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Amplifying mitochondrial function rescues adult neurogenesis in a mouse model of Alzheimer's disease

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ABSTRACT

Adult hippocampal neurogenesis is strongly impaired in Alzheimer's disease (AD). In several mouse models of AD, it was shown that adult-born neurons exhibit reduced survival and altered synaptic integration due to a severe lack of dendritic spines. In the present work, using the APPxPS1 mouse model of AD, we reveal that this reduced number of spines is concomitant of a marked deficit in their neuronal mitochondrial content. Remarkably, we show that targeting the overexpression of the pro-neural transcription factor *Neurod1* into APPxPS1 adult-born neurons restores not only their dendritic spine density, but also their mitochondrial content and the proportion of spines associated with mitochondria. Using primary neurons, a *bona fide* model of neuronal maturation, we identified that increases of mitochondrial respiration accompany the stimulating effect of *Neurod1* overexpression on dendritic growth and spine formation. Reciprocally, pharmacologically impairing mitochondria prevented *Neurod1*-dependent trophic effects. Thus, since overexpression of *Neurod1* into new neurons of APPxPS1 mice rescues spatial memory, our present data suggest that manipulating the mitochondrial system of adult-born hippocampal neurons provides neuronal plasticity to the AD brain. These findings open new avenues for far-reaching therapeutic implications towards neurodegenerative diseases associated with cognitive impairment.

1. Introduction

In mammals, new neurons are produced in the hippocampus throughout adult life (Altman and Das, 1965; Eriksson et al., 1998). These neurons become structurally and functionally integrated into the hippocampal circuitry where they contribute to hippocampal-dependent memory processes (Aimone et al., 2011; Gu et al., 2012). Recent data indicate that regulation of mitochondrial function and morphology through fusion and fission events, *i.e.* mitochondrial dynamics, is part of the cellular program that controls the development and sustains the maturation of new neurons during adulthood (Steib et al., 2014), suggesting that, reciprocally, mitochondrial defects could be associated with altered adult neurogenesis.

Due to neurons distinctive arborescent morphology and high compartmentalization (dendrites, axon, synapses), their functions and survival present a challenge for mitochondria, which must be highly adaptive and move within and between the subcellular compartments involved in neuroplasticity. By generating energy and contributing to calcium and redox homoeostasis, mitochondria are imperative in the fundamental processes that control neuroplasticity, such as neural differentiation (Hagberg et al., 2014), neurite outgrowth and synaptic maturation (Bertholet et al., 2013), neurotransmitter release (Vos et al., 2010), and dendritic remodeling (Cheng et al., 2010; Li et al., 2004; Mattson, 2008). Moreover, as circulating signaling platforms, mitochondria integrate various signals, including deleterious ones, and can ultimately precipitate neurons into apoptosis (Magnifico et al., 2013).

Among neurodegenerative pathologies, Alzheimer Disease (AD) is one of the most common causes of dementia and cognitive impairment in the elderly. Mitochondrial alterations have been reported in the brains of AD patients (Hirai et al., 2001), as well as in transgenic mouse models of AD (Pedrós et al., 2014; Trushina et al., 2012; Xu et al., 2017). More recently, the expression of genes essential for mitochondrial biogenesis, like NRF1 and PGC-1alpha was found to be strongly down-regulated in the hippocampus of the transgenic APPxPS1

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mouse model, as early as 3 and 6 months of age (Pedrós et al., 2014). Notably, several studies, including ours, have reported alterations of adult hippocampal neurogenesis in these AD mice (Demars et al., 2010; Hamilton and Holscher, 2012; Taniuchi et al., 2007; Verret et al., 2007). We recently established that morphological development and synaptic integration of adult-born hippocampal neurons of the double transgenic APPxPS1 mouse model of AD could be enhanced by the targeted overexpression of the basic helix–loop–helix transcription factor (bHLH) *Neurod1*, favoring its pro-neural differentiating effects (Richetin et al., 2015). Remarkably, the presence of such highly connected adult-born neurons was sufficient to rescue to normal spatial memory in APPxPS1 mice (Richetin et al., 2015), emphasizing the need for further investigations of the underlying cellular mechanisms.

