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Amyloid β Peptide Compromises Neural Stem Cell Fate by Irreversibly Disturbing Mitochondrial Oxidative State and Blocking Mitochondrial Biogenesis and Dynamics

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Abstract

Alzheimer's disease (AD) is the most common neurodegenerative disease and is characterized by the accumulation of amyloid β peptide (Aβ). Although most AD mouse models present a decline in neurogenesis, they express mutated genes which regulate neurogenesis per se and are not present in most AD patients, thus masking the real impact of Aβ on adult neurogenesis. Mitochondrion, a well-known target of Aβ in neurons, is a main regulator of neural stem cell (NSC) fate. Here, we aimed to investigate the impact of Aβ on NSC mitochondria and cell fate decisions, namely whether and how Aβ affects neurogenesis. NSC fate and mitochondrial parameters, including biogenesis, dynamics, and oxidative stress, were evaluated. Our results showed that Aβ impaired NSC viability and proliferation and indirectly blocked neurogenic differentiation, by disrupting mitochondrial signaling of self-renewing NSCs. Importantly, Aβ decreased ATP levels, generated oxidative stress, and affected the radical scavenger system through SOD2 and SIRT3. Aβ also reduced mtDNA and mitochondrial biogenesis proteins, such as Tfam, PGC-1α, and NRF1, and inhibited activation of PGC-1α-positive regulator CREB. Moreover, Aβ triggered mitochondrial fragmentation in self-renewing NSCs and reduced mitochondrial fusion proteins, such as Mfn2 and ERRα. Notably, Aβ compromised NSC commitment and survival by irreversibly impairing mitochondria and thwarting any neurogenic rescue through mitochondrial biogenesis, dynamics, or radical scavenger system. Altogether, this study brings new perspective to rethink the molecular targets relevant for endogenous NSC-based strategies in AD.

Keywords Alzheimer's disease · Aβ · Mitochondria · Neurogenesis · Neural stem cells · Oxidative stress

Abbreviations

Aβ	Amyloid β
AD	Alzheimer's disease
APP	Amyloid precursor protein
BrdU	Bromodeoxyuridine
CREB	cAMP-responsive element-binding protein
DRP1	Dynamin-related protein 1
ERRα	Estrogen-related receptor α
FBS	Fetal bovine serum
FGF	Fibroblast growth factor
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GFAP	Glial fibrillary acidic protein
GFP	Green fluorescence protein
MAP2	Microtubule-associated protein 2
Mfn2	Mitofusin 2
mtDNA	Mitochondrial DNA
mtROS	Mitochondrial ROS
NRF1	Nuclear respiratory factor 1
NSC	Neural stem cell
PBS	Phosphate-buffered saline
PGC-1α	Peroxisome proliferator-activated receptor γ coactivator-1 α
PSEN1	Presenilin 1
ROS	Reactive oxygen species

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Sirt3	Sirtuin-3
SOD2	Superoxide dismutase 2
Tfam	Mitochondrial transcription factor A

Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder, being characterized by extracellular accumulation of amyloid β peptide (Aβ), intracellular aggregation of tau protein, and consequent neuronal loss in hippocampus and cortex [1, 2]. Adult neurogenesis has been described to play an important role in hippocampal-dependent learning and memory. Inhibition of both neural stem cell (NSC) proliferation and generation of newborn neurons in the hippocampus causes a decline in hippocampal-dependent spatial memory [3, 4]. Therefore, maintaining the NSC pool and stimulating survival and generation of newborn neurons may provide a putative strategy to counteract aging or neurodegenerative diseases, such as AD. Although still controversial, recent findings from post-mortem studies point that neurogenesis is disturbed in AD brain [5–7]. Indeed, a significant decline in the NSC pool and neurogenesis has also been described in AD mouse models [8–12], although the mechanisms are still unclear. On the other hand, most in vivo AD models mimic the familiar form of the disease with mutated genes, including amyloid precursor protein (*APP*) and presenilin 1 (*PSEN1*), which in turn also regulate neurogenesis process [13–16] and mask the Aβ impact on this physiologic process. Of note, most AD patients present with the sporadic form of the disease without *APP* or *PSEN1* mutations, prompting strategies to understanding the real impact of Aβ peptide in NSCs.

While little is known on how Aβ peptide affects the NSC pool, amyloid effects are well described in mature neurons. Aβ peptide leads to neuronal death and reduction of spatial learning [17, 18], through mitochondrial changes, including electron transport disruption, generation of reactive oxygen species (ROS), decreased ATP production, increased mitochondrial Ca^{2+} uptake, and opening of the mitochondrial permeability transition pore [19–21]. Importantly, mitochondria are also main regulators of NSC self-renewal and fate decision [22–25] by establishing a dynamic regulated network, alternating between fusion and fission cycles, to control cell cycle components and assure mitochondrial function, energy supply, and cell homeostasis [26]. During cell division, mitochondria go through fusion to maximize ATP production and further fission to sort mitochondria to daughter cells and delete damaged mitochondria through mitophagy [27]. Mitochondrial biogenesis is increased during neuronal differentiation along with oxidative phosphorylation and concomitant ROS to deal with higher energy demand and activate transcription of several differentiation-associated genes [22, 26, 28].

Here, we explore the role of Aβ seeding in NSC fate and identify its putative mitochondrial effects. We demonstrate that Aβ peptide impairs viability, proliferation, and differentiation of NSCs, contributing for a decline of neuronal regeneration in AD. Moreover, Aβ irreversibly modifies the oxidative state of mitochondrial of self-renewing NSCs, including other regulatory networks of ROS homeostasis, such as mitochondrial biogenesis and dynamics, to compromise mitochondrial activity and neurogenesis.

Methods

Ethics Statement

The mouse NSC line used in this study was obtained from Dr. Smith's Laboratory, University of Cambridge, Cambridge, UK, and provided by Dr. Henrique, University of Lisbon, Lisbon, Portugal [29].

Neural Stem Cell Cultures and Cell Treatments

NSCs were derived from 14.5-dpc mouse fetal forebrain as previously described [30, 31]. Forebrain progenitor cells in the embryo are clonally related to post-natal NSCs and are quiescent until they are activated in adulthood [32], thus modeling the quiescent pool of adult NSCs. These NSCs continuously expand by symmetrical division and are capable of tripotential differentiation [31, 33, 34]. Briefly, NSCs were maintained in a monolayer and in self-renewal conditions in Euromed-N medium (EuroClone®S.p.A., Pavia, Italy), at 37 °C in humidified atmosphere of 5% CO_2. The self-renewal medium was supplemented with 1% penicillin-streptomycin, 1% N-2 supplement, 20 ng/mL epidermal growth factor (EGF), and 20 ng/mL basic fibroblast growth factor (βFGF). Penicillin-streptomycin and N-2 supplements were purchased from Gibco™, Life Technologies Corp. (Carlsbad, CA, USA). Both EGF and βFGF were purchased from PeproTech® EC (London, UK). $Aβ_{1-42}$ (H-1368; Bachem AG; Bubendorf, Switzerland) was resuspended in 50 mM Tris buffer, pH 10. The concentration of Aβ stock solution was determined using the Coomassie (Bradford) Protein Assay (Bio-Rad Laboratories, Hercules, CA, USA), as previously described [35]. In self-renewal medium, NSCs were plated with a density around 1×10^5 cells/cm^2, and 24 h after platting incubated with 1.5, 5 or 10 μM $Aβ_{1-42}$ at different time points, according to the assay. Aβ peptide was incubated for either 24 h to assess cell viability and proliferation; 2 h to measure mitochondrial ROS, mitochondrial DNA copy number, and mRNA expression levels of mitochondrial regulators; or 2, 6, and 24 h to determine ATP and protein levels.

For neural differentiation, NSCs were plated in self-renewal medium onto uncoated culture plates at 1×10^5

cells/cm^2. Twenty-four hours after plating, culture medium was changed for differentiating medium. Neurogenic medium was prepared with Euromed-N medium supplemented with 1% penicillin-streptomycin, 0.5% N-2 supplement, 1% B-27 supplement (Gibco™), and 10 ng/mL βFGF. To assess amyloid impact on neurogenic differentiation, 10 μM $A\beta_{1\text{-}42}$ was incubated at the same time of neuronal differentiation induction, for 48 h, with or without a 24-h 10 μM $A\beta_{1\text{-}42}$ pre-incubation treatment in self-renewal conditions. The gliogenic differentiation medium included Euromed-N medium supplemented with 1% penicillin-streptomycin, 0.5% N-2 supplement, 10% fetal bovine serum (FBS; Gibco™), and 10 ng/mL βFGF. $A\beta_{1\text{-}42}$ (10 μM) was incubated for 48 h in NSCs at the same time of glial differentiation induction. Cells were collected and processed for flow cytometry, immunocytochemistry, immunoblotting, and quantitative real-time PCR assays.

