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Research Paper

Genetic ablation of tau improves mitochondrial function and cognitive abilities in the hippocampus

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ABSTRACT

Tau is a key protein for microtubule stability; however, post-translationally modified tau contributes to neurodegenerative diseases by forming tau aggregates in the neurons. Previous reports from our group and others have shown that pathological forms of tau are toxic and impair mitochondrial function, whereas tau deletion is neuroprotective. However, the effects of tau ablation on brain structure and function in young mice have not been fully elucidated. Therefore, the aim of this study was to investigate the implications of tau ablation on the mitochondrial function and cognitive abilities of a litter of young mice (3 months old). Our results showed that tau deletion had positive effects on hippocampal cells by decreasing oxidative damage, favoring a mitochondrial pro-fusion state, and inhibiting mitochondrial permeability transition pore (mPTP) formation by reducing cyclophilin D (Cyp-D) protein. More importantly, tau deletion increased ATP production and improved the recognition memory and attentive capacity of juvenile mice. Therefore, the absence of tau enhanced brain function by improving mitochondrial health, which supplied more energy to the synapses. Thus, our work opens the possibility that preventing negative tau modifications could enhance brain function through the improvement of mitochondrial health.

1. Introduction

Tau is a protein that associates with microtubules and is found prominently in the axons of neurons [34]. In physiological conditions tau is involved in neuronal morphogenesis, especially promoting axonal growth, stabilization, elongation, and neuronal polarity [34]. In addition, tau promotes vesicular transport and is important for synaptic function [6]. These and other functions of tau are modulated by post-translational modifications in specific residues [6,79].

Abnormal modifications of tau are involved in a number of neurodegenerative diseases, known as tauopathies, which are characterized by the formation of pathological deposits of tau [51,56]. Hyper-phosphorylated or cleaved forms of tau are the principal components of neurofibrillary tangles, one of the neuropathological hallmarks of Alzheimer's disease (AD) [96]. Pathological forms of tau generate serious alterations in neuronal activity, affecting synaptic transmission and learning and memory processes, which finally leads to neurodegeneration [101]. Moreover, previous *in vitro* and *in vivo* studies have shown that pathological forms of tau are likely to form tau aggregates and these events appear to play an important role in early synaptic dysfunction and memory deficits [39,43].

Genetic deletion of tau could be protective [73,98]. Studies in a mouse model of AD have shown that ablation of tau expression prevents neurotoxicity induced by the Amyloid-β peptide and improves cognitive damage [63,71,73]. Similarly, tau deletion protects against the effects of stress on neuronal structure and working memory [49].

Abbreviations: WT, wild-type; KO, knockout; tau-/-, homozygous tau KO; mPTP, Mitochondrial permeability transition pore; AD, Alzheimer's disease; GR, Glucocorticoid Receptor; HO, heme oxygenase; GCS, γ-glutamine cysteine synthase; ROS, reactive oxygen species; Mfn1, Mitofusin 1; Mfn2, Mitofusin 2; OPA1, optic atrophy 1; Drp1, Dynamin-related protein 1; Fis1, Fission 1; Cyp-D, cyclophilin D; VDAC, voltage-dependent anion channels; ANT, adenine nucleotide translocase; PGC-1α, Peroxisome proliferator-activated receptor-gamma coactivator; SYP, synaptophisin; VAMP, Vesicle-associated membrane protein; PSD95, Post synaptic density 95; NR2B, N-methyl D-aspartate receptor subtype 2B; NOR, Novel Object Recognition; MWM, Morris Water Maze

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However, other reports suggest that the absence of tau could have a negative effect on normal brain function [42].

In neurons, mitochondria are critical for synaptic function [50,78,93]. Mitochondria are highly dynamic organelles that are necessary to supply energy to the synapse [50]. Also, they are involved in Ca^{2+} regulation, playing a pivotal role in cytoprotection and oxidative damage reduction [26]. Pathological forms of tau can impair mitochondrial function, including mitochondrial morphology, transport, and bioenergetics [34,43]. Interestingly, we found that the expression of pathological tau species, in particular truncated tau, induces mitochondrial fragmentation and bioenergetics failure in immortalized cortical neurons and primary neuronal cultures [67,70]. Similarly, phosphorylated tau induces mitochondrial fragmentation and affects the bioenergetics function of mature neurons [22,71]. Thus, the absence of tau protein in neural cells could prevent the effects on mitochondrial structure and function produced by post-translationally-modified tau.

Considering that limited research has used tau-deficient mouse models and the role of tau on the regulation of mitochondrial function and the resulting implications on cellular and cognitive processes are not entirely clear, a study examining the impact of tau ablation will contribute to the understanding of the physiological function of tau protein *in vivo*. The present study was conducted in litters of young mice (3 months old) to investigate the effects of tau reduction in hippocampal tissue, to identify the implications of tau on mitochondrial function and behavior during youth. We used a well-known homozygous tau knockout (KO) (tau-/-) mouse line (B6.129 × 1-Mapt^{tm1Hnd}/J, Stock No: 007251, The Jackson Laboratory) [26], which is phenotypically normal and reproductively viable [26,54]. Our findings suggest that tau ablation improves mitochondrial function through the regulation of mitochondrial structure and dynamics, indicated by increased levels of proteins involved in fusion processes and decreased expression of proteins that forms the mitochondrial permeability transition pore (mPTP). In addition, tau -/- KO mice presented a significant reduction in oxidative damage, accompanied by the activation of Nrf-2 signaling and PGC-1α in the hippocampus. These effects generated an improvement in the mitochondrial bioenergetics state, indicated by increased ATP production in the hippocampus. Finally, we observed that tau deletion improved cognitive abilities, including exploration capacity against a stimulus, recognition memory, social behavior, and most significantly, attention. In conclusion, tau ablation in mice resulted in enhanced mitochondrial function in the hippocampus and improved cognitive capacities.

2. Materials and methods

2.1. Animals

Three month old wild-type (WT) and homozygous tau KO (tau-/-) mice were obtained from The Jackson Laboratory (strain name: B6.129-Mapttm1Hnd/J Bar Harbor, ME, Stock no 007251). Tau -/- mice are viable and successful reproducible, and have a normal lifespan reaching 20 month-old [26,82]. The animals were handled according to the guidelines of the National Institute of Health (NIH, Baltimore, MD). Animals were maintained at the Bioterio Central de la Universidad Autónoma under a sanitary barrier and in closed colonies. All mice were housed up and grouped to four per cage and keeping them to a monitored room temperature of 23 °C. Animals were kept on a 12-h light/dark cycle and were given ad libitum access to food and water according to Jackson lab recommendations (The Jackson Laboratory, USA). Experimental procedures were approved by the Bioethical and Biosafety Committee of the Universidad Autónoma de Chile. A total of 8 wt and 7 tau-/- mice were used to perform cognitive test. Also, for biochemical studies, we used an n = 4 of different animals for the WT group and n = 5 for tau -/- group. For mitochondrial isolation and analysis of function, we used an n = 5 of different animals for each group.

2.2. Reagents and antibodies

The primary antibodies used were: rabbit anti-β-tubulin (sc-9104, Santa Cruz Biotechnology, Inc., 1:2000), mouse anti-Total OXPHOS Human WB Antibody Cocktail (ab110411, Abcam, Inc. dilution 1:2000), mouse anti-COX IV (4D11-B3-E8) (11967S, Cell Signaling, 1:1000), mouse anti-β-actin (sc-47778, Santa Cruz Biotechnology, Inc., 1:1000), rabbit anti-Opa1 (PA1–16991, Thermo Scientific, 1:1000), rabbit anti-Cyclophilin D (PA3–022, Thermo Scientific, 1:1000), rabbit anti-Fis1 (sc-98900, Santa Cruz Biotechnology, Inc., 1:500), rabbit anti-Mfn1 (H-65) (sc-50330, Santa Cruz Biotechnology, Inc. 1:1000), rabbit anti-Mfn2 (H-68) (sc-50331, Santa Cruz Biotechnology, Inc., 1:1000), mouse anti-VDAC1 (B-6) (sc-390996, Santa Cruz Biotechnology, Inc., 1:1000), rabbit anti-phospho-DRP1 (Ser616) (4494, Cell Signaling, 1:1000), mouse anti-DRP1 (C-5) (sc-271583, Santa Cruz Biotechnology, Inc., 1:1000), rabbit anti-nitrotyrosine (n-tyr) (141682-AP, US Biological Life Sciences, 1:500), and goat anti-4HNE (H6275-02, US Biological, Life Sciences, 1:1000). Anti-Total OXPHOS Human WB Antibody Cocktail has been previously used with mice samples, but is necessary add anti-COX IV mouse to the correct detection, because OXPHOS Human Cocktail not detect this mouse mitochondrial complex [24]. The fluorescent dyes used were MitoTracker™ Red CM-H2Xros (Catalog number: M7513, Thermo Fisher Scientific), MitoTracker™ Green FM (Catalog number: M7514, Thermo Fisher Scientific), CM-H2DCFDA (Catalog number: C6827, Thermo Fisher Scientific), and VECTASHIELD Antifade Mounting Medium with DAPI (Catalog number: H1200, Vector Laboratories, Inc).

2.3. Behavioral tests

All behavioral tests were monitored using an automatic tracking system (Any-MAZE Behavioral software).

2.3.1. Morris water maze (MWM) tests

The MWM task was performed as previously described [85]. Briefly, the mice were trained in a 1.2-m-diameter circular pool (opaque water, 50 cm deep) filled with 19–21 °C water. A submerged 9-cm platform (1 cm below the surface of the water, invisible to the animal) was used for training, with a maximum trial duration of 60 s. The mice remained on the platform for 10 s at the end of each trial. Each animal was trained to locate the platform. The test was performed with 3 trials per day and for each trial, the latency time required to reach the platform and the time spent in each quadrant was measured. After testing, the mouse was gently removed from the maze and returned to its cage.

2.3.2. Memory flexibility (MF)

MF tests were performed as previously described [83,86]. Briefly, a circular white pool was prepared with non-toxic white paint and a hidden platform (diameter: 9 cm) in 4 quadrants. The animals were pretrained in this pool for 60 s (s) 1 day before the actual testing began. The water temperature was kept between 18 and 20 °C. To acclimate the animals to the room and the swimming strategy, the platform was removed from the pool. Then, animals were subjected to testing for 4 consecutive days with a maximum of 15 trials per day. Every day, the platform position in a quadrant was changed. Testing stopped when the animal reached the platform on 3 consecutive trials with an average of 20 s or less. Data are presented as the number of trials after which the animals met this criteria.

2.3.3. Open field (OF) tests

OF tests were performed 2 days after the MWM test as previously described [86]. The animals were individually placed at the center of a 72 × 72 × 32 cm white acrylic box and were allowed to move freely

within it for 10 min. For all behavioral tests, data were gathered and analyzed with a video tracking system (HVS Imagen, UK). The room temperature was maintained at 20 °C.

2.3.4. Novel object recognition (NOR) tests

NOR tests were performed in a 38 × 38 × 32 cm white acrylic box one day after the OF test as previously described [86,91]. The animals were habituated to the box without any objects present for 2 consecutive days. For testing, each animal was placed at the center of the box which contained 2 identical objects (old objects) for 10 min. Then, the box and objects were cleaned with a 50% methanol solution. Two hours later, the animal was exposed to an old object and a new object of a different shape and color than the old object. The box and objects were cleaned again and the next animal was tested. The recognition index was calculated as the time spent exploring the new object divided by the time spent exploring both objects.