Of note, another neurogenic bHLH transcription factor from the NeuroD family, Neurod6, was found to confer tolerance to oxidative stress to differentiated stably transfected PC12 cells, by sustaining neuronal mitochondrial biomass (Uittenbogaard et al., 2010) and ATP levels during the very early stages of neuronal differentiation (Baxter et al., 2009). Similarly, PGC-1alpha-dependent enhancement of mitochondrial biogenesis was demonstrated to play an *sine qua non* role during the formation and maintenance of neuronal dendritic spines (Cheng et al., 2012). Therefore, we hypothesized that the beneficial effect of *Neurod1* overexpression on adult-born neurons could be mediated, at least in part, by an action on mitochondrial biogenesis and/or function. We thus tested this hypothesis in maturating neurons *in vitro* as well as in the APPxPS1 mouse model of AD. In this study, we conclusively identify mitochondria dysfunction as an important contributor of adult neurogenesis alteration in the context of AD pathology, and we demonstrate that this effect can be reversed by *Neurod1* gene delivery through an increase of mitochondrial biogenesis as well as of mitochondria-endowed spines.

2. Material and methods

2.1. Primary culture of cortical neurons and transfection

All animal procedures were approved by the CNRS/Fédération de Recherche de Biologie de Toulouse Animal Experimentation Ethics Committee (C2EA-01) under the protocol number 01024-01.7. As previously described (Bertholet et al., 2013), cortical neurons were prepared from Day 17 embryos from pregnant Wistar rats (Janvier) dissected after intraperitoneal Pentobarbital (Sigma) anesthesia. Briefly, cortices were microdissected, enzymatically dissociated with papain (10 U/ml, Sigma), dissociated by trituration and filtered through a membrane (70 μm, BD Falcon). Cells were then purified through a BSA solution (8%, Sigma) diluted in Neurobasal A-25 (Invitrogen). Cells (5×10^6) were electroporated after dissociation using the Rat Neuron Nucleofector Kit (Amaxa, Lonza) and three micrograms of pCMMP-IRES2eGFP (to express GFP alone) or pCMMP-*Neurod1*-IRES2eGFP (to co-express GFP and *Neurod1*) plasmids. According to our previous work, transfection efficiency around 36–37% (Bertholet et al., 2013). Cells were plated on 35 mm dishes or 12 mm glass coverslips in 24 well plates previously coated with poly-D-lysine (0.1 mg/ml, Sigma). Cells were grown in Neurobasal A-25 supplemented with B27 (Invitrogen), 1 mM glutamine, 1% penicillin and streptomycin (1000 U/ml, Gibco), 250 U/ml amphotericin (Invitrogen) and 1 mM lactic acid (Sigma) at a density of 1.25×10^6 per dish or 2.5×10^5 per well. Alternatively, neurons were treated at DIV2 with carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP, Sigma) at a 1.5 μM final concentration, or with the vehicle MeOH (Sigma) at equivalent dilution, for 24 h.

2.2. Immunocytochemistry

Primary cortical neurons cultured on glass coverslips were fixed with PBS containing 3.7% formaldehyde for 20 min at 37 °C. They were permeabilized for 15 min in a PBS solution containing 0.3% Triton X-100 and 10% normal goat serum. Unless otherwise stated, the following incubations and rinsing steps were done in PBS supplemented with 10% goat serum, 5% BSA and 0.5% Tween20. The following primary antibodies were used overnight at 4 °C: anti-ATP synthase (mouse, 1:500, A21351, Life Technologies) or anti-Map2 (mouse, M3696, 1:1000, Sigma) plus anti-GFP (chicken, 1:500, ab16901, Millipore). Alexa Fluor 594 goat anti-mouse (Invitrogen, A11005) and Alexa Fluor 488 goat anti-chicken (Invitrogen, A11039) were applied (1:1000) for 1 h at room temperature. After a final incubation with 0.25 μg/ml Hoechst in PBS over 5 min, coverslips were rinsed and mounted in Mowiol and stored at 4 °C until analysis under a Leica SP5 confocal microscope.