Evaluation of Cell Death and Viability

Cell death and viability were assessed by Guava Nexin® assay (Guava Technologies, Millipore Corp., Billerica, MA, USA) according to the manufacturer's instructions. After 24 h of amyloid incubation in self-renewal and neurogenic conditions, the cell culture medium containing dead cells was collected together with adherent cells previously detached with StemPro Accutase (A11105-01; Gibco™). Cells were centrifuged for 5 min at 600*g* and resuspended in PBS with 2% FBS. Cell suspension was mixed with Guava Nexin reagent (1:1) and incubated for 20 min at room temperature. Sample acquisition was performed using Guava easyCyte 5HT flow cytometer (Guava Technologies). FlowJo software (Tree Star, Inc.) was used for data analysis.

Determination of Proliferation

Proliferation was determined by the incorporation of BrdU, a synthetic thymidine analogue. The APC BrdU Flow Kit (BD Pharmingen, BD Biosciences, San Jose, CA, USA), was used according to the manufacturer's instructions. Briefly, cells were seeded in self-renewal medium for 24 h, in the presence or absence of Aβ peptide. BrdU was incubated for 4 h in the culture medium, 20 h after Aβ treatment. Cells were subsequently collected and stained. Sample acquisition was performed using the LSRFortessa™ (BD Biosciences). Data were analyzed using FlowJo version X 10.0.7., FlowJo software (Tree Star, Inc.).

Analyses of NSC Differentiation

Neural differentiation was evaluated through flow cytometry accordingly to the expression levels of nestin, βIII-tubulin, microtubule-associated protein 2 (MAP2), and glial fibrillary acidic protein (GFAP). Nestin is a marker for stem cells, βIII-tubulin, and MAP2 for early and late neuronal differentiation, respectively, and GFAP for glial differentiation. Cells were collected and processed as previously described [36, 37]. Briefly, cells were detached with Accutase and collected by centrifugation for 5 min at 600*g*. Cells were then fixed with paraformaldehyde (4% *w/v*) in PBS for 20 min at 4 °C, washed with washing solution of 0.1% saponin (Fluka, Biochemika, Switzerland) in PBS, and incubated for 20 min at 4 °C in blocking solution of 0.25% saponin and 5% FBS in PBS. Then, cells were incubated for 30 min with mouse primary antibodies reactive to nestin (1:800; MAB353, Millipore Corp.), βIII-tubulin (1:250; Tuj1; Covance, Princeton, NJ, USA), GFAP (1:200; MAB360, Millipore Corp), or with rabbit primary antibody reactive to MAP2 (1:500; AB5622; Millipore Corp.). Next, cells were washed and incubated for 30 min with the respective secondary antibody, anti-mouse antibody conjugated to DyLight 488 (1:100; 35502; Thermo Fisher Scientific Inc., Rockford, IL, USA) or anti-rabbit antibody conjugated to Alexa 488 (1:200; A-21206 Thermo Fisher Scientific Inc.). All antibodies were diluted in 0.1% saponin and 5% FBS in PBS. Cells were washed twice and resuspended in PBS with 2% FBS. Finally, samples were acquired with flow cytometer Accuri C6 (BD Biosciences), and data statistically analyzed using FlowJo software (Tree Star).

Total RNA Extraction

Total RNA was extracted using the RIBOZOL™ reagent (AMRESCO, LLC, Solon, OH, USA) according to manufacturer's instructions. Each sample was homogenized on 0.5 mL RIBOZOL™ reagent. After mixing with 0.1 mL chloroform, each sample was centrifuged at 12,000*g* for 15 min at 4 °C and the aqueous phase collected. Total RNA was precipitated by incubation with 0.25 mL isopropyl alcohol at − 20 °C for 1 h. Samples were centrifuged at 12,000*g* for 10 min at 4 °C and the RNA pellet was washed with 75% ethanol and centrifuged at 7500*g* for 5 min at 4 °C. RNA pellets were air-dried and resuspended in 40 μL RNase-free water. The purity of RNA was checked by confirming the ratio of the absorbance readings at 260 and 280 nm in a range of 1.9–2.1. Finally, DNA contaminations were eliminated with DNase I recombinant (04716728001; Roche Applied Science, Mannheim, Germany) following manufacturer's instructions.

Quantitative RT-PCR (qRT-PCR)

cDNA synthesis was performed using the NZY Reverse Transcriptase (NZYTech, Lisbon, Portugal) according to manufacturer's instructions. Real-time RT-PCR was performed using SensiFast™ SYBR® Hi-ROX Kit (Bioline USA Inc., Taunton, MA, USA) in the Applied Biosystems 7300 System (Thermo Fisher Scientific Inc.). Primer sequences can be

found in Table 1. Relative gene expression was calculated based on the standard curve and normalized to the level of glyceraldehyde 3-phosphate dehydrogenase (*Gapdh*) or β-actin (*Actb*) housekeeping genes and expressed as fold change from controls.

Total Protein Extraction

NSCs were collected and lysed using ice-cold lysis buffer (10 mM Tris-HCl, pH 7.6, 5 mM $MgCl_2$, 1.5 mM potassium acetate, 1% Nonidet P-40, 2 mM DTT) and 1X Halt Protease and Phosphatase Inhibitor Cocktail (Thermo Fisher Scientific, Inc.) for 30 min at 4 °C. Samples were subsequently sonicated and centrifuged at 10,000*g* at 4 °C for 10 min. The supernatant was recovered and stored at − 80 °C. Protein content was measured by the Bio-Rad protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA), according to the manufacturer's specifications. Bovine serum albumin was used as standard.

Immunoblotting

Forty micrograms of total protein extracts were separated on a 7.5 or 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred onto nitrocellulose membrane and blocked with 5% milk solution. Blots were incubated overnight with anti-c-myc-peroxidase (1:1000; 9E10; Roche); or mouse primary antibodies against total CREB (1:1000; 86B10; Cell Signaling Technology, Inc., Danvers, MA, USA), PGC-1α (1:1000; ST1202; Millipore Corp.), Mfn2 (1:1000; ab56889; Abcam Plc, Cambridge, UK), GAPDH (1:2500; sc-32233; Santa Cruz Biotechnology Inc.), and β-actin (1:20000; A5541; Sigma-Aldrich Co.); or rabbit primary antibody against p-CREB (1:200; sc-101663; Santa Cruz Biotechnology Inc.) and DRP1 (1:200; sc-32898; Santa Cruz Biotechnology Inc.); SOD2 (1:200; sc-30080 Santa Cruz Biotechnology Inc.); Sirt3 (1:1000; D22A3; Cell Signaling Technology, Inc.); and with the secondary antibody conjugated with horseradish peroxidase (1:5000, Bio-Rad Laboratories) for 2 h at room temperature. Membranes were processed for protein detection using SuperSignal substrate (Pierce, Thermo Fisher Scientific). β-Actin was used as loading control. Finally, the relative intensities of protein bands were analyzed using ImageLab Version 5.1 densitometric analysis program (Bio-Rad Laboratories).

ATP Measurement

ATP was detected by using Mitochondrial ToxGlo™ assay Kit (G8001; Promega Co., Madison, WI, USA). Cells were plated and further lysated with water during 1 h. Cell lysate was diluted 1:10 and incubated with ATP Detection Reagent, according to the manufacturer's instruction of the kit. Luminescence, that is proportional to the amount of intracellular ATP, was measured by using FB12 Luminometer (Berthold detection system). Finally, ATP levels were normalized with total proteins quantified by colorimetric Bradford assay. The absorbance was read at 595 nm using GloMax® 96 Microplate Luminometer (Promega Co.).

