate adult NSC differentiation towards a neuronal phenotype, but also have major roles in mitochondrial metabolism. As NSC metabolism relies mainly on glycolysis, whereas mature cells preferentially use oxidative phosphorylation, we studied how THs and mitochondrial metabolism interact on NSC fate determination.

Methods: We used a mitochondrial membrane potential marker in vivo to analyze mitochondrial activity in the different cell types in the SVZ of euthyroid and hypothyroid mice. Using primary adult NSC cultures, we analyzed ROS production, SIRT1 expression, and phosphorylation of DRP1 (a mitochondrial fission mediator) as a function of TH availability.

Results: We observed significantly higher mitochondrial activity in cells adopting a neuronal phenotype in vivo in euthyroid mice. However, prolonged hypothyroidism reduced not only neuroblast numbers but also their mitochondrial activity. In vitro studies showed that TH availability favored a neuronal phenotype and that blocking mitochondrial respiration abrogated TH-induced neuronal fate determination. DRP1 phosphorylation was preferentially activated in cells within the neuronal lineage and was stimulated by TH availability.

Conclusions: These results indicate that THs favor NSC fate choice towards a neuronal phenotype in the adult mouse SVZ through effects on mitochondrial metabolism.

1. INTRODUCTION

Neurogenesis persists in the brain of adult mammals in two well-defined niches, the hippocampus and the sub-ventricular zone (SVZ) [1,2]. In both areas, NSCs can differentiate to form neurons or glial cells (oligodendrocytes and astrocytes) [3]. NSCs produce transit amplifying progenitors that predominantly differentiate to neuroblasts which migrate to the olfactory bulb forming interneurons [4]. Progenitors can also acquire an oligodendroglial phenotype, becoming oligodendrocyte precursor cells (OPCs) that mature to oligodendrocytes (OLs) [5]. Diverse internal and external signals modulate NSC differentiation, with thyroid hormones (THs) having a crucial role in promoting neuroblast determination [6]. Understanding homeostatic controls of neuron/glial cell fate decisions is necessary to address how adult neurogenesis is modified in physiological and pathological conditions.

It is well established that NSCs principally rely on aerobic glycolysis before differentiation, contrasting with mature cells, in which metabolism is mainly based on mitochondrial oxidative phosphorylation (OXPHOS) [7,8]. Though generating less ATP, high glycolysis can provide proliferating cells with increased production of nucleotides and lipids [9]. Hypoxia also contributes to maintaining an undifferentiated state, influencing proliferation and cell-fate commitment [10,11]. In turn, changes in metabolic status influence NSCs pluripotency and differentiation [12,13]. According to energy requirements, mitochondria change shape, number and connections, through mitochondrial biogenesis, fission, and fusion. These processes modulate mitochondrial production, growth, interconnections, intracellular dissemination, and destruction of damaged mitochondria [14]. Factors enhancing OXPHOS will involve an increase in these processes. Amongst factors regulating mitochondrial dynamics, DRP1 induces mitochondrial fission. When phosphorylated, DRP1 interacts with FIS1...
Figure 1: Thyroid hormones favor neuronal over glial fate decision in vivo and in vitro. (A) Coronal sections of dorsal SVZ in euthyroid versus hypothyroid adult mice (two and three weeks, see materials and methods) treated by IHC (OLIG2, in green and DCX, in red). Representative images. Scale bar: 50 μm. LV: Lateral ventricle. (B) Quantification of DLX2+ cell numbers in the adult SVZ of euthyroid and hypothyroid mice. n=4 euthyroid, hypothyroid 2 weeks and hypothyroid 3 weeks. (C–D) Quantification of DCX+ (integrative...
at the mitochondrial surface to induce division [15]. Triiodothyronine (T3), the active form of THs, promotes DRP1 localization to mitochondria [16], where the protein activates fission. Finally, it is well-documented that THs are major regulators of cell and mitochondrial metabolism [17,18].

Given the twin roles of THs in NSC biology and mitochondrial metabolism, we hypothesized that TH determination of NSC fate could implicate modulation of the glycolysis to OXPHOS metabolism transition. To test this hypothesis, we studied NSC determination both in vivo and in vitro as a function of TH availability. We provide a number of novel results on the role of TH in activating mitochondria and inducing a neuronal, as opposed to a glial, cell fate. First, we show that TH influences mitochondrial activity and ROS production, being higher in cells adopting a neuronal fate. Second, we find that the mitochondrial fission-inducer DRP1 is activated preferentially in cells differentiating toward a neuroblast phenotype, a process influenced by TH availability. Finally, if mitochondrial activity is blocked by oligomycin, neuronal determination is dramatically reduced. In conclusion, the data show that TH signaling increases mitochondrial dynamics and activates mitochondrial respiration during NSC differentiation, thus directing adult NSC determination to a neuronal fate.

2. MATERIAL AND METHODS

2.1. Animals

C57BL/6 wild-type male mice, 8 weeks old, were purchased from Janvier (Le Genest St. Isle, France). Food and water were available ad libitum. All procedures were conducted according to the principles and procedures in Guidelines for Care and Use of Laboratory Animals and validated by local and national ethical committees.

2.2. In vivo studies

2.2.1. Hypothyroid treatments

To induce hypothyroidism, 8 week-old male mice were given iodine-deficient food containing 6-n-propyl-2-thiouracil (PTU) at 0.15% (Harlan Tekland, Madison, WI) for two to three weeks. This method of inducing hypothyroidism is recommended by the American Thyroid Association (ATA) in their rodent guide [19]. It is possible that inducing long-term hypothyroidism or hyperthyroidism affects food intake and body weight. We did not measure the amount of food eaten by hypothyroid mice, but we saw no obvious changes in body weight during the time span of treatment. Hypothyroidism was checked by measuring serum T4 concentrations at sacrifice, using a T4 ELISA test (Labor Diagnostika Nord (LDN), Nordhorn, Germany) (Figure S1A) or using RIA (carried out by Academic Medical Center, University of Amsterdam, Netherlands) (Figure S1B).