2.3.5. Social interaction test

For this task, a protocol previously described was used [84]. Briefly, mice were habituated for 10 min in a 3-chamber box, with each chamber measuring 20 × 40 × 22 cm. Then, an object and an unknown mouse were placed inside individual lateral chambers (the same distance from the wall) and presented to the experimental mouse. Experimental mice started at the center chamber and were allowed to explore freely for 10 min. Finally, the object was replaced with another unknown mice. The experimental mouse was allowed to explore freely for an additional 10 min. Data were collected using a video tracking system coupled to the Honestech TVR 2.5 program and analyzed offline using the ANY-MAZE software.

2.3.6. Object-based attention test

For this task, a protocol previously described was used [3]. Briefly, the test was performed in a rectangular apparatus containing 2 chambers, the exploring chamber (40 × 40 × 22 cm) and the test chamber (40 × 20 × 22 cm). First, during the habituation phase, each mouse was subjected to a 10-min familiarization session, where they explored both empty chambers. Later, in the acquisition phase, mice were subjected to a 3-min session where they explored five objects (1, 2, 3, 4 and 5) separated within the chamber. Finally, a retention phase was performed; immediately following the acquisition phase (< 15 s), an old object (used in the acquisition phase) was placed in its original position and a sixth novel object was placed in the test chamber. The mice explored both objects for 3 min. A recognition index of the retention session was calculated as the ratio (T6 × 100)/(T2 + T6), where T2 and T6 are the times spent at objects 2 and 6, respectively.

2.4. Total RNA extraction

Total RNA was isolated from 100 mg of tissue using TRIzol® reagent (Invitrogen Life Technologies, USA) following the manufacturer's instructions. The residual DNA was removed with RNase free-DNase I, Amplification Grade (Invitrogen). RNA yield and purity were determined by TECAN (Infinite 200 PRO series).

2.5. Reverse transcription cDNA synthesis

One μg of total RNA was subjected to reverse transcription using the ImProm-II Reverse Transcription System (Promega) following the manufacturer's protocol. cDNA was stored at − 20 °C for future use. For qPCR analysis, each cDNA sample was diluted 10 times with nuclease-free water.

2.6. Real-time PCR

The real-time PCR reaction was performed in triplicate in the LightCycler® 96 System (Roche Diagnostics GmbH, Roche Applied Science, Mannheim, Germany) using KAPA SYBR FAST qPCR Master Mix (2×) in a final volume of 10 μl. Amplification conditions consisted of an initial denaturation at 95 °C for 10 min followed by amplification of 40 cycles (95 °C for 15 s, 60 °C for 20 s, and 72 °C for 20 s). A melting curve analysis was performed immediately after amplification from 55 to 95 °C. Values were normalized to 18S expression levels using the ΔCT method.

Gene	Forward primer	Reverse primer
18S	5′-GCCGCTAGAGGTGAAATTCTTGGA-3′	5′-ATCGCCAGTCGGCATCGTTTAT-3′
Vdac1	5′-CGGCCACACATGATCACAGA-3′	5′-ACCAGTCTCGGGGTCTTCTT-3′
Cyp- D	5′-AGGAGATAGCCCCAGGAGAT-3′	5′-TTGCATACACGGCCTTCTCTT-3′
ANT	5′-CCACCCAGGCTCTCAACTTT-3′	5′-AAGCACAAGGATGTAGCCCC-3′
mT32-t138 (tau)	5′-GTCCTCGCCTTCTGTCGATTATC-3′	5′-GCTGTGGGGGAGACTCTTTTAAG-3′
NRF2	5′-ACCCGAAGCACGCTGAAGGC-3′	5′-GTCACTGAACCCAGGCGGTGG-3′
GR1	5′-GGGGTGACGAGGTGGAGTA-3′	5′-GATCTGGCTCTCGTGAGGAA-3′
GCS	5′-GGGGTGACGAGGTGGAGTA-3′	5′-GTTGGGGTTTGTCCTCTCCC-3′
HO1	5′-CACAGCACTATGTAAAGCGTCT-3′	5′-TGTGCAATCTTCTTCAGGACC-3′

2.7. Immunoblotting

The hippocampus of WT and tau -/- mice were dissected on ice and immediately processed as previously described [84,85]. Briefly, the hippocampal tissue were homogenized in RIPA buffer (10 mM Tris-Cl, pH 7.4, 5 mM EDTA, 1% NP-40, 1% sodium deoxycholate, and 1% SDS) supplemented with a protease inhibitor mixture and phosphatase inhibitors (25 mM NaF, 100 mM Na3VO4, and 30 μM Na4P2O7) using a Potter homogenizer and then sequentially passed through syringes of different calibers. The protein samples were centrifuged twice at 14,000 rpm for 15 min at 4 °C. The protein concentrations were determined using the BCA Protein Assay Kit (Pierce). The samples were resolved by SDS-PAGE, followed by immunoblotting on PVDF membranes.

2.8. Hippocampal slices and staining with mitochondrial fluorescent dyes

Coronal 20 μm slices of unfixed tissue were obtained from the brain of WT and tau -/- mice. Slices were mounted on slides and incubated with both MitoTraker Green FM, a marker of mitochondrial mass [65], and MitoTraker Red CM-H2Xros, an indicator of mitochondrial membrane potential [33], in Krebs-Ringer-Hepes-bicarbonate (KRH) buffer for 45 min at 37 °C. After incubation, slices were washed 3 times for 5 min in PBS and mounted with DAPI fluorescent mounting media. Images were acquired with a high-resolution fluorescence microscope (Leica 6000×) and analyzed using Image J software.

2.9. Isolation of hippocampal mitochondria

Hippocampal mitochondria were isolated as previously described [41]. Briefly, n = 5 animals for group (wild type and tau -/-) were euthanized and the hippocampus was rapidly removed and suspended in MSH buffer (230 mM mannitol, 70 mM sucrose, 5 mM Hepes, pH 7.4) supplemented with 1 mM EDTA and protease inhibitor cocktail. Homogenates were centrifuged at 600 g for 10 min at 4 °C to discard nuclei and cell debris. The supernatant was centrifuged at 8000 g for 10 min; the new mitochondrial pellet was washed twice in MSH without EDTA. Protein concentration was determined using a standard BCA kit.

2.10. Estimation of mitochondrial complexes activity

Mitochondrial complex activity I and III were estimated through the indirect evaluation of the Reactive Oxygen Species (ROS) production in hippocampal mitochondrial enriched preparations [14,41]. Mitochondrial ROS production was measured using 25 μM DCF (485 nm, 530 nm) in the Biotek Synergy HT plate reader as previously described [14]. Isolated mitochondria (25 μg of protein) were added to 100 μl of KCl respiration buffer with 5 mM pyruvate and 2.5 mM malate as oxidative substrates at 37 °C. ROS production was calculated as the maximum DCF fluorescence following 2 h of incubation, expressed in arbitrary fluorescence units.

2.11. Measurement of ATP concentration

ATP concentration was measured in the hippocampal lysate and in the supernatant of isolated mitochondria after the incubation with oxidative substrates, using a luciferin/luciferase bioluminescence assay kit (ATP determination kit #A22066, Molecular Probes, Invitrogen) as previously described [84]. For ATP determinations in isolated mitochondria, we used Trifluoromethoxy carbonylcyanide phenylhydrazone (FCCP), as an uncoupler of the oxidative phosphorylation in mitochondria [9]. The amount of ATP in each sample was calculated from standard curves and normalized to the total protein concentration.

2.12. Statistical analysis

The data are expressed as the mean ± standard error of the mean (SEM), with the number of experiments indicated in the corresponding figures. All samples included in these studies were analyzed to normality distribution using the Kolmogorov-Smirnov test. Later, the obtained data were analyzed using Student's *t*-test with Dunnett's post hoc test or, if analyzing more than 2 groups, ANOVA followed by Bonferroni's post hoc test. $P < 0.05$ was considered statistically significant. All statistical analyses were performed using Prism software (GraphPad Software Inc.). No statistical methods were used to predetermine the sample size.

3. Results

3.1. Tau ablation reduced oxidative damage in the hippocampus

To determine if the absence of tau has a significant effect on hippocampal cells, we used litters of 3 months old male WT or tau KO (tau -/-) mice. The hippocampus of WT and KO mice were dissected and total protein or mRNA was extracted. We performed a PCR analysis to identify tau mRNA (Fig. 1A) and a western blot assay to detect the tau protein (Fig. 1B). In both results, tau was not present in the hippocampus of tau -/- mice, confirming its ablation in KO mice. Later, we evaluated oxidative stress-mediated damage in the hippocampus of WT and tau -/- mice (Fig. 1). We determined the levels of oxidized proteins by western blot using an anti-4HNE antibody that recognizes sulfhydryl or histidine and lysine groups of proteins to form stable HNE-protein adducts [64] (Fig. 1C), and anti-nitrotyrosine antibody, a nitric oxide production marker that detect proteins containing nitrotyrosine (Fig. 1D). Our results showed that the levels of oxidative damage were significantly reduced in the hippocampus of tau -/- mice, as indicated by a decrease in the levels of lipid peroxidation products and nitrotyrosinated proteins (Figs. 1C and 1D). A potential signaling pathway that is activated under oxidative conditions is the Nrf-2 transcriptional pathway [59], which controls the expression of some antioxidants, thus detoxifying enzymes and improving mitochondrial function [28]. Interestingly, tau -/- animals presented a significant decrease in the total levels of Nrf-2 protein (Fig. 1E), as indicated by the densitometry analyses (Fig. 1F). However, we also found that in the hippocampus of tau -/- animals, there are increased Nrf2 mRNA levels (Fig. 1G) as well as increased expression of downstream genes of Nrf-2-dependent signaling, including the Glucocorticoid Receptor (GR) (Fig. 1H), heme oxygenase (HO) (Fig. 1I), and γ-glutamine cysteine synthase (GCS) (Fig. 1J). Altogether, our results indicate that tau ablation reduces cellular damage induced by oxidative stress in the hippocampus, possibly by increasing the activation and transcription of Nrf-2 signaling genes which finally could lead to Nrf-2 protein degradation.

3.2. The absence of tau positively regulates mitochondrial dynamics in the hippocampus

Since the mitochondria is the main producer of reactive oxygen species (ROS) in the cell [89] and considering that the Nrf2 pathway also regulates mitochondrial function [28], we next investigated whether the absence of tau modifies the expression of proteins that are fundamental for mitochondrial structure and function. Mitochondria are dynamic organelles that normally undergo fusion/fission processes to maintain mitochondrial function and optimize bioenergetics capabilities [27,60,99]. Fusion events are controlled by the dynamin-related GTPases, Mitofusins (Mfn1 and Mfn2) and optic atrophy 1 (OPA1), which stimulate the fusion of outer and inner mitochondrial membranes, respectively [27,90]. When we measured these protein levels, we observed that Mfn2 was increased, whereas Mfn1 and Opa1 showed no significant changes in tau -/- animals (Fig. 2A). In contrast, fission is mediated by Dynamin-related protein 1 (Drp1), which is recruited to the outer membrane to constrict mitochondria and induce its division [4,61], a process that is mainly stimulated by Drp1 phosphorylation at Ser616 [95]. Additionally, Drp1 interacts with other mitochondrial receptor proteins, such as Fission 1 (Fis1), to complete this process [16,46,99]. Our results show that Fis1 decreased its expression while simultaneously, total Drp1 or its phosphorylated form at Ser616, remained unaffected (Fig. 2B). Therefore, the absence of tau protein in the hippocampus increases the expression of a fusion protein and decreases expression of a fission protein, suggesting that a pro-fusion state could be beneficial to mitochondrial function.