Quantification of Mitochondrial DNA Copy Number

Total DNA was extracted using QIAamp DNA Mini Kit (51304; Qiagen), according to manufacturer's protocols. Quantitative real-time PCR analysis was performed using Maxima SYBR Green/ROX qPCR Master Mix (2×) in Applied Biosystems 7300 System (both from Thermo Fisher Scientific Inc.), as previously described [38]. Mitochondria-encoded gene *mt-Co1* was used to determine mtDNA copy number and nuclear *Rn18s* gene to determine the number of cells. The relative amounts of each gene were calculated based on the standard curve. Mitochondrial DNA (mtDNA) copy number was determined by the ratio of *mt-Co1* gene amplification to *Rn18s* gene and expressed as fold change from controls. Primer sequences can be found in Table 1.

Mitochondrial ROS Measurement

Mitochondrial superoxide anion was measured using MitoSOX™ Red mitochondrial superoxide indicator (M36008; Molecular Probes™, Life Technologies Corp.), according to manufacturer's protocols. After 2 h of Aβ treatment, cells were incubated with 5 μM MitoSOX™ Red in Hank's balanced salt solution (HBSS, 14025; Gibco™), for 10 min at 37 °C. Then, cells were washed, collected with Accutase and resuspended in DPBS with 2% FBS. Samples were subsequently acquired with Accuri C6 Flow Cytometer (BD Biosciences). Data analysis was performed in FlowJo version X 10.0.7., FlowJo software (Tree Star, Inc.).

Transfection

To overexpress PGC-1α and Mfn2, NSCs were transfected with the following plasmids: pcDNA3.1-PGC-1α-Flag (provided by Dr. Jorge Ruas, Karolinska Institutet) and pcDNA3.1-Mfn2-Myc (provided by Dr. David Chan; Addgene plasmid # 23213) [39]. Empty plasmid pcDNA3.1(−) harboring a CMV promoter (pCMV) was used as control. Sirt3 was overexpressed using pCMV6-AC-GFP as control, and the same plasmid-encoding human GFP-tagged Sirt3 (RG217770). We used Lipofectamine® 3000 (Invitrogen, Thermo Fischer), according to manufacturer's instructions. Briefly, Opti-MEM® (Gibco™) containing the mixture of Lipofectamine® 3000 and DNA (2 μL:1 μg) was added and cells were incubated for 24 h at 37 °C in a humidified atmosphere of 5% CO_2. To assess transfection efficiency,

Table 1 List of primers used for PCR

Gene	Sequence (5′-3′)
Sod2	5′ CAGACCTGCCTTACGACTATGG 3′ (fwd)
	5′ CTCGGTGGCGTTGAGATTGTT 3′ (rev)
Sirt3	5′ TGCTACTCATTCTTGGGACCT 3′ (fwd)
	5′ CACCAGCCTTTCCACACC 3′ (rev)
Tfam	5′ CACCCAGATGCAAAACTTTCAG 3′ (fwd)
	5′ CTGCTCTTTATACTTGCTCACAG 3′ (rev)
Ppargc1a (for PGC-1α)	5′ GGACATGTGCAGCCAAGACTCT 3′ (fwd)
	5′ CACTTCAATCCACCCAGAAAGCT 3′ (rev)
Esrra (for ERRα)	5′ GGGGAGCATCGAGTACAGC 3′ (fwd)
	5′ AGACGCACACCCTCCTTGA 3′ (rev)
Nrf1	5′ AGCACGGAGTGACCCAAAC 3′ (fwd)
	5′ TGTACGTGGCTACATGGACCT 3′ (rev)
Mfn2	5′ CAGAGCAGAGCCAAACTGCT 3′ (fwd)
	5′ AACATGTTGAGTTCGCTGTCC 3′ (rev)
Nes (for Nestin)	5′ CTCAGATCCTGGAAGGTGGG 3′ (fwd)
	5′ GCAGAGTCCTGTATGTAGCCA 3′ (rev)
Tubb3 (for βIII-tubulin)	5′ GCGCCTTTGGACACCTATTCA 3′ (fwd)
	5′ TTCCGCACGACATCTAGGACTG 3′ (rev)
Map2	5′ GTTCAGGCCCACTCTCCTTC 3′ (fwd)
	5′ CTTGCTGCTGTGGTTTTCCG 3′ (rev)
Mki67 (for Ki67)	5′ CCTTTGCTGTCCCCGAAGA 3′ (fwd)
	5′ GGCTTCTCATCTGTTGCTTCCT 3′ (rev)
Gapdh	5′ ATTCAACGGCACAGTCAAGG 3′ (fwd)
	5′ TGGATGCAGGGATGATGTTC 3′ (rev)
Actb (for β-actin)	5′ GTGGGCCGCTCTAGGCACCAA 3′ (fwd)
	5′ CTCTTTGATGTCACGCACGATTTC 3′ (rev)
mt-Co1 (for CO1)	5′ ATCTGTTCTGATTCTTTGGGCAC 3′ (fwd)
	5′ AGCCTAGAAAGCCAATAGACATTA 3′ (rev)
Rn18s (for 18S)	5′ TAGAGGGACAAGTGGCGTTC 3′ (fwd)
	5′ CGCTGAGCCAGTCAGTGT 3′ (rev)

protein levels of PGC-1α, Mfn2, and Sirt3 were determined by Western blotting. After 24 h of transfection, cells were treated with Aβ peptide, as previously described, and collected to assess viability and differentiation by PCR. Of note, in PGC-1α transfection assays, cells were also treated with proteasome inhibitor MG132 (10 μM) 6 h before the end of the experiment, to accumulate PGC-1α and efficiently overexpress it.

Immunocytochemistry and Confocal Microscopy

For visualization of mitochondrial morphology and intracellular distribution of Aβ, namely its mitochondria co-localization in NSCs, cells were incubated with 2 μM 5-FAM-$A\beta_{1\text{-}42}$ (H-7444, Bachem AG), a green-stained amyloid peptide, for 24 h. Before harvesting, cells were re-incubated for 30 min at 37 °C with 0.5 μM MitoTracker Red CMXRos (M-7512; Molecular Probes), which preferentially accumulates in mitochondria. Cells were washed twice and fixed with paraformaldehyde (4%, *w/v*) in PBS. Nucleic acids were stained with Hoechst 33342 dye (50 μg/mL in PBS; Sigma-Aldrich Corp) and samples were mounted using Mowiol 4-88 (Calbiochem). For Aβ and mitochondria co-localization, samples were single-layer scanned with a Zeiss LSM 880 with Airyscan, a confocal point-scanning microscope (Carl Zeiss, Jena, Germany) equipped with a 63×/1.4 oil differential interference contrast plan-apochromat objective. Images were further processed using Fiji software. In addition, NSC morphology was evaluated using bright field with Zeiss Primo Vert microscope (Carl Zeiss Microscopy GmbH, Jena, Germany), equipped with a Leica DFC490 camera (Leica Microsystems, Wetzlar, Germany). Images were processed using Fiji software.