2.2.2. JC-1 in vivo staining

JC-1 dye (2 µL 1 µg/µL) was stereotaxically injected in the lateral ventricle (anterior: 3.85 mm, lateral: 1 mm and depth: 2.1 mm relative to lambda) of 2 month-old euthyroid and hypothyroid mice anesthetized with isoflurane. Mice were sacrificed 4 h after injection and brains fixed in 4% paraformaldehyde (PFA) in PBS (0.1 M, pH 7.4). JC-1 red and green fluorescence were quantified in the cytoplasm of EGFR+-, NG2+ and DCX+ cells located in the dorso-lateral part of the SVZ after specific immunostainings. JC-1 red on green fluorescence values were standardized with the global SVZ red on green signal.

2.2.3. Immunohistochemistry (IHC) in vivo

Mice were anesthetized with Pentobarbital (130 mg/kg, Centravet) and perfused rapidly through the left heart ventricle with PBS, then with 4% PFA in PBS (0.1 M, pH 7.4). Brains were harvested and post-fixed at 4 °C overnight in the same fixative solution. Brains were cryoprotected in 30% sucrose in PBS at 4 °C, embedded in OCT, frozen, and stored at −80 °C until processed. Brain coronal sections (30 µm thick) were incubated for 1 h in a blocking solution of 10% normal donkey serum (Sigma) and 1% BSA (Sigma) in PBS at room temperature (RT), then incubated with primary antibody diluted in blocking solution at 4 °C overnight. After three 10 min washes in PBS at RT, sections were incubated with fluorescent secondary antibodies (1/500, Invitrogen) in 1% donkey serum and 1% BSA in PBS for 2 h RT. Sections were washed three times 10 min in PBS at RT, incubated with DAPI for 5 min at RT, and mounted onto SuperFrost glass slides (Fisher) Prolong Gold with antifade reagent (Invitrogen). Fluorescence images were acquired using a Leica TCS-SP5 confocal microscope. Images were processed using the FIJI software [20]. All in vivo quantifications were done on brain slices located between bregma ±0.4 mm and ±0.9 mm stereotaxic coordinates.

2.2.4. Characterization of DLX2 as specific marker of adult SVZ neuronal precursors

In the adult SVZ, well-defined markers of oligodendrocyte precursor cells (OPC) are SOX10, NG2, and OLG2, the latter two being maintained in immature OL [4,21]. If localized in the SVZ, OLG2+ and NG2+ cells can be considered OPCs as these markers are expressed prior to migration. A well-established marker for differentiating neuroblasts is DCX [22]. DLX2 over-expression acts as a pro-neurogenic factor in the adult SVZ [23], making it a good candidate for being specific to early neuronal commitment in the adult. To ascertain this point, we immunoo-assayed adult SVZ for DLX2, OLG2 and for SOX2, a pluripotency marker expressed in NSC and progenitor cells, the expression of which decreases during differentiation [6]. DLX2 and OLG2 signals in the SVZ showed sharp mutual exclusion (Figure S2A-C). The only cells expressing both markers also displayed high SOX2 levels (Figure S2C), suggesting that the rare co-expression of OLG2/DLX2 occurs prior to differentiation. Inversely, nearly all SVZ DCX+ neuroblasts express low levels of DLX2 (Figure S2D). Thus, DLX2 expression decreases with neuronal differentiation enabling DLX2 to be used as a marker of adult SVZ neuronal precursors.

2.3. In vitro studies

2.3.1. Primary adult NSC neurosphere culture

Five male mice were sacrificed per neurosphere culture. Brains were removed and lateral SVZ dissected under a binocular dissection microscope in DMEM-F12-glutamax 1:50 Glic 45% and incubated with papain for 30 min at 37 °C with pipette dissociation every 10 min to obtain a single-cell suspension. Cells were collected by centrifugation...
Figure 2: Mitochondrial activity and ROS production are higher in cells adopting a neuronal fate and are influenced by thyroid hormones. (A–D) Mitochondrial activity is higher in cells adopting a neuronal fate and is decreased by hypothyroidism specifically in neuroblasts in vivo. (A) Schematic representation of JC-1 dye mechanism of action. Excited at a wavelength of 488 nm, JC-1 monomers outside of mitochondria exhibit green fluorescence, whereas JC-1 aggregates inside mitochondria exhibit a red fluorescence.
(1000 rcf, 5 min). After resuspension, cells were cultured in complete culture medium (DMEM-F12 [Gibco], 40 μg/ml insulin [Sigma], 1/200 B-27 supplement [Gibco], 1/100 N-2 supplement [Gibco], 0.3% glucose, 5 mM Heps, 100 U/ml penicillin/streptomycin) containing 20 ng/ml of EGF and 20 ng/ml of FGF2 (Peprotech), in a 5% CO2 environment at 37 °C for 7 days to obtain primary neurospheres. To analyze cell differentiation, neurospheres were dissociated into single cells and 50,000 cells were plated per well on Poly-o-Lysine-coated glass coverslips in 24-well plates in complete culture medium without EGF/FGF2 for 7 days maximum.

To test the effect of T3 on cell differentiation, T3 was added to the differentiation medium (5 nM or 50 nM). Medium was renewed every two to three days during differentiation. Cells were then fixed with 4% paraformaldehyde for 10 min RT at different differentiation time points and used for immunocytochemistry (see below).

To inhibit mitochondrial superoxide production, MitoTEMPO (Sigma) 20 μM was added to culture medium during 7 days of cell differentiation. To measure cellular ROS production, cells were exposed to Hydroethidin (HEt) (ThermoFisher) 40 μM 0.4% DMSO in culture medium during 30 min before fixation. HET transformation to ethidium bromide in presence of superoxide ions has been quantified in cells nuclei. To inhibit ATP synthase and OXPHOS, oligomycin (Sigma) 1 μM was added to culture medium during cell differentiation.