In addition, we evaluated the main protein components of the mPTP, a mega protein channel formed in the inner membrane of the mitochondria in response to mitochondrial calcium overload that permits the release of small molecules into the cytoplasm [36,66]. The opening of the mPTP promotes uncoupling of the respiratory chain, blocks ATP production, and can ultimately lead to apoptosis [36]. mPTPs are composed of cyclophilin D (Cyp-D), voltage-dependent anion channel (VDAC), and adenine nucleotide translocase (ANT) proteins [35]. The levels of these three proteins were measured in the hippocampi of WT and tau -/- mice (Fig. 2C). Interestingly, we observed that the levels of Cyp-D and ANT proteins, but not VDAC, were significantly reduced in tau KO mice. Surprising, mRNA levels of Cyp-D and ANT were increased in tau -/- animals (Fig. 2D-F). Since that mRNA and protein levels correlation in mPTP components is unknown, we only could suggest possible explanations to this phenomenon. One possibility is that increased mRNA levels act a compensatory mechanism to the reduced protein levels, due that when comparing protein and mRNA levels during dynamic processes, there is a delay

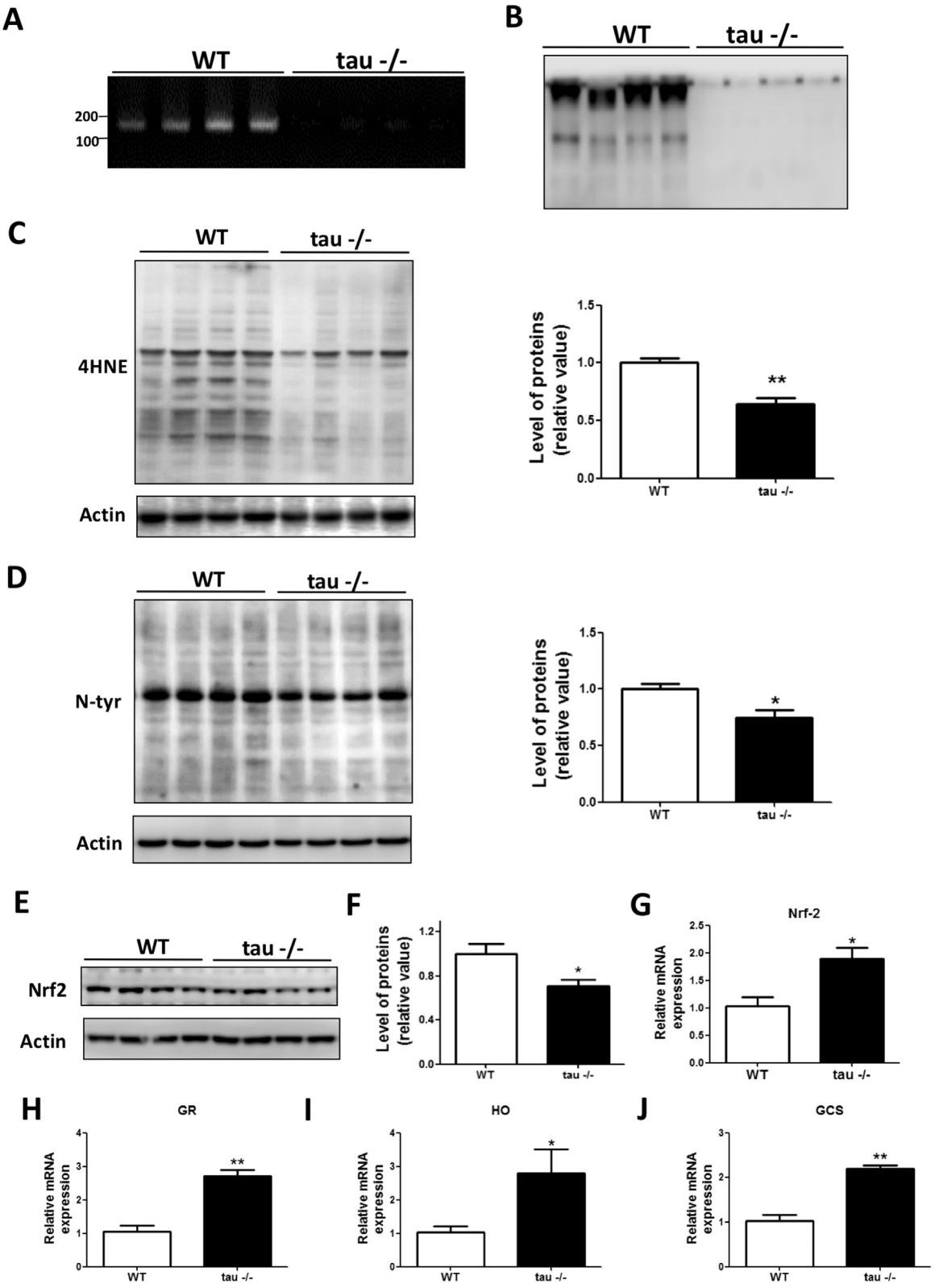


Fig. 1. Tau ablation reduces oxidative stress in the hippocampus. (**A**) Representative detection of the levels of tau mRNA in the hippocampus of WT and tau KO (-/-) mice by RT-PCR. (**B**) Representative western blot of tau protein in hippocampal lysates from WT and tau KO (-/-) mice; tau was measured using a specific tau antibody. Western blot of hippocampal lysates and densitometric analyses of (**C**) 4HNE and (**D**) n-Tyr. (**E**) Western blot of hippocampal lysates of Nrf2 and (**F**) its densitometric analyses. (**G**) Relative mRNA expression of Nfr2 and (**H-J**) its downstream mRNA of target genes. *$p < 0.05$, **$p < 0.01$; data are presented as the mean ± SE. n = 4 different animals for WT group and n = 5 different animals for tau -/- group.

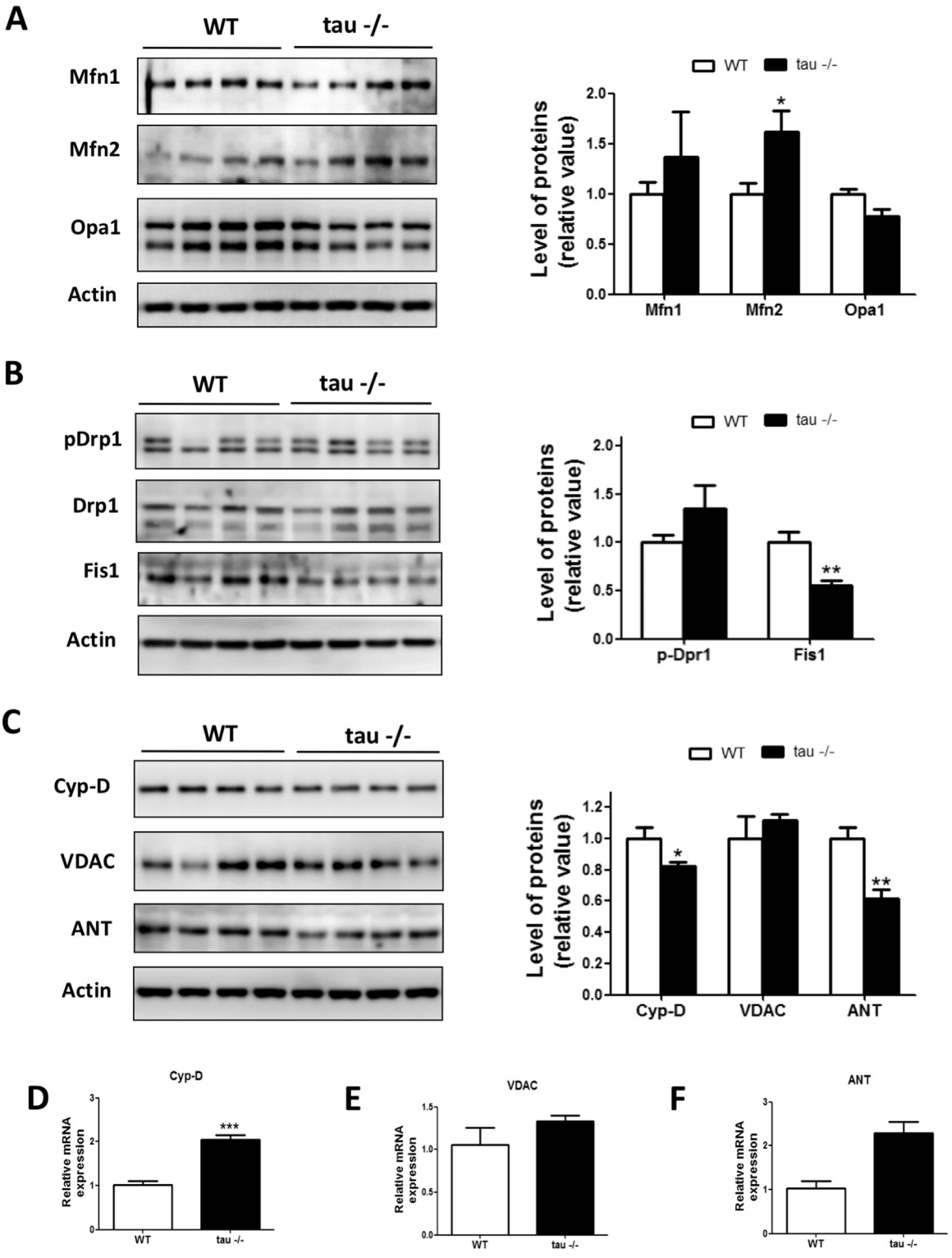


Fig. 2. Effects of tau deletion on the levels of proteins involved in mitochondrial dynamics and structure. (**A**) Representative western blot of hippocampal lysates and densitometric analysis of proteins involved in mitochondrial fusion, such as Mnf1, Mnf2, and Opa1. (**B**) Representative western blot of hippocampal lysates and densitometric analysis of proteins involved in mitochondrial fission, including phospho-Drp1, total Drp1, and Fis1. (**C**) Western blot of hippocampal lysates and densitometric analysis of proteins that form the mitochondrial permeability transition pore (mPTP), such as Cyp-D, VDAC, and ANT. (**D-F**) Relative mRNA expression of Cyp-D, VDAC, and ANT in the hippocampus of WT and tau -/- animals. *$p < 0.05$, **$p < 0.01$; data are presented as the mean ± SE. n = 4 different animals for WT group and n = 5 different animals for tau -/- group.

between transcription and translation events in the cell [48]. Other possibility is that mRNA and protein levels no shown positive correlation as consequence of differences in the regulation of the steps in the synthesis of a protein or post-transcriptional defect [48,74]. Despite this results, our observations indicate that tau deletion promotes changes in the proteins that could favoring a pro-mitochondrial fusion state and reduce Cyp-D expression, this last event could be related with a reduction in the mPTP activation in tau -/- mice [29,35,75].