Statistical Analysis

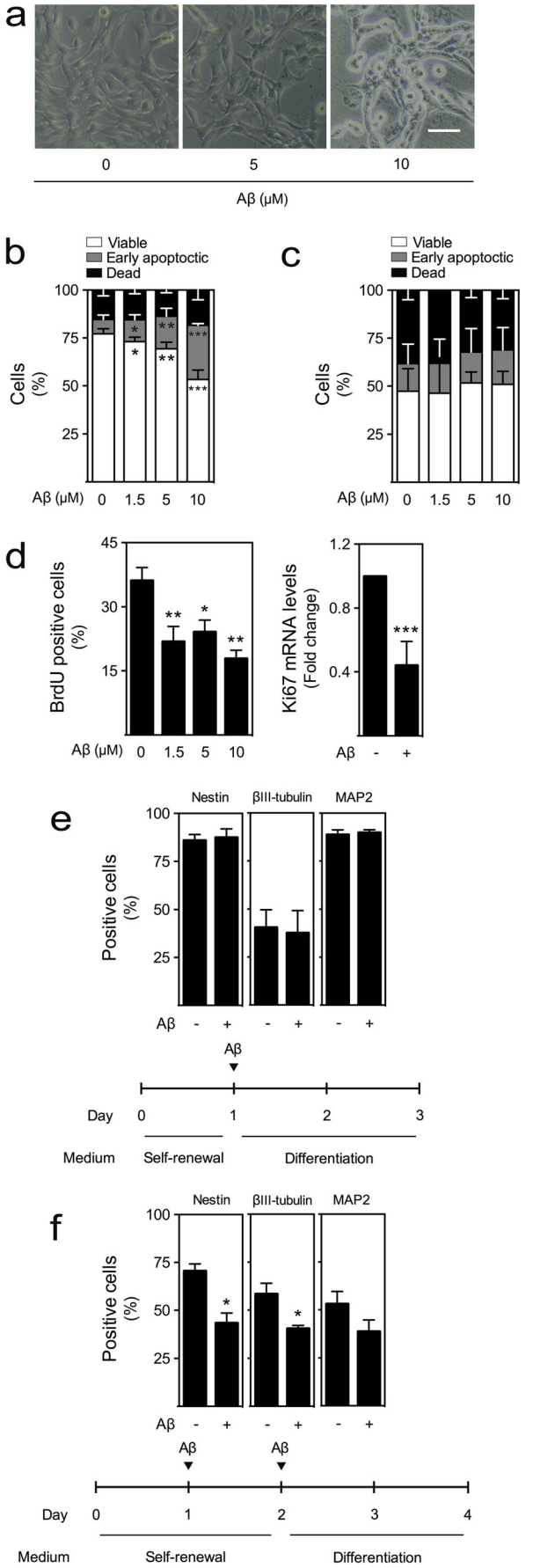
Statistical analysis was performed using the Student's *t* test when two groups where compared, one-way ANOVA with a Dunnett's post-test for multiple comparisons to one control and with a Tukey post-test for multiple comparisons. All analyses were performed using the GraphPad Prism version 5.0, GraphPad Software (San Diego, CA USA, www.graphpad.com). Values of $p < 0.05$ were considered significant.

Results

Aβ Peptide Inhibits Viability and Proliferation of Self-renewing NSCs and Indirectly Blocks Neurogenic Differentiation

A mouse NSC line was cultured in self-renewal conditions and incubated with Aβ peptide at different concentrations (1.5, 5, and 10 μM) to test the impact of Aβ on NSC viability. Remarkably, NSCs treated with Aβ displayed an appearance of cellular aggregates with a reduction in the total number of cells, more evident at 10 μM Aβ (Fig. 1a). In addition, Aβ peptide significantly decreased viable undifferentiated NSCs and increased early apoptotic cells at all concentrations (at least, $p < 0.05$; Fig. 1b). The number of dead NSCs, including necrotic and late apoptotic cells, was not significantly affected at any concentration. Of note, the Tris buffer used to resuspend Aβ peptide did not interfere with NSC viability (Suppl. Fig. 1). Furthermore, the impact of Aβ peptide on viability of differentiating NSCs was also tested by inducing neurogenic differentiation and concomitantly incubating NSCs with Aβ peptide. Intriguingly, Aβ exposure did not significantly affect the viability of NSCs at early stages of differentiation (Fig. 1c).

Since NSCs have the ability to self-renew to assure the maintenance of the NSC pool [40], we also ascertained the effect of Aβ peptide on NSC proliferation. In self-renewal



◀ **Fig. 1** Aβ inhibits viability and proliferation of NSCs and indirectly blocks neurogenic differentiation. Mouse NSCs were treated with 1.5, 5, or 10 μM $Aβ_{1-42}$ in self-renewal conditions. After 24 h of treatment, cells were collected to assess viability and proliferation as described in *Materials and Methods*. In self-renewal conditions, cells were incubated with 10 μM $Aβ_{1-42}$ for 24 h. In differentiation conditions, 10 μM Aβ was incubated at the same time of neuronal differentiation induction, for 48 h, with or without 10 μM $Aβ_{1-42}$ pre-incubation in self-renewal conditions. Samples were collected for bright-field microscopy, flow cytometry, and qPCR analysis. **a** Morphology of self-renewing NSCs exposed to 5 or 10 μM Aβ. Representative images of bright-field microscopy. Scale bar, 50 μm. **b**, **c** Quantification of NSC viability, by Guava Nexin flow cytometry in self-renewal (**b**) or neurogenic differentiation (**c**) conditions, after 24 h of amyloid treatment. Data is expressed by percentages of viable, early apoptotic, and dead cells. **d** Quantification of proliferation by BrdU incorporation (*left*) and qRT-PCR of Ki67 expression *right*) in self-renewal conditions, 24 h after amyloid treatment. BrdU incorporation was analyzed by flow cytometry and data is expressed by percentages of cells that incorporate BrdU. Ki67 expression was measured for 10 μM Aβ treatment. **e** Flow cytometry analysis (*top*) of percentage of positive cells for nestin, βIII-tubulin, and MAP2 after 48 h of neurogenic differentiation without amyloid pre-incubation in self-renewal conditions. Experimental scheme representing the protocol of treatment (*bottom*). **f** Flow cytometry analysis (*top*) of percentage of positive cells for nestin, βIII-tubulin, and MAP2 after 48 h of neurogenic differentiation with amyloid pre-incubation in self-renewal conditions. Experimental scheme representing the protocol of treatment (*bottom*). Data represents mean values ± SEM for at least three independent experiments. Asterisk indicates $p < 0.05$, double asterisks indicate $p < 0.01$, and triple asterisks indicate $p < 0.001$ compared to untreated cells

conditions, Aβ peptide significantly arrested NSC proliferation at several concentrations (at least $p < 0.05$), as assessed by a reduction in BrdU incorporation (Fig. 1d). In agreement, mRNA levels of Ki67, a proliferation marker, were strongly diminished by the highest levels of Aβ peptide after 24 h ($p < 0.001$; Fig. 1d) and 48 h ($p < 0.01$; Suppl. Fig. 2) of Aβ exposure, indicating that this peptide indeed blocks the cell cycle re-enter.

As the most prominent effects on viability and proliferation were observed with 10 μM of Aβ peptide, this concentration was used to study Aβ-induced NSC fate deregulation and underlying mechanisms in subsequent experiments.

Since NSCs can differentiate into neurons and glia lineage, we further explored the role of Aβ seeding in regulating neurogenic and glial differentiation of NSCs [41]. Neuronal differentiation was investigated by flow cytometry through the expression of stemness (Nestin), early (βIII-tubulin), and late (MAP2) neuronal markers. Aβ peptide was first incubated concomitantly with the induction of neurogenic differentiation. However, Aβ exposure did not reduce the number of positive cells for βIII-tubulin and MAP2, suggesting that it does not directly interfere with neuronal differentiation process (Fig. 1e). Aiming a more pathophysiological context, NSCs were then incubated with 10 μM Aβ peptide before and at the beginning of differentiation. Aβ strikingly reduced the number of βIII-tubulin-positive cells ($p < 0.05$) and slightly decreased MAP2-stained cells (Fig. 1f). Curiously, Aβ

peptide also reduced Nestin-positive cells ($p < 0.05$; Fig. 1f). Similar effects on βIII-tubulin- and MAP2-positive cells were observed with 5 μM Aβ peptide (Suppl. Fig. 3), excluding the effect of Aβ dose on neurogenesis deregulation. On the other hand, in gliogenic conditions, Aβ treatment of NSCs resulted in a marked reduction of GFAP-positive NSCs ($p < 0.05$; Suppl. Fig. 4).

Collectively, these results reveal that Aβ does not affect neuronal differentiation per se. Instead, Aβ deregulates cellular events in self-renewing NSCs compromising further neuronal differentiation. Future experiments to determine amyloid targets were performed in self-renewal conditions.

Aβ Peptide Co-localizes with Mitochondria, Reduces ATP Supplies, and Increases Mitochondrial Oxidative Stress in NSCs

Mitochondria regulate energy production and control ROS levels, thus modulating cell fate decisions [23, 24]. We determined whether Aβ-induced changes in NSC fate arose from mitochondrial alterations in self-renewing NSCs. Our results showed that Aβ peptide significantly reduced total ATP levels from 2 h of treatment ($p < 0.001$; Fig. 2a). Conversely, Aβ significantly increased mitochondrial ROS (mtROS) levels ($p < 0.01$; Fig. 2b). In fact, the increased mitochondrial oxidative stress can result from either an increase in ROS production or a decline in mtROS elimination. Thus, we further assessed the role of Aβ peptide over the mtROS scavenging system, namely on the expression of both the superoxide dismutase 2 (SOD2), a scavenging enzyme only expressed in mitochondria, and its positive activator sirtuin-3 (Sirt3), a NAD^+-dependent mitochondrial deacetylase [42, 43]. Accordingly, amyloid treatment significantly decreased mRNA and protein levels of *Sod2* and *Sirt3* (at least $p < 0.05$; Fig. 2c, d), precluding the elimination of NSC mtROS and contributing to increased oxidative stress.