2.3.2. Immunostaining in vitro

Cells fixed on glass coverslips were blocked with 10% donkey serum and 1% BSA in PBS for 30 min at RT. Incubation with primary antibody was performed in blocking solution overnight at 4 °C or for 2 h at RT. After three 5 min washes in PBS at RT, cells were incubated with fluorescent secondary antibodies in 1% donkey serum and 1% BSA in PBS for 1 h at RT. Cells were washed three times in PBS, incubated with DAPI for 5 min at RT and mounted on SuperFrost glass slides with Prolong Gold antifade reagent. Fluorescence images were acquired using a Leica TCS-SP5 confocal microscope and processed using the FIJI software [20]. All in vitro quantifications were done on Max Intensity Z projection of 4 μm stack images. At least 10 40× images were quantified per slide per experiment.

2.4. Statistical analysis

Statistical analyses were carried out with R and GraphPad Prism. Statistical differences were analyzed with appropriate tests, indicated in figure legends, to compare control and treated groups.

2.5. Antibodies and reagents

A complete list of antibodies and reagents used is presented in supplemental experimental procedures.

3. RESULTS

3.1. Thyroid hormone availability differentially modulates mitochondrial activity and ROS production in neuronal and glial lineages

To understand the implication of mitochondrial metabolism in thyroid hormone (TH) control of NSC fate, we analyzed mitochondrial activity in vivo and in vitro in neuronal and glial lineages. A first step was to verify that TH favor neuronal over glial fate decision in a dose and time dependent manner in vivo and in vitro. This objective required an unambiguous marker of neuronal precursor, shown in Figure S2 (see details in Experimental procedures). We quantified cell populations in mice fed control or hypothyroid diet for two (2w) or three weeks (3w). Hypothyroidism time-dependently decreased both DLX2+ neuronal precursor cell (NPC) and DCX+ neuroblast numbers, whilst increasing OLIG2+ OPCs in the latero-dorsal SVZ (Figure 1A–D). Thus, low TH availability in vivo favors adult NSCs determination to an oligodendrocyte fate, at the expense of DLX2+ NPCs and DCX+ neuroblasts. To test the consequences of TH excess on NSC fate, we used an in vitro assay, in which adult SVZ-derived proliferating NSCs and progenitors form neurospheres in the presence of growth factors. After 7 d, neurospheres were dissociated and differentiation induced without growth factors (Figure 1E). We quantified DCX+ and OLIG2+ cells after 24 h and 7 d in differentiating conditions ±T3 (5 or 50 nM). After 24 h, T3 (50 nM) decreased the proportion of OLIG2+ cells (p < 0.05).

This decrease in OLIG2+ cells was highly significant (p < 0.01) at 7 d differentiation with T3 (50 nM), accompanied by increased proportions of DCX+ neuroblasts (Figure 1F and G). Thus, in vivo and in vitro T3 availability controls neuronal vs glial fate in the adult SVZ.

To quantify mitochondrial activity in cells committed towards a glial or a neuronal fate in the SVZ, we used JC-1. This dye enters mitochondria as a function of their membrane potential. In active mitochondria, the nuclear membrane potential and favoring JC-1 entry. JC-1 particles emit a green fluorescence in the cytoplasm, and aggregate to emit a red fluorescence inside mitochondria (Figure 2A). Following intracerebro-ventricular injection (ICV), we determined JC-1 ratios (red/green) as a function of cell type using cytoplasmic immuno-staining for proliferating cells (EGFR), for NPCs and neuroblasts (DCX) and for OPCs (NG2) (Figure 2B and C). Figure 2C shows a representative example of JC-1 red/green signals in DCX+ SVZ cells in the niche. To determine how TH availability affected JC-1 ratios in different cell types, we compared euthyroid to hypothyroid mice (2 or 3 weeks) (Figure 2D). In euthyroid mice, quantifying JC-1 ratio showed that mitochondrial activity was significantly higher in DCX+ neuroblasts than in EGF+ proliferating NSCs and progenitors (p < 0.001) or in NG2+ OPCs that is proportional to mitochondrial membrane potential. (B) Membrane and cytoplasmic markers were used to quantify red and green JC-1 signals in the SVZ in respectively, neural stem cells and progenitors (EGFR), neuronal precursor cells and neuroblasts (DCX), oligodendrocyte precursor cells and immature oligodendrocytes (NG2). (C) Representative image of JC-1 red and green fluorescence after ICV injections of the dye into the lateral ventricle of a euthyroid mice. Coronal sections were treated for immunohistochemistry using antibodies against DCX (purple). Scale bar: 10 μm (D) JC-1 red on green fluorescence values for EGFR−, NG2− and DCX− cells located in the lateral-dorsal part of the SVZ of euthyroid and hypothyroid (2 or 3 wks) adult mice. EUTHYROID: n = 16, HYPOTHYROID 2 wks: n = 14, HYPOTHYROID 3 wks: n = 9, p-Value: ***<0.001, **<0.01, *<0.05, ****<0.0001, Kruskal–Wallis test followed by a Dunn’s multiple comparison post-hoc test. E–D ROS production is increased by T3 in vitro and is higher in DCX+ cells. (E) HET nuclear intensity after 7 differentiation of adult NSC in different conditions of T3 availability and in the presence or absence of MitoTEMPO (20 μM). Administration of MitoTEMPO counteracts T3-induced increase in ROS production. (F) HET nuclear intensity after 24 h of adult NSC differentiation in control medium or with T3 (5 or 50 nM) in DCX+ and OLIG2+ cells. (G) HET nuclear intensity after 7 d of adult NSC differentiation in medium or with addition of T3 (5 or 50 nM) in DCX+ and OLIG2+ cells. (H) Relative proportion of OLIG2+ and DCX+ cells after 24 h differentiation for each dose of T3, in presence or absence of oligomycin (1 μM). (I) Representative immunochemistry on cells differentiated for 24 h in control conditions (DMSO) or oligomycin (1 μM) (OLIG2: green, and DCX: red). p-Value: ***<0.001, **<0.01, *<0.05, ****<0.0001, 0.0001: ns: non-significant; Kruskal–Wallis followed by Mann–Whitney tests.
p < 0.01) (Figure 2D, left panel). The significantly higher level of JC-1 ratios in DCX+ neuroblasts versus EGFR + NSC/progenitors or NG2 + OPCs was lost in hypothyroid mice (3w, Figure 2D right panel), due to a strong decrease in mitochondrial activity in the DCX+ cells that was more marked as a function of duration of hypothyroidism. Three weeks hypothyroidism significantly reduced JC-1 ratios in DCX+ cells compared to 2 w (p > 0.5) (Figure 2D).