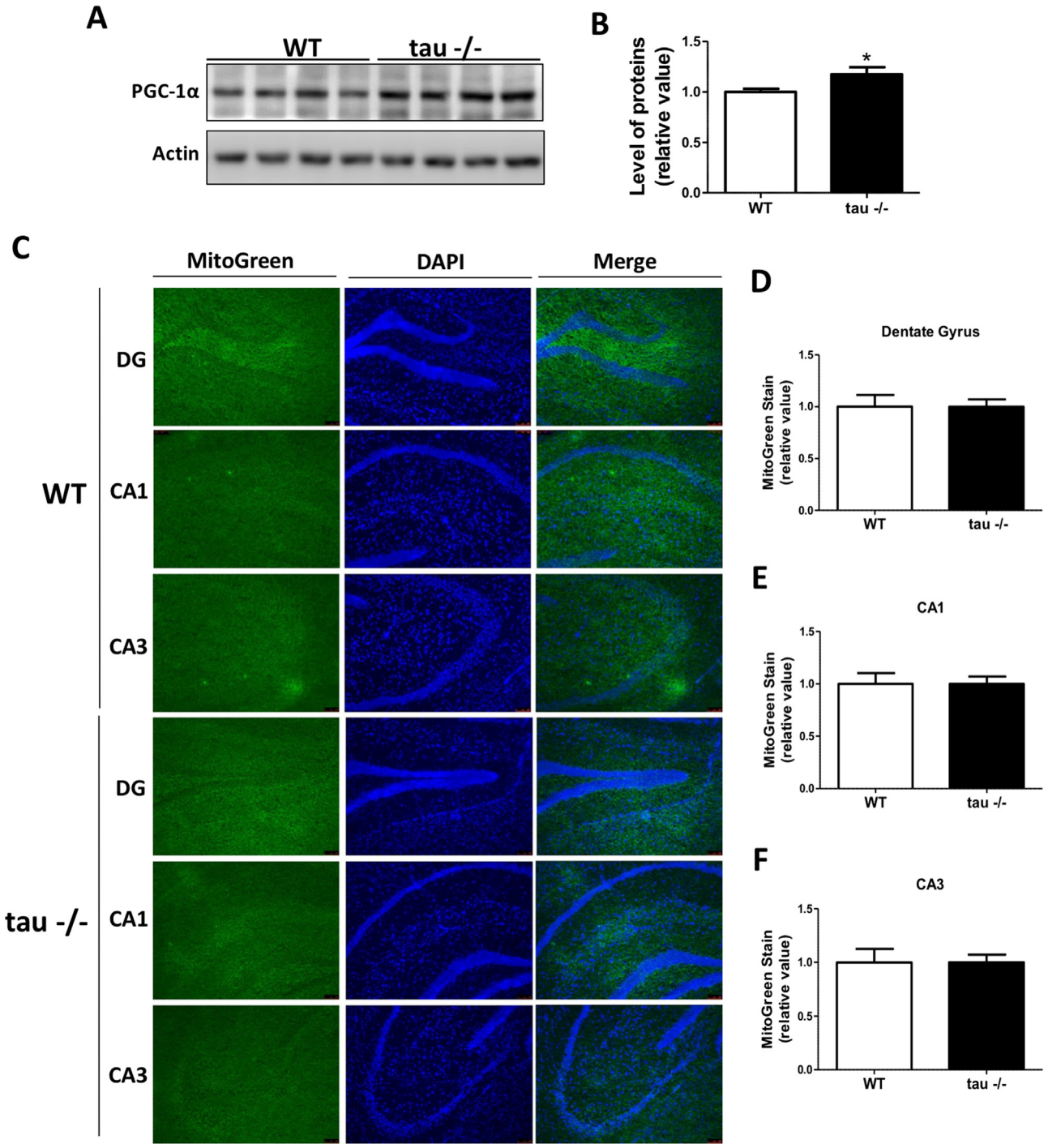
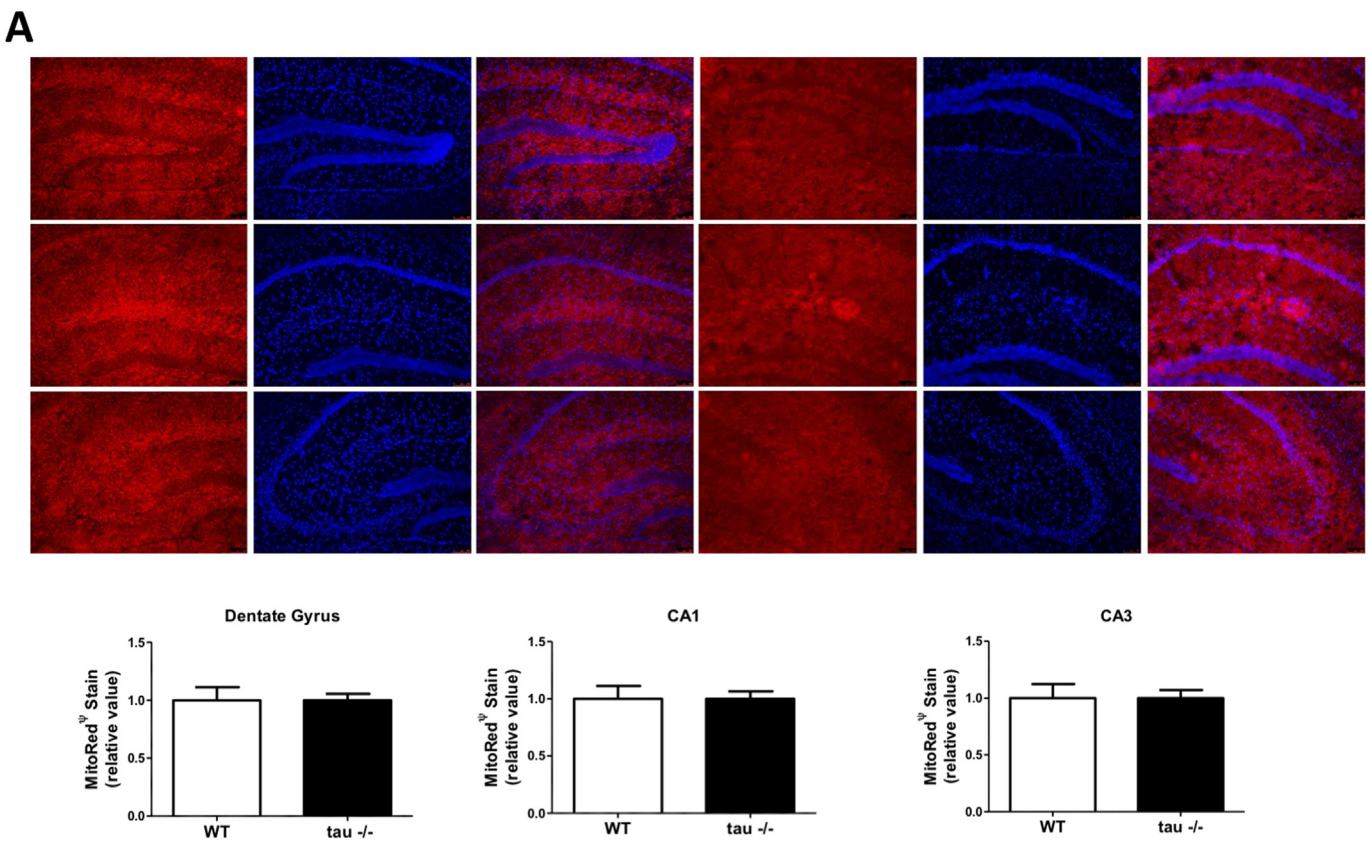
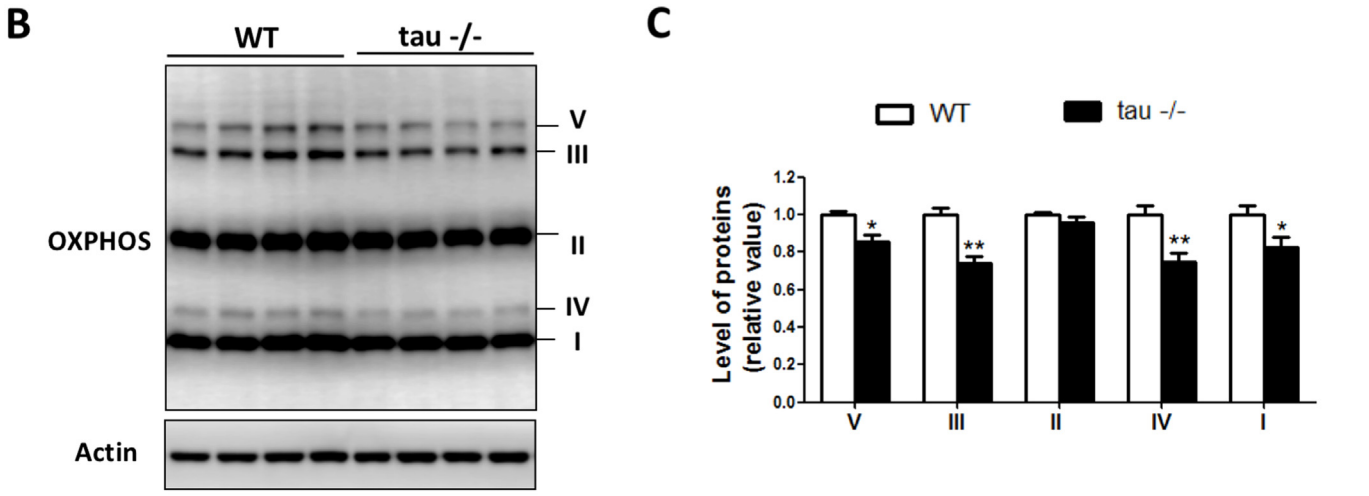


Fig. 3. Tau -/- animals have increased levels of PGC-1α without affecting mitochondrial mass. (**A**) Representative western blot of hippocampal lysates and (**B**) densitometric analyses of PGC-1α. (**C**) Representative images of unfixed hippocampal slices from WT and tau -/- mice stained with MitoGreen at 10×. (**D-F**) Quantitative analysis for MitoGreen dye in DG, CA1, and CA3 hippocampal regions. *$p < 0.05$, **$p < 0.01$; data are presented as the mean ± SE. n = 4 different animals for WT group and n = 5 different animals for tau -/- group.

3.3. Tau deletion increase hippocampal PGC1α levels

Peroxisome proliferator-activated receptor-gamma coactivator (PGC)**-1α** is a member of a family of transcription coactivators that plays a key role in regulating cellular energy metabolism [45]. PGC-1α promotes mitochondrial biogenesis by regulating both carbohydrate and lipid metabolism and modulating mitochondrial function [80,97]. In contrast, the absence of PGC-1α induces mitochondrial dysfunction and neurodegeneration [15]. Therefore, we investigated the effect of tau depletion on PGC-1α protein levels and observed that tau -/- mice have increased levels of this protein compared to WT mice (1.000 ± 0.03204 WT; 1.176 ± 0.06912 tau -/-) in approximately a 18% (Fig. 3A, B). To corroborate whether increased PGC-1α protein levels results in increased mitochondrial biogenesis, we incubated unfixed hippocampal slices of WT and tau KO animals with the mitochondrial dye MitoTracker Green, a cell-permeable probe that passively diffuses across the plasma membrane and binds to lipids in the mitochondria [30]. Our analysis revealed similar mitochondrial mass between WT and tau -/- mice (Fig. 3C) in all the analyzed regions in the hippocampus, including the dentate gyrus (DG) (Fig. 3D), CA1





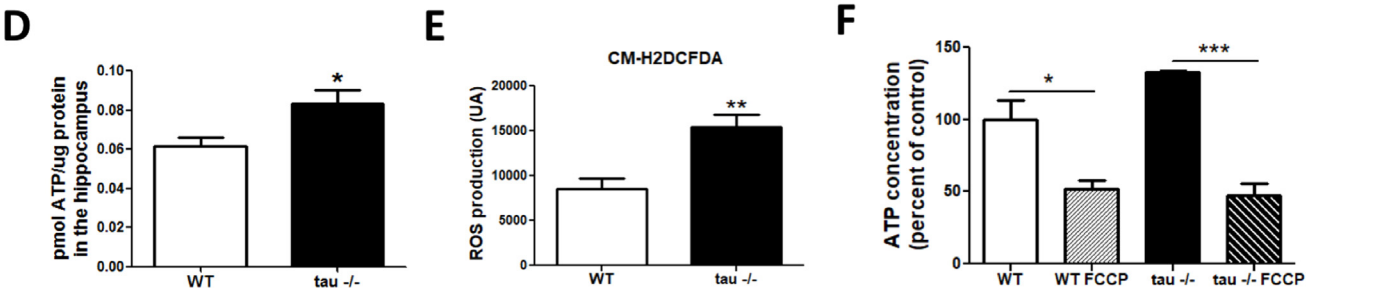


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Fig. 4. Absence of tau improves the bioenergetics capacity of hippocampal mitochondria. (**A**) Representative images of unfixed hippocampal slices stained with MitoTracker Red dye sensitive to mitochondrial membrane potential at 10× and its quantitative analysis for DG, CA1, and CA3. (**B**) Representative western blot and (**C**) densitometry analyses for mitochondrial OXPHOS complexes I-V levels. (**D**) Whole hippocampal extracts from lysates of WT and tau KO -/- mice. ATP levels were expressed as pmol ATP/μg of total protein extract (**E**) ROS production of isolated mitochondria measured by the fluorescent dye CM-H2DCFDA after exposition to oxidative substrates, to evaluate indirectly the mitochondrial activity of I and III complexes. **(F)** Isolated mitochondria of WT and tau KO -/- mice were exposed to malate and pyruvate substrates and then, the ATP levels were measured, expressed as ATP concentration (% of WT mice mitochondria). *$p < 0.05$, **$p < 0.01$; data are presented as the mean ± SE. n = 4 different animals for WT group and n = 5 different animals for tau -/- group in western blot analysis. n = 5 different animals for WT and tau -/- groups to mitochondrial studies.