We also investigated the cellular distribution of Aβ peptide in NSCs and evaluated the putative co-localization of Aβ peptide with mitochondria by confocal laser scanning microscopy. After 24 h of amyloid treatment, Aβ peptide clearly co-localized with the mitochondrial network in NSCs (Fig. 2e), suggesting a possible direct effect on mitochondria integrity and function. As not completely co-localized with mitochondria, Aβ peptide may also have additional indirect effects on this organelle.

Aβ-Treated NSCs Display Less Mitochondrial Mass and Disrupted Regulation of Mitochondrial Biogenesis

During differentiation of embryonic and pluripotent stem cells, mitochondrial biogenesis and oxygen consumption increase [44, 45]. Indeed, the energy supply and function of

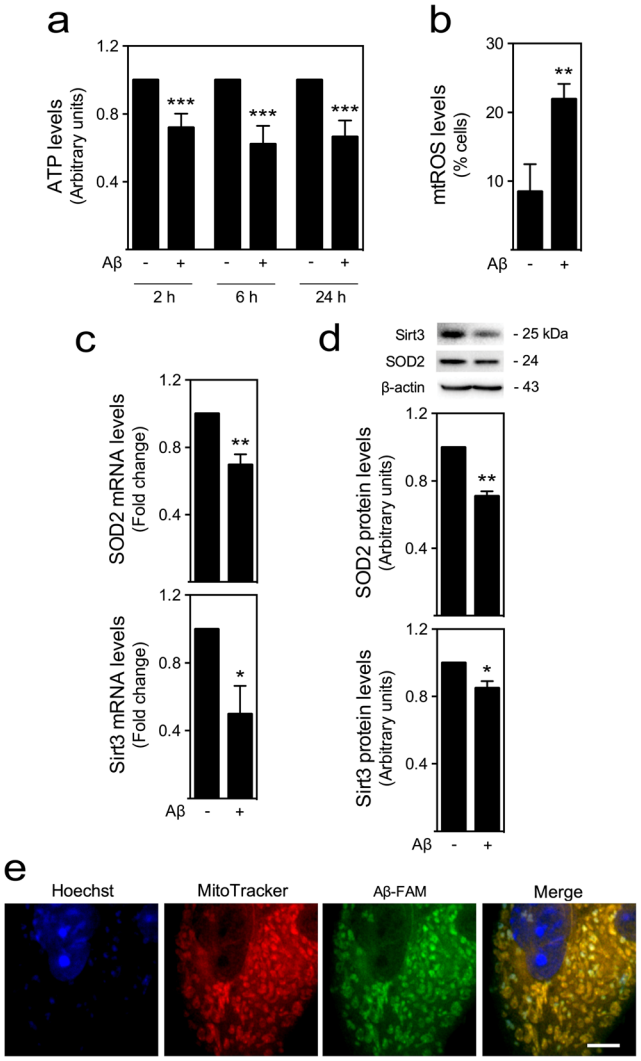


Fig. 2 Aβ reduces ATP supplies, increases mitochondrial oxidative stress, and co-localizes in mitochondria of self-renewing NSCs. Mouse NSCs were treated with 10 μM $Aβ_{1-42}$ in self-renewal conditions. Cells were collected for luminescence detection, flow cytometry, qRT-PCR, immunoblotting, and immunocytochemistry analysis as described in *Materials and Methods*. **a** Representative quantification of ATP levels in self-renewing NSCs, at 2, 6, and 24 h of Aβ treatment. Total ATP levels were determined by Mitochondrial ToxGlo™ assay, and normalized to total protein amount. Data is expressed as fold change over control. **b** Representative quantification of mtROS levels in self-renewing NSCs, at 2 h of Aβ treatment. mtROS were measured by flow cytometry. Data is expressed by percentages of cells stained with MitoSOX™ Red reagent. **c** qRT-PCR analysis of *Sod2* and *Sirt3* in self-renewing NSCs treated with Aβ for 2 h. *Gapdh* was used as loading control. Data is expressed as fold change over control. **d** Immunoblotting and respective densitometry of SOD2 and Sirt3 in self-renewing NSCs treated with Aβ for 2 h. β-actin was used as loading control. Data is expressed as fold change over control. **e** Representative images of fluorescence detection of Aβ (green) co-localized with mitochondria (red), by confocal microscopy and Airyscan processing. Nuclei were counterstained with Hoechst 33258 (blue). Scale bar, 5 μm. Self-renewing NSCs were incubated with 2 μM Aβ-FAM for 24 h. Data represents mean values ± SEM for at least three independent experiments. Asterisk indicates $p < 0.05$, double asterisks indicate $p < 0.01$, and triple asterisks indicate $p < 0.001$ compared to untreated cells

mitochondria is tightly controlled by its biogenesis. We next explored whether Aβ peptide impinges on the number of mitochondria, by assessing mtDNA copy number. In fact, Aβ peptide significantly reduced mtDNA copy number ($p < 0.01$; Fig. 3a), suggesting a decrease on mitochondrial mass in NSCs. Moreover, Aβ peptide also decreased mRNA expression of the mitochondrial transcription factor A (Tfam) ($p < 0.01$; Fig. 3a), which is required for both transcription and replication of mtDNA [46].

Mitochondrial biogenesis is a fine-tuned process regulated by both the nuclear respiratory factor 1 (NRF1) and the peroxisome proliferator-activated receptor γ coactivator-1 α (PGC-1α), which form a complex to induce Tfam expression [47, 48]. After 2 h of treatment, Aβ peptide diminished mRNA levels of *Ppargc1a* and *Nrf1* in self-renewing NSCs (at least $p < 0.05$; Fig. 3b). Accordingly, PGC-1α protein levels were also significantly reduced after 6 and 24 h of amyloid incubation (at least $p < 0.01$; Fig. 3c).

Moreover, the *Ppargc1a* gene has a binding site for the transcription factor cAMP-responsive element-binding protein (CREB), which in turn is activated by phosphorylation at Ser133 [49]. In agreement, although total levels of CREB remained unchanged, the phosphorylated and active form of CREB (p-CREB) was shown to be markedly reduced in NSCs by Aβ peptide throughout time (at least $p < 0.05$), (Fig. 3d). Thus, the activation of CREB and consequently its activity were significantly decreased by Aβ peptide as verified by the p-CREB/CREB ratio (at least $p < 0.05$; Fig. 3d).

Taken together, these data show that Aβ-induced decline of mitochondrial mass in self-renewing NSCs is partially dependent on its upstream effects in modulating key regulators of mitochondrial biogenesis.