Increased mitochondrial activity drives reactive oxygen species (ROS) production. We used hydroethidine (HE) to measure ROS production within different cell types derived from adult NSCs in vitro. In the presence of the superoxide anion (O₂⁻), the main species of ROS, HEI is oxidized to form ethidium bromide (EB), which intercalates with DNA, staining nuclei red [24]. To confirm that HEI signal relates to mitochondrial activity, we quantified nuclear HEI in cells differentiated in the presence of MitoTEMPO, a mitochondria-specific ROS-scavenger, ±T3 (50 nM). ROS scavenging by MitoTEMPO did not significantly reduce HEI nuclear intensity in control cells but significantly abrogated the T3-dependent increase in ROS production (Figure 2E). Thus, T3-dependent mitochondrial activity increases ROS production in neurons that have been dissociated and allowed to differentiate. We used HEI to quantify ROS production in OLIG2+ and DCX+ cells after 24 h and 7 d in vitro differentiation. DCX+ neuroblasts produced more ROS than OLIG2+ OPCs and immature OL at both differentiation times in control conditions (Figure 2F and G), confirming results obtained with JC-1 in vivo (Figure 2D). Adding T3 to the differentiation medium increased ROS production in both lineages, at each time point, but DCX+ cells always displayed significantly higher ROS production in neurosphere cultures that have been dissociated and allowed to differentiate. We used HEI to quantify ROS production in OLIG2+ and DCX+ cells after 24 h and 7 d in vitro differentiation. DCX+ neuroblasts produced more ROS than OLIG2+ OPCs and immature OL at both differentiation times in control conditions (Figure 2F and G), confirming results obtained with JC-1 in vivo (Figure 2D). Adding T3 to the differentiation medium increased ROS production in both lineages, at each time point, but DCX+ cells always displayed significantly higher ROS production (Figure 2F and G). Additionally, neuroblasts were more T3 sensitive: DCX+ cells responding significantly to 5 nM T3 at 24 h whereas OLIG2+ OPCs did not (Figure 2F).

To investigate whether blocking mitochondrial activity affected cell fate choice, we used oligomycin [25]. Figure 2H and I shows that oligomycin significantly abrogated the T3-dependent increase in ROS production (Figure 2D). Thus, T3-dependent mitochondrial activity increases ROS production in the neuronal lineage, again in a fate-specific manner during the glycolysis to OXPHOS metabolic switch. We first analyzed which cell types express the Ser616 phosphorylated form of DRP1 (pDRP1S616), usually associated with fission, pDRP1S616 was mainly found in a sub-population of SOX2low committed progenitors and in neuronal lineage cells (DLX2+ and DCX+), but not in SOX10+ or OLIG2+ OPCs (Figure 4A). Further, there was an overall decrease in the pDRP1S616 signal in hypothyroid mice (Figure 4B), revealing a global reduction of pDRP1S616 cells, consistent with decreased neuroblast numbers (Figure 1B and C).

CDK1 mediates Ser616 activating DRP1 phosphorylation [29,30] while CDK5-mediated Ser616 phosphorylation of DRP1 inhibits mitochondrial fission during neuronal maturation [31]. To assess the activating or inhibiting nature of pDRP1S616 in the adult SVZ cells in vivo, we analyzed CDK5 and CDK1 expression. While CDK1 was found in DCX+ neuroblasts, CDK5 was not, confirming the fission-activating nature of pDRP1S616 in differentiating neuroblasts (Figure S4).

To characterize more precisely phosphorylation-dependent DRP1 activation in each cell lineage, we used in vitro studies to quantify total DRP1 and pDRP1S616 in differentiating cells. After 24 h, pDRP1S616 was low in SOX2+ progenitors and increased significantly as a function of differentiation in both lineages (Figure 4C). Although significant, the increase was only 20% during differentiation from SOX2+/OLIG2-to SOX10+, and from to OLIG2+ OPCs and immature OLs. In contrast, during SOX2+ cell determination to DLX2+ NPCs and DCX+ neuroblasts it was >300% (Figure 4C–E and Figure S5A–C). Quantifying total DRP1 in different cell types showed that DRP1 protein levels were opposite to changes in pDRP1S616 signal, underlining that increased DRP1 phosphorylation correlates with differentiation (Figure 4F and Figure S5D–F). After 7 d differentiation, pDRP1S616 levels were reduced in DCX+ neuroblasts, showing no difference with those in OLIG2+ cells (Figure 4H). Thus, DRP1 activation peaks during neuronal determination, and decreases with maturation. T3 effects on DRP1S616 in DCX+ neuronal versus OLIG2+ glial cells at 24 h and 7 d differentiation were assessed next. At 24 h differentiation, T3 (5 nM) significantly increased pDRP1S616 in DCX+ cells, but not in OLIG2+ cells (Figure 4G). After 7 d differentiation, the only increase in pDRP1S616 intensity was seen in the neuronal lineage, again in response to T3 (5 nM) (Figure 4H). Differences in pDRP1S616 signal were not accounted for by increased DRP1 expression as there were no major differences in DRP1 levels in OLIG2+ and DCX+ cells. Similarly, smaller responses to T3 (5 nM) were limited to neuroblasts (Figure S5H and I). Overall, these results demonstrate that T3 specifically induces DRP1 phosphorylation at early stages of NSC/progenitor cell determination toward a neuronal fate.
4. DISCUSSION

Adult NSC-driven neurogenesis generates both neurons and glial cells [4], processes that require precise control of NSC fate decisions. THs commit NSCs to a neuronal phenotype [6]. Here we show that TH signaling favors neuronal fate in the adult SVZ through induction of mitochondrial respiration. Studying how TH signaling and mitochondrial metabolism affect NSC decisions requires specific markers of differentiation.