(Fig. 3E), and CA3 (Fig. 3F), indicating that tau deletion may not affect mitochondrial mass. Therefore, our observations suggest a more active state of PGC-1α in tau -/- mice, which could be possibly regulating mitochondrial function without modulating mitochondrial mass in the hippocampus. This is further supported by reports which highlight that PGC1α can modify the composition and function of individual mitochondria independent of mitochondrial biogenesis [5].

3.4. Mitochondrial function is improved in the hippocampus of tau -/- mice

To determine how tau proteins affect mitochondrial bioenergetics, we first measured mitochondrial membrane potential using the fluorescent dye MitoTracker Red $CMXH_2Ros$ [65,69]. This dye specifically detects functional mitochondria, with the florescence intensity proportional to mitochondrial membrane potential [65,69]. Fig. 4A shows that unfixed slices from the hippocampus of WT and tau -/- mice incubated with MitoTracker Red $CMXH_2Ros$ presented with similar mitochondrial potential levels in the DG, CA1, and CA3 regions of the hippocampus (Fig. 4A). Later, we evaluated the levels of protein involved in oxidative phosphorylation using an antibody cocktail (OXPHOS) that contains all 5 mitochondrial complexes [24,37]. We observed that tau -/- mice present with significantly reduced levels of V, III, IV, and I complexes in the hippocampus compared to WT animals (Fig. 4B). Finally, we measured the bioenergetics capacity of the mitochondria by determining total ATP levels using a luminescence assay (see methods). Using this assay, we determined that tau -/- mice have significantly higher ATP levels compared to WT mice (Fig. 4D). Altogether, our results suggest that despite OXPHOS protein are decreased in the tau -/- mice, the oxidative phosphorylation of these animals is more active, producing higher ATP levels. To validate this possibility, we isolated hippocampal mitochondria from WT and tau -/- mice. These mitochondria were exposed to malate and pyruvate substrates during two hours to 37 °C, in presence of CM-H2CDFA dye, to measure the ROS production and thus, indirectly the activity of mitochondrial complexes I and III [14]. Later, the supernatant was used to detect the ATP concentration produced. Fig. 4E indicate that tau -/- mitochondria produce increased levels of ROS compared with mitochondria isolated of WT mice, suggesting a higher activity of the mitochondrial respiratory chain. Furthermore, Fig. 4F demonstrate that tau -/- mitochondria produce significantly more ATP levels, effect that is reduced by the presence of FCCP (a mitochondrial uncoupler) in both experimental groups. Interestingly, the increased ROS production in tau -/- mitochondria could be beneficial to hippocampal cells; due that although very high concentrations of mitochondrial ROS (mROS) are detrimental to cell, normal levels of mROS are necessary to the cell homeostasis and act as a signal to activate pathways of response to stress [72,77]. This could explain the activation of Nrf-2 and PGC-1α pathways in tau -/- mice [80] and the resulting decreased oxidative damage in tau -/- mice (Fig. 1). Therefore, our results strongly suggest that tau KO mice have an enhanced mitochondrial bioenergetics.

3.5. Genetically reducing tau modifies the expression of synaptic proteins in hippocampal tissue

Considering that mitochondria are fundamental for correct synaptic function [21,38], we measured the levels of different synaptic proteins by western blot (Supplementary Fig. 1). As pre-synaptic markers, we evaluated the levels of synaptophisin (SYP) and vesicle-associated membrane protein (VAMP). We detected that VAMP protein levels are significantly decreased in tau -/- mice, compared to WT hippocampi (Supplementary Fig. 1A). In contrast, we observed that tau KO mice had increased expression of the post-synaptic markers Post synaptic density 95 (PSD95) and N-methyl D-aspartate receptor subtype 2B (NR2B) in the hippocampi. Specifically, these mice had significantly higher expression of PSD95 compared to WT mice (Supplementary Fig. 1B). Altogether, these results indicate that tau deletion induces changes in synaptic protein levels, mainly by increasing a key scaffold protein necessary to maintain the correct function of receptors in the post-synaptic cells [8,20].

3.6. Tau reduction enhances exploratory capacity and recognition memory

In order to investigate whether tau deletion influences the learning process and hippocampus-dependent memory, we performed the Novel Object Recognition (NOR) task [13]. To carry out this test, we first exposed the animals to a habituation phase, in which each animal explored the test chamber for 10 min on 2 consecutive days [86]. On the test day, mice were subjected to the familiarization phase. In this stage, each animal had 10 min to explore the chamber, which contained 2 identical objects (Fig. 5A). The differences in the behavior of WT mice compared to tau -/- mice are reported in the heat map (Fig. 5B) and the paths travelled (Fig. 5C). We observed that all tau - /- mice (n = 7) explored more time the objects than WT mice, as indicated by the amount of time that the animal's head spent in object zones 1 (Figs. 5D) and 2 (Fig. 5E), as well as by the number of times the animal's head entered into object zones 1 (Figs. 5F) and 2 (Fig. 5G). This is further supported by the amount of time that the animals spent exploring the objects, as tau -/- animals explored both objects for a significantly longer amount of time than WT mice (Fig. 5H). These results suggest that tau -/- mice have more explorative behaviors than WT mice. After 2 h, the recognition phase (Testing phase) was performed. In this phase, for 5 min, animals explored an old object as well as a novel object that replaced one of the former objects (Fig. 5I). Our results showed that 2 subpopulations within the litter of tau -/- mice exists. These subpopulations differ in their behaviors toward the novel object, which also differs from the conduct of WT mice (Fig. 5J). Five tau KO animals spent more time exploring the novel object compared to WT mice (Fig. 5J, K and L), while 2 tau -/- animals had the opposite behavior, in which they explored the old object longer than the other tau KO mice and spent a similar amount of time exploring the novel object as WT mice (Fig. 5J and L). Interestingly, knocking out tau improved recognition memory for the majority of the animals, but also encouraged them to spend more time exploring objects. This could explain why the recognition index was not significantly different between the mice (Fig. 5M), as this is calculated as the time that mice spent exploring the new object versus the time spent exploring both objects.

To determine whether the observed differences tau -/- animal behavior could be associated, at least in part, with stress and/or anxiety, processes related to cortex-hippocampus circuit, we developed the open field test (Supplementary Fig. 2) [76]. We found that the same animal subpopulations observed in the NOR test were detected in this task, as indicated by the path travelled and heat map (Supplementary Fig. 2A-

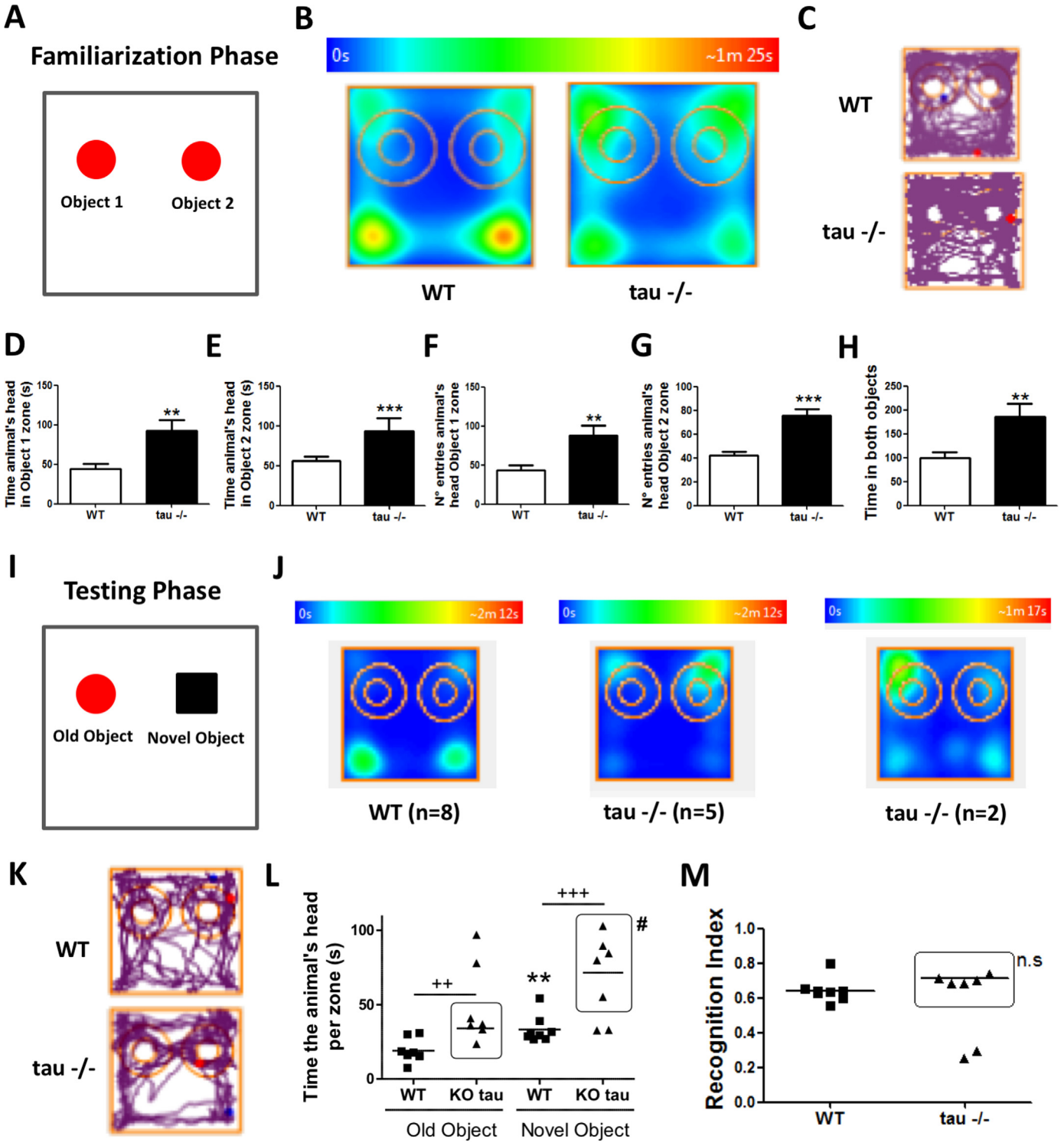

Fig. 5. Recognition memory is improved in tau -/- mice. (A) Schematic representation of the novel object recognition (NOR) test. Mice are allowed to explore two identical objects for 10 min (familiarization phase). (**B**) Heat maps show mean data for an entire test group. **(C)** Representative tracks of WT and tau KO (-/-) mice in the NOR test familiarization phase. Familiarization phase; the time the animal's head spent exploring (**D**) object zone 1 and (**E**) object zone 2. Familiarization phase; the number of head entries in (**F**) object zone 1 and (**G**) object zone 2. (**H**) Familiarization phase; time animals spent exploring both objects 1 and 2. (I) Schematic representation of the NOR testing phase, which occurred 2 h after the end of the familiarization phase with a familiar object and a novel object. (J) Heat maps for the mean data for an entire test group. (K) Representative tracks of WT and tau (-/-) mice in the NOR testing phase. (L) Testing phase; the exploration time in the old and novel object zone. (M) Recognition Index, indicated as the time spent exploring the novel object divided by the time spent exploring both objects. *p < 0.05, **p < 0.01; data are presented as the mean ± SE. n = 8 different animals for WT and n = 7 different animals for tau -/-.