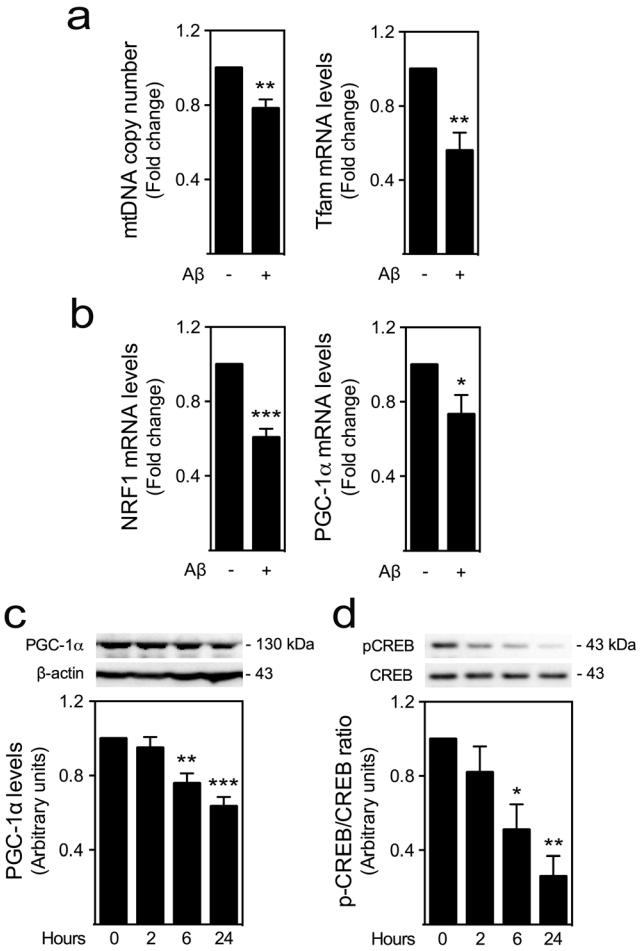


Fig. 3 Aβ reduces mitochondrial mass and disrupts regulation of mitochondrial biogenesis in NSCs. Mouse NSCs were treated with 10 μM Aβ in self-renewal conditions. Cells were collected for qPCR, qRT-PCR, and immunoblotting analysis as described in *Material and Methods*. **a** Measurement of markers associated to mitochondrial biogenesis. Quantification of relative mtDNA copy number assessed by qPCR analysis of mitochondria-encoded gene *mt-Co1* (*left*). Nuclear *Rn18s* was used as loading control. Self-renewing NSCs were treated with Aβ, for 2 h. qRT-PCR analysis of *Tfam* (*right*). Self-renewing NSCs were treated with Aβ, for 2 h. *Gapdh* was used as loading control. **b** qRT-PCR analysis of *Pparg1a* (for PGC-1α) and *Nrf1*. Self-renewing NSCs were treated with Aβ, for 2 h. *Gapdh* was used as loading control. **c** Immunoblotting and respective densitometry of PGC-1α in self-renewing NSCs treated with Aβ for 2, 6, and 24 h. β-actin was used as loading control. **d** Immunoblotting and respective densitometry of p-CREB and total CREB in self-renewing NSCs treated with Aβ for 2, 6, and 24 h. p-CREB was normalized by total CREB levels to determine CREB activation through p-CREB/CREB ratio. Data is expressed as fold change over control and represents mean values ± SEM for at least three independent experiments. Asterisk indicate $p < 0.05$, double asterisks indicate $p < 0.01$, and triple asterisks indicate $p < 0.001$ compared to untreated cells

Aβ Peptide Shatters Mitochondrial Network and Disturbs Regulation of Mitochondrial Dynamics in NSCs

Mitochondrial dynamics regulate events of self-renewal, differentiation and viability [22, 50]. By increasing fusion events, mitochondria can elongate its network, maintain ROS levels, and promote self-renewal. In contrast, mitochondria fragmentation or fission has been associated with cellular apoptosis and differentiation, depending on the stimulus and its magnitude. We then evaluated the transcription factor estrogen-related receptor α (ERRα) that binds to PGC-1α and induces the transcription of mitofusin 2 (Mfn2), a protein involved in mitochondrial fusion [51, 52]. Notably, Aβ treatment significantly reduced *Esrra* and *Mfn2* mRNA levels in NSCs ($p < 0.001$; $p < 0.01$, respectively; Fig. 4a, b). Accordingly, Mfn2 protein levels were also reduced after 6 and 24 h of amyloid treatment (at least $p < 0.01$; Fig. 4c), suggesting a decreased mitochondrial fusion rate. Also, the impact of Aβ peptide in mitochondrial fission of NSCs was assessed by determining the levels of the dynamin-related protein 1 (Drp1) [50]. Curiously, DRP1 protein levels persisted unchanged comparing to untreated cells (Fig. 4c). However, since the mitochondrial network is in constant remodeling and its dynamics depend on the ratio of both fusion and fission mediators, amyloid-induced changes in Mfn2

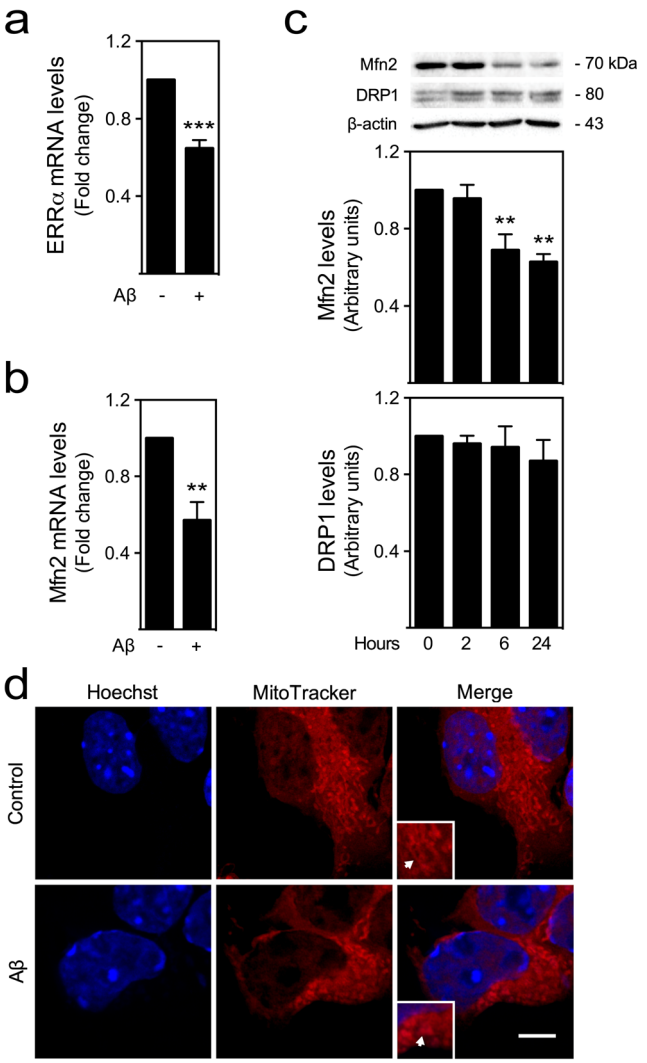


Fig. 4 Aβ triggers mitochondria fragmentation and disturbs regulation of mitochondrial dynamics in NSCs. Mouse NSCs were treated with 10 μM Aβ in self-renewal conditions. Cells were collected for qRT-PCR, immunoblotting, and immunocytochemistry analysis as described in *Material and Methods*. **a**, **b** qRT-PCR analysis of *Esrra* (for ERRα) and *Mfn2*, respectively, in self-renewing NSCs treated with Aβ, for 2 h. *Gapdh* was used as loading control. **c** Immunoblotting and respective densitometry of Mfn2 and Drp1 in self-renewing NSCs treated with Aβ for 2, 6, and 24 h. β-actin was used as loading control. **d** Representative images of fluorescence detection of mitochondrial morphology (red), by confocal microscopy and Airyscan processing. The insets show enlarged views of the boxed area. Arrows mark examples of tubular and elongated mitochondria in control and small and fragmented mitochondria with Aβ. Nuclei were counterstained with Hoechst 33258 (blue). Scale bar, 5 μm. Self-renewing NSCs were incubated with 2 μM Aβ for 24 h. Data is expressed as fold change over control and represents mean values ± SEM for at least three independent experiments. Double asterisks indicate $p < 0.01$ and triple asterisks indicate $p < 0.001$ compared to untreated cells

protein levels suggest that Aβ peptide favors fission of mitochondria. To confirm amyloid-induced alterations in mitochondrial dynamics, we examined qualitatively mitochondrial morphology through confocal microscopy. In agreement, Aβ-treated NSCs displayed fragmented mitochondria compared to control (Fig. 4d).

Overall, Aβ peptide unbalances mitochondrial dynamics in self-renewing NSCs, favoring mitochondrial fission, by acting upstream to this organelle.

High Levels of Aβ Irreversibly Impair Mitochondria Precluding Any Recover of NSC Cell Fate

Since mitochondria play a pivotal role in NSC fate decision and Aβ peptide precludes neurogenesis, we hypothesized that NSC viability, proliferation, or differentiation would be recovered by restoring protein levels of PGC-1α, Mfn2, or Sirt3.

To re-establish biogenesis, NSCs were transfected with PGC-1α. Of note, since PGC-1α has a high turnover in NSCs, protein accumulation of PGC-1α only occurred in transfected cells simultaneously treated with the proteasome inhibitor MG132 (Fig. 5a). Nevertheless, overexpression of PGC-1α was not sufficient to protect against Aβ-induced NSC death (Fig. 5b). Next, we operated at mitochondrial dynamic levels and overexpressed Mfn2 protein (Fig. 5a). Again, although the reestablishment of Mfn2 was observed in NSCs, Mfn2 did not endow any protection over Aβ-induced cytotoxicity (Fig. 5b). Finally, we overexpressed Sirt3 to rescue NSCs from oxidative stress (Fig. 5a). Still, under this scenario, the percentage of viable cells was unchanged comparing to cells only treated with Aβ peptide (Fig. 5b). Indeed, increased Sirt3 could not even recover the Aβ effect on neurogenic markers (Fig. 5c). Collectively, our results demonstrate that high levels of Aβ peptide strongly and irreversibly impair mitochondrial biogenesis, dynamics, and oxidative state, affecting both NSC survival and commitment.