Figure 3: SIRT1 expression is maintained at early stages of neuroblast determination. (A) In vivo co-immuno-stainings performed on wild-type adult mice SVZ. SIRT1 (green) expression is detected in a sub-population of SOX2⁺ progenitors and is mainly excluded from SOX10⁺ OPCs. SIRT1 is positively correlated with DLX2 expression in neuronal precursor cells (R² = 0.719, p < 0.0001), and expression is maintained in most DCX⁺ neuroblasts. (B) Quantification of the number of SIRT1⁺ cells in the dorsal SVZ of euthyroid and hypothyroid (2 and 3 wks) adult mice. Four experiments were pooled, n = 12 euthyroid, n = 10 hypothyroid 2 wks, n = 6 hypothyroid 3 wks. (C) SIRT1/OLIG2 cell by cell nuclear relative fluorescence after 1 h, 24 h or 7 d of differentiation in vitro. SOX2 positive cells are represented by blue dots. (D) Proportion of DCX⁺ and OLIG2⁺ cells positive for SIRT1 after 1 h, 24 h and 7 d differentiation in control conditions. (E) Proportion of DCX⁺ and OLIG2⁺ cells positive for SIRT1 after 24 h differentiation in control medium or with T₃ (5 and 50 nM). p-Value: *<0.05, ****<0.0001. Kruskal–Wallis followed by Mann–Whitney tests.
Thyroid hormones activate DRP1 phosphorylation in cells adopting a neuronal fate.

(A) Immunohistochemistry stainings performed on wild-type adult mice SVZ using antibodies against pDRP1S616 (green), DLX2, DCX, SOX2, SOX10, and OLIG2 (red). pDRP1S616 is expressed in SOX2low more committed progenitor cells, DLX2+ neuronal precursor cells (NPC) and DCX+ neuroblasts. pDRP1 is not detectable in SOX2high early progenitors nor in SOX10+ and OLIG2+ oligodendrocyte precursor cells (OPC). (B) Immunohistochemistry showing pDRP1S616 expression in dorsal SVZ and quantification of pDRP1S616 signal in dorsal SVZ of euthyroid and hypothyroid (2 and 3 wks) adult mice. n = 12 (EUTHYROID and HYPOTHYROID 2 wks) or n = 7 (HYPOTHYROID 3 wks). Scale bar: 10 μm.

(C) Quantification of pDRP1S616 cytoplasmic signal of SOX2+ , SOX10+ , OLIG2+ , DLX2+ , and DCX+ cells after 24 h of NSC differentiation in vitro. Co-immunostainings were done with antibodies directed against pDRP1S616, OLIG2, and a third marker (SOX2, SOX10, DLX2, DCX). (D) Markers used for specific cell type detection. (E) Representative images of pDRP1S616 immunostainings at 24 h differentiation. Scale bar: 10 μm. (F) Quantification of total DRP1 cytoplasmic signal in SOX2+ , SOX10+ , OLIG2+ , DLX2+ , and DCX+ cells after 24 h and 7 d differentiation, (G–H) Quantification of the cytoplasmic signal of pDRP1S616 in OLIG2+ and DCX+ cells after 24 h and 7 d differentiation in control conditions or with T3 (5 nM or 50 nM). p-Value: *<0.05, **<0.01, ***<0.001, ****<0.0001. Kruskal–Wallis followed by Mann–Whitney tests.
early neuroblast determination. We demonstrate that DLX2 expression is specific to NPCs in the adult mouse SVZ, allowing the use of this marker to distinguish between NPC and OPC.

NSC rely primarily on glycolytic metabolism, whereas most mature neural cells use mainly OXPHOS-based energy [7]. Furthermore, oxygen availability impacts NSC differentiation capacity and whether the NSC becomes a neuron, an astrocyte, or an oligodendrocyte [13,32]. We show first, that the transition toward an OXPHOS-based metabolism occurs in the neurogenic lineage during early cell-fate commitment and, second, that it is a TH-dependent process. Further, our results show that this metabolic switch occurs simultaneously with activation of mitochondrial fission through phosphorylation of DRP1, also induced by THs. Lastly, expression of the metabolic regulator SIRT1 is preferentially maintained in cells differentiating to neuroblasts. These experiments thus underline the importance of TH-dependent activation of mitochondrial OXPHOS during adult NSC determination to a neuronal phenotype. In the glial lineage, there is also a switch to OXPHOS-based metabolism, but this occurs later in the differentiation process.