C). As observed earlier, both subpopulations of tau -/- animals had different behaviors from those of WT mice. The majority of tau KO animals (n = 5) presented with reduced total distance travelled (Supplementary Fig. 2D) and average speed (Supplementary Fig. 2E), but increased episodes and freezing time (Supplementary Fig. 2F and G respectively). Simultaneously, they spent more time and had more immobile episodes (Supplementary Fig. 2H-K), and entered/spent less time in the center of the apparatus (Supplementary Fig. 2L-N). These observations indicate that the main behavior associated with tau -/- mice is reduced movement when the field is empty, possibly due to more anxiety. However, this in contrast to the 2 tau -/- mice which presented with the opposite behavior. Altogether, these results strongly suggest that tau ablation increases the stress and/or anxiety in an empty field, but in the presence of objects, promotes the animal to become more mobile and explorative and have a higher recognition memory. It is important to highlight that several individuals within the litter of brothers had behaviors that varied from the common population. This could explain the variability of results observed in the literature.

Additionally, we evaluated spatial memory, other type of hippocampal memory [12]. We performed the classic Morris Water Maze (MWM) protocol, but observed no significant differences on any of the test days (Supplementary Fig. 3A-D). The next day after the MWM, a probe in absence of the platform was developed. We found that WT and tau -/- animals spent similar times and had similar entries onto the platform area (Supplementary Fig. 3F-H). Ten days after the initial MWM test, the escape latency was measured to evaluate long term memory and no significant differences were observed (Supplementary Fig. 3I). Finally, a memory flexibility test was performed. There were no observed differences in the number of trials required to reach the criterion (Supplementary Fig. 3J) nor differences in swimming speed (Supplementary Fig. 3K). Therefore, the absence of tau does not modify spatial memory of young animals and only has positive effects on recognition memory.

3.7. Tau -/- mice have similar social abilities to WT mice

Next, we investigated the effects of tau deletion on social abilities in mice, a behavior mediated by hippocampal and cortical circuits [10,53]. For this, a social interaction test was performed [100]. Before phase 1 of the task, for 10 min, the animals were exposed to an empty chamber divided into 3 compartments. During the first phase, the animals had 10 min to explore the chamber containing an unknown mouse in a compartment and an object in the other compartment (Fig. 6A). Fig. 6B shows a representative track and the heat maps of the WT and tau -/- groups. In these representations, one can observe how the mice had an increased preference for the unknown mouse. This was more evident when we analyzed the number of entries or the time that the mice spent in the compartment of the object versus the unknown mouse (Figs. 6C and 6D respectively). More importantly, we observed no significant differences when analyzing the number of entries of the animal's head in the area around the unknown mouse or object (Fig. 6E), however, the time that the animal's head spend in the area around the unknown mouse is higher than the object (Fig. 6F). Additionally, tau -/- mice spent significantly more time exploring the object compared to WT mice (Fig. 6F). Therefore, these results indicate that both WT and tau -/- mice showed a preference for a similar individual over an object, however, tau KO animals also spent more time exploring the object, reinforcing the idea that they are more explorative animals.

Immediately following the first stage, the object was replaced by an unknown mouse and then the animals explored the chamber for 10 min (phase 2, Fig. 6G). Representative tracks and heat maps of WT and tau -/- animals showed that both experimental groups had a preference for the novel mouse versus the old mouse (Fig. 6H). We found that both WT and tau -/- mice entered a novel mouse zone more times compared to an old mouse zone (Fig. 6I), but tau KO mice remained in this zone for more time compared to the WT group (Fig. 6J). These results demonstrate that when faced with a new individual, tau -/- mice presented with similar behavior to WT mice, suggesting that tau ablation does not modified social abilities in mice.

3.8. Absence of tau protein optimizes attention capacity

Finally, we performed a recognition-based attention test to determine whether tau deletion favors attentive behavior, a complex process mediated by the prefrontal cortex and its communication with the hippocampus [3]. For this task, 5 different objects were placed in the chamber in different positions as indicated in Fig. 7A. Then, the animals explored the chamber containing the objects for 3 min. The representative travel path and heat map of each group are presented in Fig. 7B. Fig. 7C shows the amount of time that the animal's head spent in the area of each object. With the exception of object 4, the WT and tau KO animals spent similar amounts of time exploring each object as well as the total time exploring all objects (Fig. 7D). Interestingly, tau -/- mice travelled a longer distance (Fig. 7E) and had a higher mean speed (Fig. 7F), suggesting that they were more interested in the objects compared to WT mice. Next, in the attention test phase (15 s later) the chamber size was reduced by half, where an old object remained in the chamber (object 2) and a new object was added (object 6). The representative scheme of this phase is shown in Fig. 7G. The animals were allowed to explore this chamber for 3 min Fig. 7H shows representative tracks and heat maps of WT and tau -/- groups. In these images, it is evident that tau -/- mice prefer the novel object, however, the distance travelled (Fig. 7I) and the speed of exploration (Fig. 7J) is similar in both groups. Statistical analysis revealed that tau -/- mice entered the novel object zone more often (Fig. 7K) and spent more time exploring it (Fig. 7L). This is more evident when we analyzed the recognition index (Fig. 7M). Altogether, these results indicate that tau ablation improves the attention capacity of young animals.

In conclusion, our study demonstrates that tau deletion induces relevant improvements in mitochondrial health and this could ultimately result in increased cognitive abilities associated with recognition memory and attention.

4. Discussion

In the present study, we performed a complete analysis to study the role of tau on mitochondrial function in the hippocampus of young mice, as well as on cognitive abilities, including recognition and spatial memory, social capacity, and attention. Surprisingly, our results indicate that tau ablation reduces oxidative damage of hippocampal cells and activates the pathways necessary to protect mitochondrial health, such as Nrf-2 [28] and PGC-1α pathways. In particular, we observed increased levels of proteins involved in the dynamics of mitochondrial fusion processes and at the same time, fission proteins were reduced, suggesting a pro-fusion state that could be beneficial to mitochondrial function [18]. Interestingly, these effects were also accompanied by a reduction in the expression of an important mPTP component, Cyp-D, and increased ATP production in the hippocampal tissue of juvenile tau -/- mice. Finally, we analyzed the behavior of a litter of young male animals and observed that tau -/- mice have more exploratory behavior with concomitant improved recognition memory and attention capabilities. Thus, our results indicate that the absence of tau protein has beneficial effects on brain function in the youth, increasing the cognitive capacity of mice by a mechanism implicated in improvement of mitochondrial function.

Limited production of oxidative stress is a consequence of metabolic activity, in which catabolic processing in the mitochondria forms reactive ROS [57]. The main producers of ROS in cells are the mitochondria, however, peroxisomes and the endoplasmic reticulum also contribute to the redox state [47]. Diverse reports demonstrate that mROS have physiological roles maintain the cell homeostasis and serve

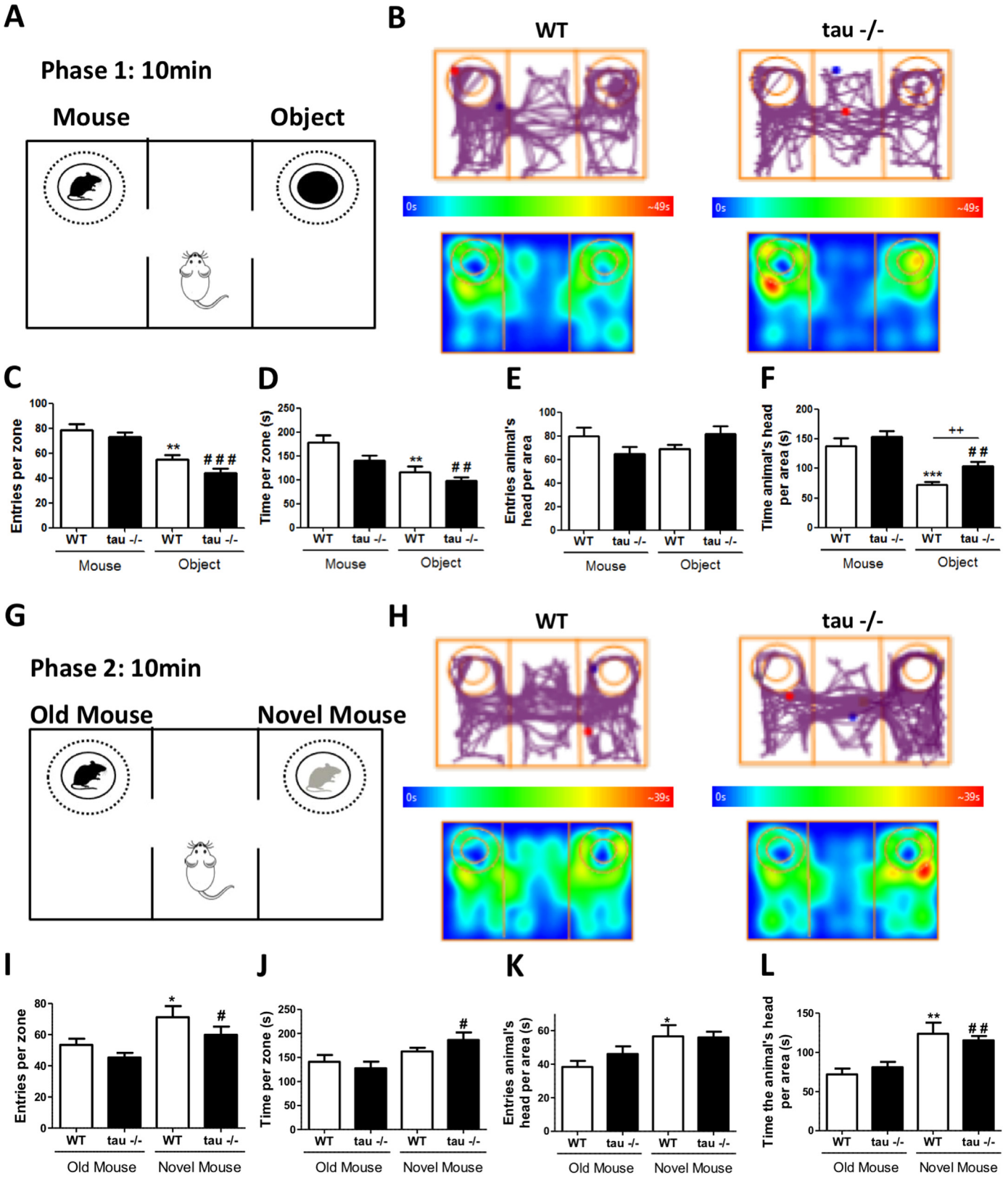

Fig. 6. Social interaction is similar between WT and tau -/- mice. (A) Mice are allowed to explore a mouse and an object for 10 min (Phase 1) as indicated in the scheme. (B) Representative tracks of WT and tau (-/-) mice and the heat map of the Social Interaction test of the entire experimental group. (C) Graphical representation of head entries per zone, (D) time the animal's head spent per zone, (E) head entries per area around the mouse and object, and (F) time the animal's head spent in each area around the mouse or object. (G) Phase 2; mice explored a familiar mouse and a novel mouse as indicated in the scheme. (H) Representative tracks of WT and tau (-/-) mice and the heat map of each animal group in the Social Interaction test phase 2. (I) Graphical representation of head entries per zone, (D) time the animal's head spent per zone, (E) head entries per area around the old and novel mice, and (F) time the animal's head spent in each area around the old or novel mouse. *p < 0.05, **p < 0.01; data are presented as the mean ± SE. n = 8 different animals for WT and n = 7 different animals for tau -/-.