Discussion

Adult neurogenesis has been increasingly recognized to affect learning and memory [3, 4, 53, 54]. Since AD patients suffer from memory loss, the maintenance and stimulation of adult NSC pool and neurogenesis may attenuate AD progression. Importantly, mouse models of AD mainly exhibited neurogenesis decline. In fact, APP/PS1 transgenic mice were shown to display reduced number, proliferation, and differentiation of NSCs [55–57]. Strikingly, some proteins central to AD familial pathology, such as APP, PS1, and amyloid precursor protein intracellular domain (AICD), a metabolite of APP, have been found to also regulate adult neurogenesis per se [13–15]. Moreover, most AD patients have the sporadic form of the disease, without expressing mutated forms of *APP* or *PSEN1*. In this study, we established the functional effect of the AD hallmark, Aβ peptide seeding, on stem cell properties. We clearly demonstrate that $A\beta_{1\text{-}42}$ peptide irreversibly

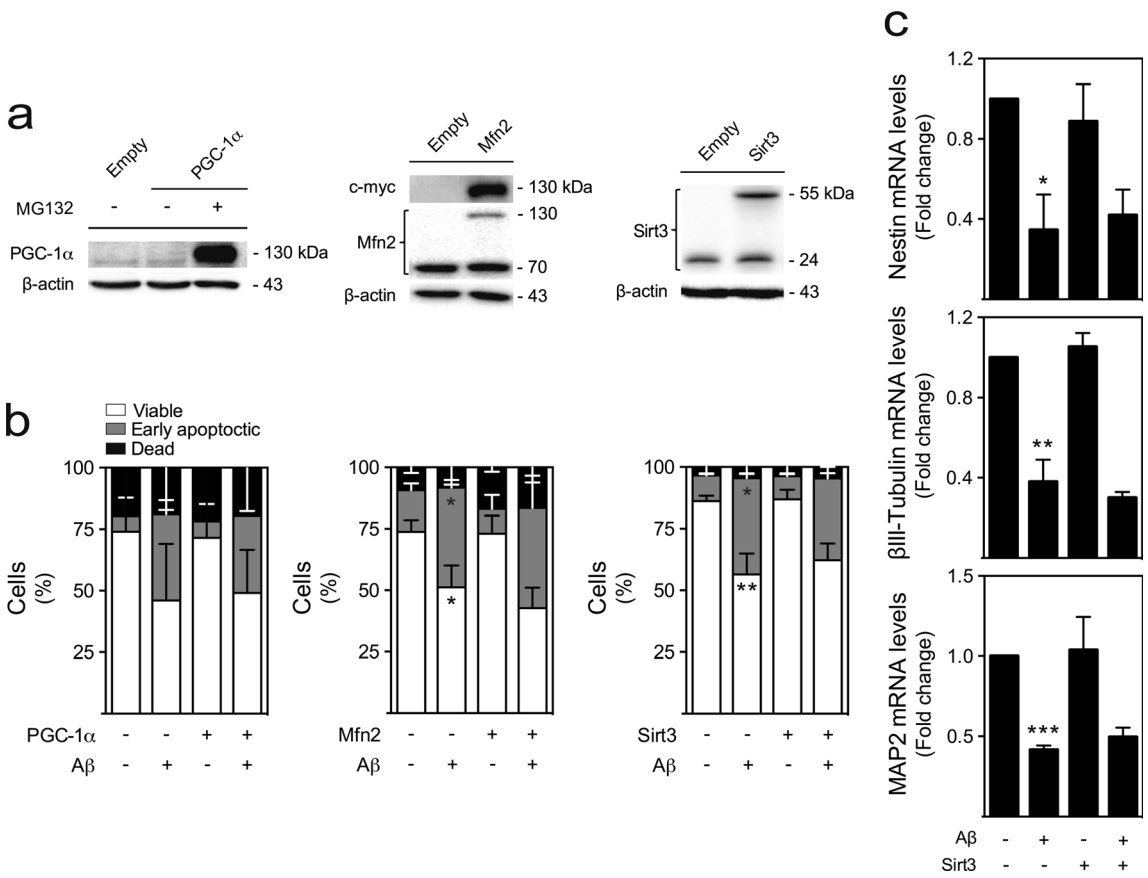


Fig. 5 Aβ irreversibly impair mitochondria precluding any recover of NSC cell fate. Mouse NSCs were transfected with PGC-1α, Mfn2, or Sirt3 as described in *Material and Methods*. After 24 h of transfection, self-renewing NSCs were treated with Aβ peptide to assess viability by flow cytometry. **a** Representative immunoblotting to assess transfection efficiency of PGC-1α, Mfn2-c-myc, and Sirt3. For PGC-1α overexpression, cells were also treated with proteasome inhibitor MG132 (10 μM) 6 h before cell collection. **b** Quantification of NSC viability, by Guava Nexin flow cytometry in self-renewal conditions, after 24 h of PGC-1α, Mfn2, or Sirt3 transfection, followed by 24 h of amyloid treatment. Data is expressed by percentages of viable, early apoptotic, and dead cells. **c** qRT-PCR analysis of *Nes*, *Tubb3*, and *Map2* for differentiation markers nestin, βIII-tubulin, and MAP2, after 48 h of neurogenic differentiation with amyloid pre-incubation and Sirt3 overexpression in self-renewal conditions. *Actb* was used as loading control. Data represents mean values ± SEM for at least three independent experiments

hampers survival, proliferation, and neurogenic differentiation of NSCs, by disturbing key regulators of mitochondrial biogenesis, dynamics, and oxidative stress.

The reduction of NSC survival and proliferation by Aβ peptide corroborates other studies [9, 58–61]. Indeed, Aβ peptide has the capacity to reduce the pool of NSCs, compromising the formation of newborn neurons, as observed in AD mouse models and AD patients [5, 7, 10, 11, 62]. However, in contrast with most in vitro studies describing the impairment of neuronal differentiation by Aβ [9, 58–60, 63], we did not detect a direct inhibition of neurogenesis by Aβ peptide. These discrepancies may result, in part, from a different amyloid concentration, form, and species of Aβ peptide but also from different protocols and type of cells used. Nonetheless, we demonstrated that neurogenic differentiation is indirectly blocked by Aβ peptide when NSCs are pre-exposed to Aβ peptide before the differentiation induction. The observed decrease in both immature and mature cells upon Aβ exposure could rely on a decrease in self-renewal and survival potential of NSCs. In fact, Park et al. reported that nestin itself promotes cell survival and self-renewal of NSCs [64].

We then hypothesized that amyloid peptide disrupts signaling pathways of undifferentiated cells important for neurogenesis progression after a neurogenic stimulus. In fact, differentiating cells are highly sensitive to mitochondrial dysfunction [65], and it is expected that neuronal differentiation will be significantly hampered when mitochondria are already compromised in self-renewing NSCs. We found that Aβ peptide strongly reduces cellular ATP supplies, while generating oxidative stress in mitochondria of proliferative NSCs. These findings are in agreement with the fact that in primary neurons, Aβ peptide interacts with mitochondrial enzymes and respiratory complexes promoting free radical formation with consequent decreased ATP production [66]. On the other hand, we show that mtROS were induced by an unbalanced radical scavenger system; Aβ exposure reduced the