We also show mitochondrial activity and ROS production to be higher in cells differentiating to a neuronal than to a glial lineage in the adult brain. This result corroborates the observation that, in contrast to neurons, mature glial cells maintain high rates of glycolysis, allowing a better adaptability to metabolic stress [33,34]. Further, the JC-1 data showed that the decreased numbers of cells differentiating to neuroblasts in hypothyroid conditions was accompanied by a diminished cellular respiration in this cell type, confirming the crucial role of TH signaling in modulating mitochondrial respiration during cell determination. Another result showing that mitochondrial activity is essential for TH-induction of NSC fate determination came from use of oligomycin, an inhibitor of mitochondrial ATP synthase. Applying this drug overrode TH induction of neuroblast determination. Thus, TH induction of neuroblast cell fate requires mitochondrial OXPHOS activity. Differential expression of TR coregulators could be another important modulator of TH influence on NSC fate choice. The NAD+--dependent deacetylase SIRT1 can act as a coactivator for TRβ and TRα [28,35]. SIRT1 is known to be involved in regulating several metabolic pathways, especially mitochondrial function through PGC1α and AMPK [36–38]. SIRT1 also has an important role in stemness maintenance by directly regulating SOX2 [39], OCT4 [40] and NANOG [41], three major transcription factors required for pluripotency and inhibition of differentiation. As expected, our results show that SIRT1 is largely expressed in adult NSC-derived SOX2+ progenitors, but also preferentially maintained in early differentiation stages of the neuronal lineage. In contrast, virtually no SOX10+/OLIG2+ engaged OPCs still expressed this deacetylase. Although a decrease in the total number of SIRT1+ cells was observed in vivo in the SVZ of hypothyroid mice, we saw no significant change in SIRT1 expression in either OLIG2+ or DCX+ cells when modifying T3 availability in vitro. The decrease seen in hypothyroid mice in vivo correlates with the observed decrease in DLX2+ and DCX+ cells, confirming SIRT1 expression maintenance in the neuronal lineage. It can be hypothesized that while SIRT1 is repressed in pluripotency of SOX2+ progenitors when differentiation starts, its roles as TR coactivator and mitochondrial metabolism modulator are boosted during early neuronal but not early OL determination. Which factors induce SIRT1 in the neuronal lineage will be an interesting question to address in future studies. Besides SIRT1, TH signaling impacts other modulators of mitochondrial metabolism, either through TH nuclear receptors or through non-genomic processes. T3 promotes DRP1 localization on mitochondria [16], where the protein can induce fission. We showed that T3 induces DRP1 activation through phosphorylation of its Ser616 residue, an event that enables DRP1 translocation to the mitochondrial membrane. Though our results showed a slight decrease in DRP1 expression during NSC differentiation, we observe a clear increase in the amount of pDRP1, that is much larger in DLX2+ NPCs than in SOX10+ OPCs. It is worth noting that repression of DRP1 in vitro is known to lead to a reduction in oxygen consumption [42]. Thus, cell fate-dependent induction of mitochondrial activity by THs occurs through changes in mitochondrial dynamics, and particularly modulation of mitochondrial fission. This result adds to previous published findings showing that T3 is required for reduction of Sox2, Oct4, and Nanog pluripotency genes during embryonic neurogenesis [43]. Furthermore, it is consistent with the increased mitochondrial dissemination observed during neuronal differentiation and maturation [44]. Fission allows mitochondria to disperse inside the cell, a process likely linked to axonal and dendritic development. These results lead to the conclusion that local control of TH signaling affects mitochondrial activity and cell fate determination. THs could impact mitochondrial metabolism of differentiating NSCs through different modes of action. Through nuclear TR, T3 can activate expression of both PGC1a and TFAM, two factors inducing mitochondrial protein expression and biogenesis, processes, which in turn increase mitochondrial dynamics [45–47]. But THs have also been shown to increase mitochondrial respiration efficiency through non-nuclear early effects [48,49]. What is more, two truncated variants of TRz, p28 and p43, are found specifically in mitochondria [50,51]. T3 induces p28 import into mitochondria inner membrane, where the respiratory chain is located, but the precise function of p28 is not yet known [52]. However, it is known that p43 activates mitochondrial protein synthesis and mitochondrial biogenesis in presence of T3 [53–56]. Thus, these processes could be important for early influence of THs on mitochondrial metabolism and further accompany nuclear receptor-mediated T3 effects on mitochondrial biogenesis during NSC determination.

5. CONCLUSIONS

Our results show that THs favor adult NSC fate choice toward a neuronal phenotype in the adult sub-ventricular zone (SVZ) through induction of mitochondrial respiration. Mitochondrial activity and ROS production are higher in cells differentiating to a neuronal than to a glial lineage in the adult brain and are influenced by THs. SIRT1 is preferentially maintained in cells engaged in a neuronal differentiation, but does not appear to be directly impacted by TH availability. We also show that THs promote DRP1 activating phosphorylation preferentially in cells acquiring a neuronal fate, in line with the idea of an increase in mitochondrial dynamics and dissemination during cell determination. Further research on how metabolic controls contribute to NSC fate decision processes in the adult brain will be critical in the context of neurodegenerative diseases, both to understand mechanisms underlying disease susceptibility and progression and to develop strategies to direct neurogenesis to neuronal or glial cell fates.

AUTHOR CONTRIBUTIONS

Conceptualization, J.-D.G., B.A.D. and S.R.; Methodology, J.-D.G., S.R. and A.S.; In vivo experiments, J.-D.G., S.R., M.L., M.P.-J.; In vitro experiments, A.S., J.-D.G., C.L., C.N.V, K.L.B.; Image acquisition, J.-D.G., A.S., M.L., C.N.V, M.P.-J.; Analyses, J.-D.G., A.S., M.L., C.N.V.; Writing, J.-D.G., B.A.D. and S.R.
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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.molmet.2017.08.003.