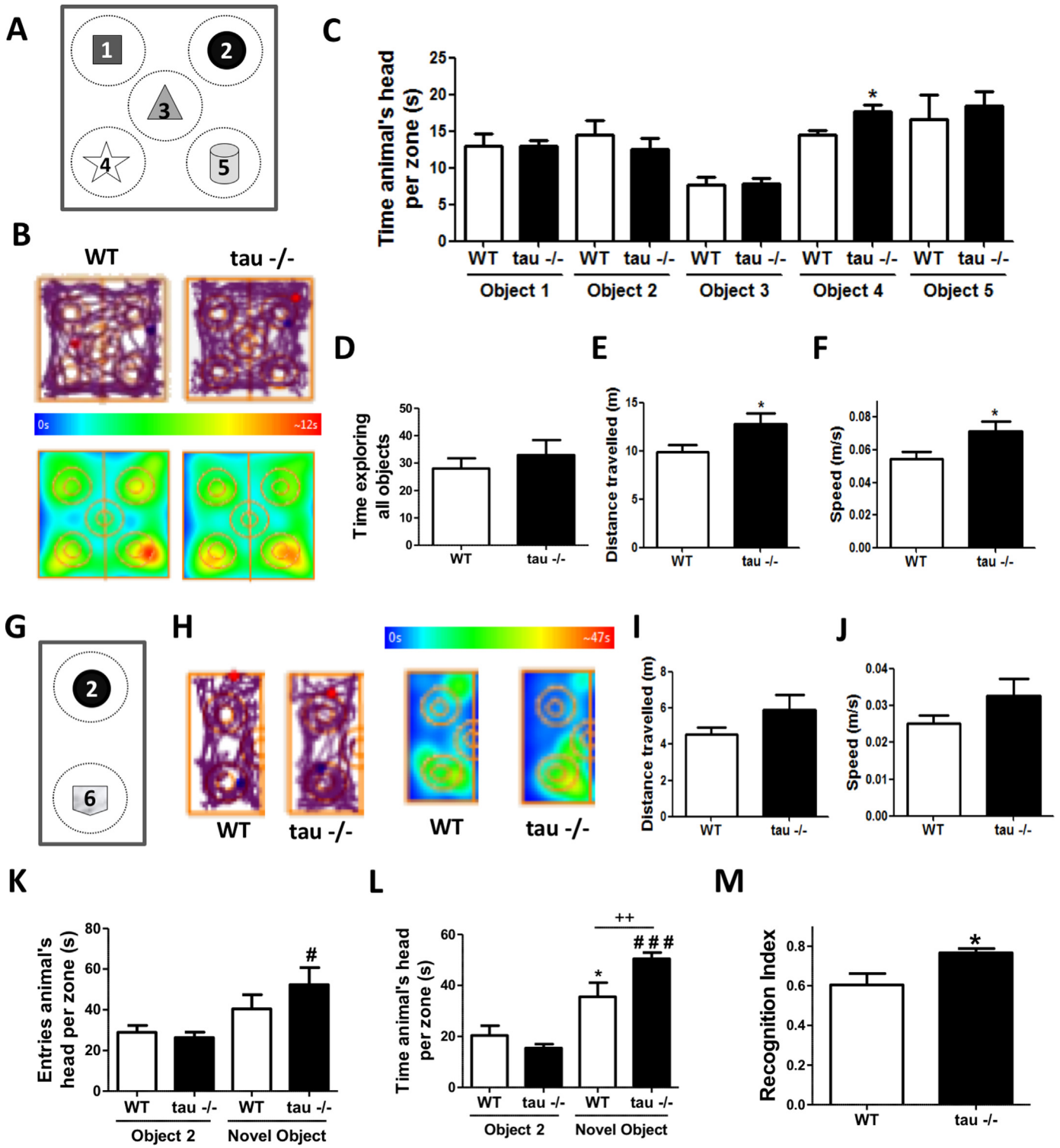

Fig. 7. Tau ablation enhances the attention of mice. **(A)** Representative scheme of the object-based attention test. Mice were exposed to five objects (1–5) for 3 min (exploration phase). **(B)** Representative track and heat maps showing mean data for each entire experimental group. **(C)** Graphical representation of the time that the animals spent exploring all the objects. (D) Quantification of the total time exploring all the objects. (E) Graphical representation of distance travelled (m) and (F) mean speed (m/s) in the exploration phase. (G) Representative scheme of the testing phase after a 15 s interval; the mice were exposed to one old object (2) and a novel object (6) for 3 min (H) Representative track and heat maps showing mean data for an entire group in the testing phase. (I) Graphical representation of distance travelled (m) and (J) mean speed (m/s) in the testing phase. (K) Graphical representation of the head entries per zone and (L) the amount of time the head spent per zone. (M) Recognition index, indicated as the time exploring the novel object divided by the time exploring both objects. *p < 0.05, **p < 0.01; data are presented as the mean ± SE. n = 8 different animals for WT and n = 7 different animals for tau -/-.

such as signaling molecules that modulate the function of pathways adaptive to stress [72,77]. In contrast, higher levels of ROS are toxic to neurons, as they damage crucial biomolecules in the cell, such as DNA, RNA, proteins, and membrane lipids [88]. Pathological forms of tau, including phosphorylated tau, have been associated with increased oxidative stress; however, the increased production of ROS could also

promote tau hyperphosphorylation [2]. This suggests that there is a positive feedback loop between oxidative stress and phosphorylated tau protein that could result in neurodegeneration [2]. Therefore, preventing the occurrence of this vicious cycle could protect neurons. In fact, in this study, we demonstrate that tau ablation reduces oxidative damage in the hippocampus of young tau KO mice compared to WT mice, as indicated by a reduction in the levels of nitrotyrosinated proteins and proteins containing 4-hydroxynonenal adducts. Thus, these findings confirm the relationship between tau protein and oxidative balance, in which the presence of tau favors an oxidative state. A signaling pathway that could contribute to the reduced redox state is the Nrf-2 pathway. This signaling pathway is activated in response to ROS by stimulating the transcription of antioxidant genes and promoting the reestablishment of mitochondrial function [58,59]. Here, we reported that tau deletion favors the activation of the Nrf-2 pathway and induces the transcription of its target genes, including GR, HO, and GCS (Fig. 1). Interestingly, we also observed decreased levels of Nrf2 protein in the hippocampus of tau -/- mice. Apparently the differences between Nrf2 mRNA and protein levels in tau -/- mice are contradictory, but could be explained considering that Nrf2 is a highly unstable protein ($t_{1/2} \sim$ 15 min), which translocate directly to the nucleus following its synthesis and induce the transactivation of its genes [59,81]. Immediately after, its ubiquitylated and targeted for degradation by a mechanism Keap1-dependent in the nucleus. In contrast, under cellular stress condition, Nrf2 is stabilized by reducing its access to Keap1 [59,81], and for this reason WT mice may have more Nrf2 protein, considering that present more oxidative damage compared with tau -/- animals.

In the mitochondria, ROS are formed constantly by oxidative phosphorylation as a consequence of ATP production. The reduced oxidative damage in the hippocampus of tau -/- mice could be responsible, in part, for the improved mitochondrial function. To evaluate this possibility, we first measured the levels of proteins that are important for mitochondrial structure and function. Generally, mitochondria undergo fission in the presence of toxic agents, which could result in neuronal death [23,25]. In contrast, mitochondrial fusion is thought to be a rescue mechanism against damage and could prevent apoptosis [17,18]. Interestingly, we found that the absence of tau protein in the hippocampus increases Mfn2 levels, a protein involved in mitochondrial fusion, with a simultaneous reduction in Fis1 levels, a pro-fission protein. These results strongly suggest a mitochondrial profusion state that could promote the health of mitochondria in the hippocampus [55]. The formation and opening of the mPTP, which allows the release of mitochondrial components that can result in mitochondrial swelling and cell death [52,66], is also indicative of mitochondrial damage. VDAC, ANT, and Cyp-D are among the proteins that compose the mPTP. Studies reveal that Cyp-D is necessary for mPTP opening and interestingly, we observed that tau -/- mice had reduced levels of this protein, suggesting that in the absence of tau protein, mitochondria are less prone to activate the mPTP. Additional studies investigating mitochondrial swelling in the presence of higher calcium concentrations are needed to evaluate this hypothesis.

PGC-1α is a protein that modulates the genes involved in energy metabolism. It is the main regulator of mitochondrial biogenesis, and it was recently demonstrated that it regulates mitochondrial remodeling by changing its intrinsic properties and promotes ROS elimination by inducing the transcription of antioxidant enzymes [5]. Therefore, increased expression of PGC-1α improve the respiratory capacity of individual mitochondria and remove mROS produced, minimizing the impact of ROS on cell physiology [5,80]. We measured the levels of PGC-1α protein in the hippocampus of both experimental groups and our results revealed that tau ablation significantly increased the expression of this protein, however, we did not detect alterations in general mitochondrial mass. Therefore, we suggest that PGC-1α is activated in tau -/- mice and could be removing ROS and elevating antioxidative metabolism, thus contributing to oxidative homeostasis, as indicated in Fig. 1. Also, PGC-1α could be control cellular mitochondrial respiration without alter the mitochondria mass, enhancing the respiratory capacity of individual mitochondria. To validate that increased levels of PGC-1α are involved in mitochondrial metabolism in tau -/- mice, in future studies we could measure the levels of threonine-177 and serine-538 phosphorylation in PGC-1α-dependent AMPK [5,40]. In contrast, to evaluate the participation of PGC-1α in mitochondrial biogenesis we may detect the levels of Arginine methylation-mediated PRMT1 [5,87].

Increased mitochondrial metabolism is associated with enhanced bioenergetics function, which could result in higher concentrations of ATP in the cell [44,62]. We found that Nrf-2 and PGC-1α signaling pathways are activated in tau -/- mice, and both pathways could contribute to improved ATP production. In fact, Nrf-2 target genes are increased, as indicated by increased mRNA levels of GR, HO, and GCS, as shown in Fig. 1. The observed reduction in the protein expression of several mitochondrial complexes in the absence of mitochondrial potential changes suggest that tau -/- mice could have increased mitochondrial respiratory chain activity, resulting in a higher concentration of ATP in hippocampal cells. This possibility was demonstrated isolating hippocampal mitochondria of WT and tau -/- mice, where we observed increased ATP production in tau -/- mitochondria. On the other hand, it is interesting that WT mice presented with similar mitochondrial membrane potential as tau -/- animals, considering that they have significantly reduced ATP production. Interestingly, it has been described on several reports that the high oxidative damage is formed predominantly from complex I, which results in deficient ATP production in conditions of high mitochondrial membrane potential (proton-motive force) [57]. In fact, these changes could be occurring in the hippocampus of WT mice, as we observed increased oxidative damage accompanied by normal mitochondrial membrane potential and reduced ATP production.