2.3. Morphometric analyses on primary cortical neurons

Morphology of primary cortical neurons was analyzed from z-series of 15–20 optical sections at 0.13 μm interval, with either a 40× oil lens or a 63× oil lens, with or without a digital zoom of 5, acquired on a TCS SP5 confocal system (Leica Microsystem). Three-dimensional reconstructions of series of confocal images were conducted using Imaris XT (Bitplane AG) on deconvoluted images (Huygens SVI). Map2-immunostained dendritic length and branching were measured for ten neurons per well using the NeuronJ add-on to the ImageJ software (http://rsbweb.nih.gov/ij/) and digitized images (40× magnification, three wells per treatment per experiment, $n = 3$ independent experiments). Spines density and mitochondria analysis of GFP-labeled (GFP+) neurons at DIV9 were performed into proximal dendritic segments located at 20 μm from the soma, according to the same method as described below for GFP+ adult-born neurons.

2.4. Immunoblotting of proteins extracted from primary cortical neurons

Cells were harvested in lysis buffer (50 mM Tris-HCL pH 7.5, 250 mM NaCl, 5 mM EDTA, 5 mM EGTA, 1 mM dithiothreitol, 0.1% Triton X-100, 0.1% SDS, 1% deoxycholate, 1% NP40) and a protease inhibitor cocktail (cOmplete protease inhibitor mixture, Roche). Lysates were sonicated and protein concentration was determined using a Bradford assay. Proteins (50–100 μg) were separated by SDS-PAGE on a 15% polyacrylamide gel, and analyzed by immunoblotting. The following primary antibodies were used: anti-OPA1 (1:300, BD Biosciences), anti-TFAM (rabbit, 1:2000, ab131607, Abcam), anti-TOM20 (rabbit, 1:25000, sc-11415, Santa Cruz Biotechnology, anti-synapsin (mouse, 1:2000, 106,001, Synaptic Systems), anti-actin (mouse, $1{:}10^5$, MAB1501, Millipore). After 1 h incubation with primary antibodies, the following secondary antibodies were applied (1:5000): polyclonal goat anti-mouse (AbCam, ab6789) or anti-rabbit (AbCam, ab6721) IgG conjugated with horseradish peroxidase. After enhanced chemiluminescent detection of HRP-labeled secondary antibodies, scanned photographic films were analyzed using ImageJ software.

2.5. Measurement of oxygen consumption of primary cortical neurons

Oxygen consumption rates (OCR) were assayed using the XF24 Extracellular Flux Analyser (Seahorse Bioscience, North Billerica, MA). Primary cortical neurons ($3 \cdot 10^5$) were plated on poly-D-Lysine (Sigma Aldrich) coated XF24 microplates, 9 days before OCR measurements. Dual-analyte sensor cartridges were soaked in XF Calibrant Solution (Seahorse Biosciences) in 24-well cell culture microplates overnight at 37 °C to hydrate. Approximately 1 h prior to experimentation, injection ports on the sensor cartridge were filled with drugs from XF Cell Mito Stress Test Kit (Seahorse Bioscience): oligomycin (0.6 μM), Carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone (FCCP) (6 μM) and rotenone (50 nM) with antimycin A (0.182 μM). For oxygen consumption measurement, neuronal growth medium was replaced 1 h prior experimentation with incubation media: DMEM (Sigma Aldrich) supplemented with NaCl (143 mM) (Sigma Aldrich), Phenol Red (3 mg/ml) (Sigma Aldrich), glucose (10 mM) (Sigma Aldrich),