REFERENCES

[1] Altman, J., 1962. Are new neurons formed in the brains of adult mammals? Science 135(3509):1127—1128.
[2] Doetsch, F., Caille, I., Lim, D.A., Garcia-Verdugo, J.M., Alvarez-Buylla, A., 1999. Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. Cell 97(3):703—716.
[3] Doetsch, F., Petreanu, L., Caille, I., Garcia-Verdugo, J.M., Alvarez-Buylla, A., 2002. EGF converts transit-amplifying neurogenic precursors in the adult brain into multipotent stem cells. Neuro 36(6):1021—1034.
[4] Menn, B., Garcia-Verdugo, J.M., Yaschine, C., Gonzalez-Perez, O., Rowitch, D., Alvarez-Buylla, A., 2006. Origin of oligodendrocytes in the subventricular zone of the adult brain. Journal of Neuroscience 26(30):7907—7918.
[5] Suzuki, S.O., Goldman, J.E., 2003. Multiple cell populations in the early postnatal subventricular zone take distinct migratory pathways: a dynamic study of glial and neuronal progenitor migration. Journal of Neuroscience 23(10):4240—4250.
[6] López-Juárez, A., Remaud, S., Hassani, Z., Jolivet, P., Pierre Simons, J., Sontag, T., et al., 2012. Thyroid hormone signaling acts as a neurogenic switch by repressing Sox2 in the adult neural stem cell niche. Cell Stem Cell 10(5):531—543.
[7] Zhang, X., Boyer, L., Jin, M., Mertens, J., Kim, Y., Ma, L., et al., 2016. Metabolic reprogramming during neuronal differentiation from aerobic glycolysis to neuronal oxidative phosphorylation. Elife 5.
[8] Ito, K., Suda, T., 2014. Metabolic requirements for the maintenance of self-renewing stem cells. Nature Reviews Molecular Cell Biology 15(4):243—256.
[9] Lunt, S.Y., Vander Heiden, M.G., 2011. Aerobic glycolysis: meeting the metabolic requirements of cell proliferation. Annual Review of Cell and Developmental Biology 27:441—464.
[10] Shyh-Chang, N., Daley, G.Q., Cantley, L.C., 2013. Stem cell metabolism in tissue development and aging. Development 140(12):2535—2547.
[11] Mohyeldin, A., Gasz-Mudri, T., Quilines-Hinojosa, A., 2010. Oxygen in stem cell biology: a critical component of the stem cell niche. Cell Stem Cell 7(2):150—161.
[12] Pistillo, F., Chen, H.L., Schwartz, P.H., Basso, G., Panchision, D.M., 2007. Oxygen tension controls the expansion of human CNS precursors and the generation of astrocytes and oligodendrocytes. Molecular and Cellular Neuroscience 35(3):424—435.
[13] De Filippis, L., Delia, D., 2011. Hypoxia in the regulation of neural stem cells. Cellular and Molecular Life Sciences 68(17):2831—2844.
[14] da Silva, A.F., Mariotti, F.R., Máximo, V., Campello, S., 2014. Mitochondria dynamism: of shape, transport and cell migration. Cellular and Molecular Life Sciences 71(12):2313—2324.
[15] Losó, O.C., Song, Z., Chen, H., Chan, D.C., 2013. Fis1, mft, MID49, and MID51 mediate Drp1 recruitment in mitochondrial fission. Molecular Biology of the Cell 24(5):659—667.
[16] Sinha, R.A., Singh, B.K., Zhou, J., Wu, Y., Farah, B.L., Obka, K., et al., 2015. Thyroid hormone induction of mitochondrial activity is coupled to mitophagy via ROS-AMPK-ULK1 signaling. Autophagy 11(8):1341—1357.
[17] Weitzel, J.M., Iwen, K.A., 2011. Coordination of mitochondrial biogenesis by thyroid hormone. Molecular and Cellular Endocrinology 342(1—2):1—7.
[18] Vaikutis, J.A., Farrar, J.S., Cell, F.S., 2015. Thyroid hormone mediated modulation of energy expenditure. International Journal of Molecular Sciences 16(7):16158—16175.
[19] Bianco, A.C., Anderson, G., Forrest, D., Galton, V.A., Gereben, B., Kim, B.W., et al., 2014. American thyroid association guide to investigating thyroid hormone economy and action in rodent and cell models. Thyroid 24(1):88—168.
[20] Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., et al., 2012. Fiji: an open-source platform for biological-image analysis. Nature Methods 9(7):676—682.
[21] Pozniak, C.D., Langseth, A.J., Dijkgraaff, G.J., Cho, Y., Werb, Z., Pleasure, S.J., 2010. Sox10 directs neural stem cells toward the oligodendrocyte lineage by decreasing Suppressor of Fused expression. Proceedings of the National Academy of Sciences (PNAS) 107(50):21795—21800.
[22] Brown, J.P., Couillard-Després, S., Cooper-Kuhn, C.M., Winkler, J., Aigner, L., Kuhn, H.G., 2003. Transient expression of doublecortin during adult neurogenesis. Journal of Comparative Neurology 467(1):1—10.
[23] Jones, K.S., Connor, B.J., 2016. The effect of pro-neurogenic gene expression on adult subventricular zone precursor cell recruitment and fate determination after excitotoxic brain injury. Journal of Stem Cells and Regenerative Medicine 12(1):25—35.
[24] Zhao, H., Kalivendi, S., Zhang, H., Joseph, J., Nilipatirkom, K., Vázquez-Vivar, J., et al., 2003. Superoxide reacts with hydroxidoine but forms a fluorescent product that is distinctly different from ethidium: potential implications in intracellular fluorescence detection of superoxide. Free Radical Biology and Medicine 34(11):1359—1368.
[25] Lardy, H.A., Johnson, D., McMurray, W.C., 1958. Antibiotics as tools for metabolic studies. I. A survey of toxic antibiotics in respiratory, phosphorylative and glycolytic systems. Archives of Biochemistry and Biophysics 78(2):587—597.
[26] Rafalski, V.A., Ho, P.P., Brett, J.O., Ucar, D., Dugas, J.C., Pollina, E.A., et al., 2013. Expansion of oligodendrocyte progenitor cells following Sirt1 inactivation in the adult brain. Nature Cell Biology 15(6):614—624.
[27] Saharan, S., Jhaveri, D.J., Bartlett, P.F., 2013. Sirt1 regulates the neurogenic potential of neural precursors in the adult subventricular zone and hippocampus. Journal of Neuroscience Research 91(5):642—659.
[28] Suh, J.H., Siegla, D.H., Zhang, A., Xia, X., Ovora, A., Winnier, G.E., et al., 2013. SIRT1 is a direct coactivator of thyroid hormone receptor β1 with gene-specific actions. PLoS One 8(7):e70097.
[29] Yamano, K., Youle, R.J., 2011. Coupling mitochondrial and cell division. Nature Cell Biology 13(9):1026—1027.
[30] Taguchi, N., Ishihara, N., Jofuku, A., Oka, T., Mihara, K., 2007. Mitotic phosphorylation of dynamin-related GTPase Drp1 participates in mitochondrial fission. Journal of Biological Chemistry 282(15):11521—11529.
[31] Cho, B., Cho, H.M., Kim, H.J., Jeong, J., Park, S.K., Hwang, E.M., et al., 2014. CDK5-dependent inhibitory phosphorylation of Drp1 during neuronal maturation. Experimental & Molecular Medicine 46:105.
[32] Rafalski, V.A., Brunet, A., 2011. Energy metabolism in adult neural stem cell fate. Progress in Neurobiology 93(2):182—203.
[33] Zhang, Y., Chen, K., Sloan, S.A., Bennett, M.L., Scholze, A.R., O’Keefe, S., et al., 2014. An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. Journal of Neuroscience 34(36):11929—11947.
[34] Rodgers, J.T., Cui, Q.L., Fang, J., Wang, L.C., Zhang, J., Khan, D., et al., 2016. Oligodendrogliopathy in multiple sclerosis: low glycolytic metabolic rate promotes oligodendrocyte survival. Journal of Neuroscience 36(17):4698–4707.