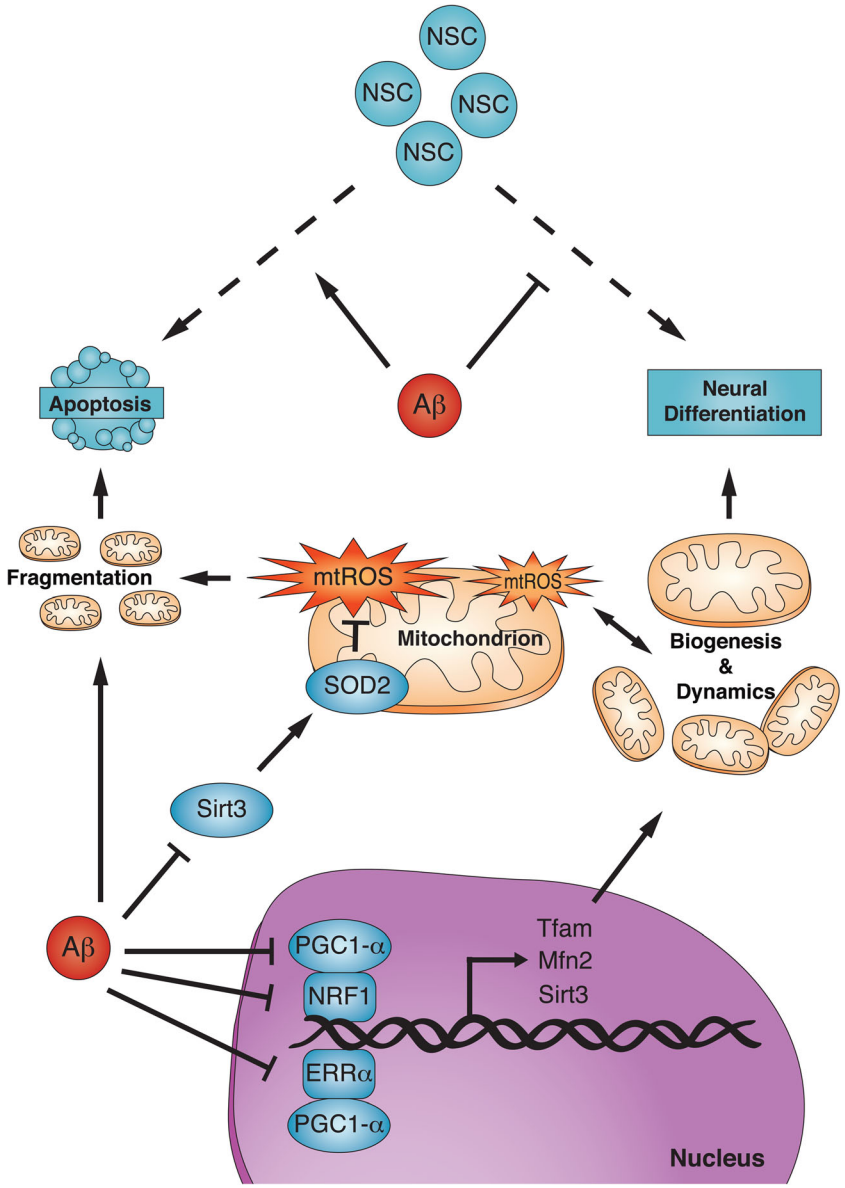


Fig. 6 Schematic model depicting the irreversible mitochondrial effects of Aβ on NSC fate. Aβ seeding directly impairs NSC viability and proliferation, possibly affecting the maintenance of in vivo NSC pool. Moreover, Aβ irreversibly changes mitochondrial pathways associated to mitochondrial biogenesis, dynamics, and oxidative state in self-renewing NSCs, compromising neurogenic differentiation

expression of the primary mitochondrial antioxidant enzyme SOD2 in self-renewing NSCs, possibly preventing the conversion of superoxide anions O_2•− into hydrogen peroxide (H_2O_2), and thus the scavenging of mtROS. In addition to SOD2 expression, Aβ peptide itself may affect SOD2 activity. In fact, we describe that Aβ peptide diminished the expression of SIRT3, which directly deacetylates and consequently activates SOD2 [63]. Accordingly, AD brains display decreased amounts of SOD2 [67], indicating that in AD brains, endogenous NSCs might also be submitted to high levels of oxidative stress.

The balance between mitochondrial fission and fusion events has been considered a process by which physiological levels of ROS and differentiation can be fine-tuned [22, 23, 50, 68]. For instance, mitochondrial fusion constitutes a mechanism to dilute mitochondrial oxidative stress and assure long-term survival of mitochondria [69]. In fact, similarly to neurons [70], we showed that Aβ peptide favors mitochondrial fragmentation in self-renewing NSCs. Aβ-induced mitochondrial fission and fragmentation in NSCs may rely on mtROS accumulation but also on disruption of mitochondrial fusion. Indeed, although Aβ peptide did not alter the expression of the fission-associated protein, Drp1, it significantly reduced the expression of the fusion-associated protein, Mfn2 in undifferentiated NSCs. Our results are in agreement with previous studies showing that Aβ causes mitochondrial

dysfunction and neuronal loss by triggering mitochondrial fragmentation through decreased Mfn2 expression without changing Drp1 levels [71]. Remarkably, these changes in mitochondrial dynamics machinery were also observed in AD brains [70]. The amyloid effect on Mfn2 expression was also consistent with Aβ-repressed expression of ERRα and PGC-1α in this cellular context, two main factors involved in *Mfn2* transcription [72]. Fragmented mitochondria have been associated with increased ROS levels, and consequently activation of differentiation-related genes [22]. However, we showed that high levels of Aβ peptide generate chronically fragmented mitochondria that barely control oxidative stress, and thus inhibit neuronal differentiation and cause NSC death.

The increase of mitochondrial mass is required for cell cycle exit and differentiation [26]. Here, we also show that Aβ peptide blocked NSC survival and differentiation by disturbing mitochondrial biogenesis. In fact, we demonstrate that Aβ peptide significantly reduced mtDNA copy number partially by reducing *Tfam* expression, a key player of mtDNA transcription and replication [28]. Loss of viable mitochondria is also intensified by Aβ-mediated effect on mitochondrial fusion and oxidative stress [73–75]. Additionally, we show that Aβ peptide impaired several mediators of mitochondrial biogenesis in self-renewing NSCs, namely CREB, PGC-1α, and NRF1. Curiously, PGC-1α forms a complex with NRF1 to promote transcription of *Tfam* and other genes encoding for mitochondrial proteins [48, 76, 77]. PGC-1α is also a positive regulator of Sirt3 [43]. Thus, Aβ seeding may also favor mitochondrial oxidative stress of NSCs by reducing PGC-1α expression. Accordingly, low levels of PGC-1α and CREB have been described in AD brains and in primary hippocampal neurons from AD mouse models [78, 79].

As mitochondrial biogenesis, dynamics and oxidative stress were impaired by Aβ peptide in NSCs and since all these parameters are pivotal in regulating NSC fate, we finally investigated whether reestablishment of some of these cellular changes would rescue NSC fate after Aβ peptide exposure. For that, we successfully restored the protein levels of PGC-1α, Mfn2, or Sirt3 in NSCs. Nonetheless, we demonstrate that once Aβ load impairs the core regulatory networks responsible for mitochondrial redox homeostasis, viability and neurogenesis of NSCs could not be rescued neither by restoring mitochondrial biogenesis, dynamics, nor oxidative state. Recently, chemical modulation of AMP-regulated protein kinase (AMPK) was shown to protect against Aβ-induced NSC death [61, 80]. Nevertheless, AMPK activates a wide range of signaling pathways in neural cells [81, 82]. Altogether, this study brings new significant information to rethink the mitochondrial targets potentially relevant for endogenous NSC-based strategies against Aβ toxicity. Additionally, it questions whether endogenous NSCs can still be considered or recovered in the context of severe oxidative stress such as in later stages of AD.

Conclusions

Overall, we have shown that Aβ peptide itself precludes viability and proliferation of NSCs, possibly compromising the maintenance of in vivo NSC pool (Fig. 6). Also, we demonstrated that Aβ peptide does not directly impair neurogenic differentiation; instead, it modifies signaling pathways in self-renewing NSCs, further compromising the neurogenic process. Finally, we dissected the molecular mechanisms by which Aβ impairs NSC fate by demonstrating that Aβ peptide irreversibly compromises the mitochondrial network, constraining any rescue of NSC fate by acting at mitochondrial biogenesis, dynamics, or oxidative state.

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Authors' Contributions MFR, CMPR, and SS conceived and designed the experiments. MFR performed the experiments. TG contributed in establishing the in vitro model. MFR, SS, and CMPR analyzed and interpreted the data. ACR contributed with reagents. MFR wrote the manuscript. CMPR and SS critically reviewed the manuscript. All authors read and approved the final manuscript.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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