[35] Thakran, S., Sharma, P., Attia, R.R., Hori, R.T., Deng, X., Elam, M.B., et al., 2013. Role of sirtuin 1 in the regulation of hepatic gene expression by thyroid hormone. Journal of Biological Chemistry 288(2):807–818.

[36] Rodgers, J.T., Lerin, C., Haas, W., Gygi, S.P., Spiegelman, B.M., Puigserver, P., et al., 2005. Nutrient control of glucose homeostasis through a complex of PGC-1alpha and SIRT1. Nature 434(7029):113–118.

[37] Lan, F., Cacicedo, J.M., Ruderman, N., Ido, Y., 2008. SIRT1 modulation of the

[38] Williams, E.O., Taylor, A.K., Bell, E.L., Lim, R., Kim, D.M., Guarente, L., 2016.

[39] Han, M.K., Song, E.K., Guo, Y., Ou, X., Mantel, C., Brockmeyer, H.E., 2008. SIRT1 regulates apoCIII and Nanog expression in mouse embryonic stem cells by controlling p53 subcellular localization. Cell Stem Cell 2(3):241–251.

[40] Benard, G., Bellance, N., James, D., Parrone, P., Fernandez, H., Letellier, T., et al., 2007. Mitochondrial bioenergetics and structural network organization. Journal of Cell Science 120(Pt 5):838–848.

[41] Wang, L., Ye, X., Zhao, Q., Zhou, Z., Dan, J., Zhu, Y., et al., 2014. Dmp1 is dispensable for mitochondrial biogenesis in induction to pluripotency but required for differentiation of embryonic stem cells. Stem Cells and Development 23(20):2422–2434.

[42] Steib, K., Slafer, I., Jagasia, R., Ebert, B., Lie, D.C., 2014. Mitochondria modify exercise-induced development of stem cell-derived neurons in the adult brain. Journal of Neuroscience 34(19):6624–6633.

[43] Garska, H.L., Fäke, M., Escribano, J.R., Wiesner, R.J., 1994. Stoichiometry of mitochondrial transcripts and regulation of gene expression by mitochondrial transcription factor A. Biochemical and Biophysical Research Communications 200(1):619–626.

[44] Wu, Z., Puigserver, P., Andersson, U., Zhang, C., Adelmant, G., Mootha, V., et al., 1999. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. Cell 98(1):115–124.

[45] Wulf, A., Hameit, A., Kröer, M., Kebenko, M., Wetzel, M.G., Weitzel, J.M., 2009. T3-mediated expression of PGC-1alpha via a far upstream located thyroid hormone response element. Molecular and Cellular Endocrinology 287(1–2):90–95.

[46] Palacios-Romero, R., Mowbray, J., 1979. Evidence for the rapid direct control both in vivo and in vitro of the efficiency of oxidative phosphorylation by 3,5,3’-tri-iodo-L-thyronine in rats. Biochem J 184(3):527–538.

[47] Sterling, K., Brenner, M.A., Sakurada, T., 1980. Rapid effect of triiodothyronine on the mitochondrial pathway in rat liver in vivo. Science 210(4467):340–342.

[48] Wulniak, C., Cassar-Malek, I., Marchal, S., Rascle, A., Heusser, S., Keller, J.M., et al., 1995. A 43-kDa protein related to c-Erb A alpha 1 is located in the mitochondrial matrix of rat liver. J Biol Chem 270(27):16347–16354.

[49] Wulniak-Cabello, C., Casas, F., Cabello, G., 2001. Thyroid hormone action in mitochondria. Journal of Molecular Endocrinology 26(1):67–77.

[50] Wulniak-Cabello, C., Casas, F., Cabello, G., 2014. p28, a truncated form of Trz regulates mitochondrial physiology. FEBS Letters 588(21):4037–4043.

[51] Casas, F., Rochard, P., Rodier, A., Cassar-Malek, I., Marchal-Victorion, S., Wiesner, R.J., et al., 1999. A variant form of the nuclear triiodothyronine receptor c-ErbA alpha 1 plays a direct role in regulation of mitochondrial RNA synthesis. Molecular Cell Biology 19(12):7913–7924.

[52] Casas, F., Rochard, P., Rodier, A., Cassar-Malek, I., Marchal-Victorion, S., Wiesner, R.J., et al., 1999. A variant form of the nuclear triiodothyronine receptor c-ErbA alpha 1 plays a direct role in regulation of mitochondrial RNA synthesis. Molecular Cell Biology 19(12):7913–7924.

[53] Daury, L., et al., 2000. Mitochondrial activity is involved in the regulation of myoblast differentiation and slow myosin isoform expression by control of Calcineurin expression. Experimental Cell Research 317(14):2059–2071.