Mitochondrial function is fundamental for communication between neurons. We detected improved mitochondrial function in tau -/- mice; and we observed reduced levels of the pre-synaptic protein VAMP while simultaneously, PSD95 was significantly increased in tau KO animals. These changes may be important in synaptic processes, since synaptic strength can be regulated by PSD-95 [19,20,32]. Finally, we performed a battery of cognitive tests and reported that the absence of tau protein in the hippocampus of young mice improves exploratory capacity, recognition memory, and attention capabilities compared to WT mice. However, other abilities, including spatial memory and social abilities, were not affected. These last results are consistent with previous studies which show no significant differences in motor and spatial learning in the MWM during the first 5 days using 6 month-old tau KO mice [1]. Interestingly, our behavioral results could be explained, in part, by enhanced mitochondrial bioenergetics function of tau -/- mice, since mitochondrial dysfunction can affect cognition, including impaired attention, executive function, and hippocampus-dependent memory [31,68].

Attention is a complex process. Part of the prefrontal cortex known as the inferior frontal junction controls visual processing areas that are tuned to recognize a specific category of objects; this is a process known as object-based attention which involves focusing on what is happening in a particular location [7,11]. This process has a high energetic demand [92]. Interestingly, chronic stress results in tau-mediated atrophy of dendrites and spine loss in the prefrontal cortex, an effect that was protected against in tau KO animals [49], supporting the idea that tau could affect attention processes in mice. More importantly, tau deletion reduces alterations to mitochondrial transport, oxidative phosphorylation, and the synaptic localization of mitochondria in the prefrontal cortex after stress [49,94]. Therefore, our findings provide evidence that tau deletion improves the cognitive abilities of mice by optimizing mitochondrial structure and function in the hippocampus.

Our study is very important, because despite that were performed with a reduced number of animals, the variability between each experimental group is minimal, evidencing the differences of WT mice

with the tau -/- group; mainly in the attentive capacity and in the mitochondrial bioenergetics function. We used a limited number of animals, because our focus was evaluated changes in the hippocampus of litters of brother mice in both WT and tau -/- animals; however, we think that these results are reproducible and representative of the hippocampal structure and function of all WT and tau -/- animals. Our work also proposes that preventing negative tau modifications, including tau phosphorylation and tau cleavage, could enhance mitochondrial and brain function. Additionally, the improvement in the mitochondrial bioenergetics function and its adaptive response to stress, propose that ablation of tau could protect the hippocampus of physiological changes such as normal aging and of pathological conditions such as neurodegenerative diseases.

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Disclosure statement

The authors confirm that there are no conflicts of interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.redox.2018.07.010.

References

[1] T. Ahmed, A. Van der Jeugd, D. Blum, M.C. Galas, R. D'Hooge, L. Buee, D. Balschun, Cognition and hippocampal synaptic plasticity in mice with a homozygous tau deletion, Neurobiol. Aging (2014), https://doi.org/10.1016/j.neurobiolaging.2014.05.005.
[2] S.M. Alavi Naini, N. Soussi-Yanicostas, Tau hyperphosphorylation and oxidative stress, a critical vicious circle in neurodegenerative tauopathies? Oxid. Med. Cell Longev. 2015 (2015) 151979.
[3] T. Alkam, M. Hiramatsu, T. Mamiya, Y. Aoyama, A. Nitta, K. Yamada, H.C. Kim, T. Nabeshima, Evaluation of object-based attention in mice, Behav. Brain Res. 220 (2011) 185–193.
[4] R. Anand, T. Wai, M.J. Baker, N. Kladt, A.C. Schauss, E. Rugarli, T. Langer, The i-AAA protease YME1L and OMA1 cleave OPA1 to balance mitochondrial fusion and fission, J. Cell Biol. 204 (2014) 919–929.
[5] S. Austin, J. St-Pierre, PGC1alpha and mitochondrial metabolism–emerging concepts and relevance in ageing and neurodegenerative disorders, J. Cell Sci. 125 (2012) 4963–4971.
[6] J. Avila, J.S. Jimenez, C.L. Sayas, M. Bolos, J.C. Zabala, G. Rivas, F. Hernandez, Tau structures, Front. Aging Neurosci. 8 (2016) 262.
[7] D. Baldauf, R. Desimone, Neural mechanisms of object-based attention, Science 344 (2014) 424–427.
[8] J.C. Beique, R. Andrade, PSD-95 regulates synaptic transmission and plasticity in rat cerebral cortex, J. Physiol. 546 (2003) 859–867.
[9] R. Benz, S. McLaughlin, The molecular mechanism of action of the proton ionophore FCCP (carbonylcyanide p-trifluoromethoxyphenylhydrazone), Biophys. J. 41 (1983) 381–398.
[10] L.K. Bicks, H. Koike, S. Akbarian, H. Morishita, Prefrontal cortex and social cognition in mouse and man, Front. Psychol. 6 (2015) 1805.
[11] N.P. Bichot, M.T. Heard, E.M. DeGennaro, R. Desimone, A source for feature-based attention in the prefrontal cortex, Neuron 88 (2015) 832–844.
[12] C.M. Bird, N. Burgess, The hippocampus and memory: insights from spatial processing, Nat. Rev. Neurosci. 9 (2008) 182–194.
[13] N.J. Broadbent, S. Gaskin, L.R. Squire, R.E. Clark, Object recognition memory and the rodent hippocampus, Learn. Mem. 17 (2010) 5–11.
[14] M.R. Brown, P.G. Sullivan, J.W. Geddes, Synaptic mitochondria are more susceptible to Ca2+overload than nonsynaptic mitochondria, J. Biol. Chem. 281 (2006) 11658–11668.
[15] L. Cui, H. Jeong, F. Borovecki, C.N. Parkhurst, N. Tanese, D. Krainc, Transcriptional repression of PGC-1alpha by mutant huntingtin leads to mitochondrial dysfunction and neurodegeneration, Cell 127 (2006) 59–69.
[16] D.C. Chan, Mitochondria: dynamic organelles in disease, aging, and development, Cell 125 (2006) 1241–1252.
[17] H. Chen, D.C. Chan, Physiological functions of mitochondrial fusion, Ann. N. Y Acad. Sci. 1201 (2010) 21–25.
[18] H. Chen, J.M. McCaffery, D.C. Chan, Mitochondrial fusion protects against neurodegeneration in the cerebellum, Cell 130 (2007) 548–562.
[19] X. Chen, J.M. Levy, A. Hou, C. Winters, R. Azzam, A.A. Sousa, R.D. Leapman, R.A. Nicoll, T.S. Reese, PSD-95 family MAGUKs are essential for anchoring AMPA and NMDA receptor complexes at the postsynaptic density, Proc. Natl. Acad. Sci. USA 112 (2015) E6983–E6992.
[20] X. Chen, C.D. Nelson, X. Li, C.A. Winters, R. Azzam, A.A. Sousa, R.D. Leapman, H. Gainer, M. Sheng, T.S. Reese, PSD-95 is required to sustain the molecular organization of the postsynaptic density, J. Neurosci. 31 (2011) 6329–6338.
[21] A. Cheng, Y. Hou, M.P. Mattson, Mitochondria and neuroplasticity, ASN Neuro 2 (2010) e00045.
[22] Y. Cheng, F. Bai, The association of tau with mitochondrial dysfunction in alzheimer's disease, Front. Neurosci. 12 (2018) 163.
[23] E.C. Cheung, H.M. McBride, R.S. Slack, Mitochondrial dynamics in the regulation of neuronal cell death, Apoptosis 12 (2007) 979–992.
[24] D. Chin, S. Hagl, A. Hoehn, P. Huebbe, K. Pallauf, T. Grune, J. Frank, G.P. Eckert, G. Rimbach, Adenosine triphosphate concentrations are higher in the brain of APOE3- compared to APOE4-targeted replacement mice and can be modulated by curcumin, Genes Nutr. 9 (2014) 397.
[25] D.H. Cho, T. Nakamura, J. Fang, P. Cieplak, A. Godzik, Z. Gu, S.A. Lipton, S-nitrosylation of Drp1 mediates beta-amyloid-related mitochondrial fission and neuronal injury, Science 324 (2009) 102–105.
[26] H.N. Dawson, A. Ferreira, M.V. Eyster, N. Ghoshal, L.I. Binder, M.P. Vitek, Inhibition of neuronal maturation in primary hippocampal neurons from tau deficient mice, J. Cell Sci. 114 (2001) 1179–1187.
[27] S.A. Detmer, D.C. Chan, Functions and dysfunctions of mitochondrial dynamics, Nat. Rev. Mol. Cell Biol. 8 (2007) 870–879.
[28] A.T. Dinkova-Kostova, A.Y. Abramov, The emerging role of Nrf2 in mitochondrial function, Free Radic. Biol. Med. 88 (2015) 179–188.
[29] H. Du, S.S. Yan, Mitochondrial permeability transition pore in Alzheimer's disease: cyclophilin D and amyloid beta, Biochim. Biophys. Acta 1802 (2010) 198–204.
[30] S.P. Elmore, Y. Nishimura, T. Qian, B. Herman, J.J. Lemasters, Discrimination of depolarized from polarized mitochondria by confocal fluorescence resonance energy transfer, Arch. Biochem. Biophys. 422 (2004) 145–152.
[31] J. Finsterer, Cognitive dysfunction in mitochondrial disorders, Acta Neurol. Scand. 126 (2012) 1–11.
[32] P.J. Fitzgerald, C.R. Pinard, M.C. Camp, M. Feyder, A. Sah, H.C. Bergstrom, C. Graybeal, Y. Liu, O.M. Schluter, S.G. Grant, et al., Durable fear memories require PSD-95, Mol. Psychiatry 20 (2015) 901–912.
[33] C.R. Giordano, L.J. Terlecky, A. Bollig-Fischer, P.A. Walton, S.R. Terlecky, Amyloid-beta neuroprotection mediated by a targeted antioxidant, Sci. Rep. 4 (2014) 4983.
[34] T. Guo, W. Noble, D.P. Hanger, Roles of tau protein in health and disease, Acta Neuropathol. 133 (2017) 665–704.
[35] M. Gutierrez-Aguilar, C.P. Baines, Structural mechanisms of cyclophilin D-dependent control of the mitochondrial permeability transition pore, Biochim. Biophys. Acta 1850 (2015) 2041–2047.
[36] A.P. Halestrap, What is the mitochondrial permeability transition pore? J. Mol. Cell Cardiol. 46 (2009) 821–831.
[37] L. Hao, Q. Sun, W. Zhong, W. Zhang, X. Sun, Z. Zhou, Mitochondria-targeted ubiquinone (MitoQ) enhances acetaldehyde clearance by reversing alcohol-induced posttranslational modification of aldehyde dehydrogenase 2: a molecular mechanism of protection against alcoholic liver disease, Redox Biol. 14 (2018) 626–636.
[38] P.J. Hollenbeck, Mitochondria and neurotransmission: evacuating the synapse, Neuron 47 (2005) 331–333.
[39] L.M. Ittner, Y.D. Ke, F. Delerue, M. Bi, A. Gladbach, J. van Eersel, H. Wolfing, B.C. Chieng, M.J. Christie, I.A. Napier, et al., Dendritic function of tau mediates amyloid-beta toxicity in Alzheimer's disease mouse models, Cell 142 (2010) 387–397.
[40] S. Jager, C. Handschin, J. St-Pierre, B.M. Spiegelman, AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1alpha, Proc. Natl. Acad. Sci. USA 104 (2007) 12017–12022.
[41] A.G. Karadayian, J. Bustamante, A. Czerniczyniec, P. Lombardi, R.A. Cutrera, S. Lores-Arnaiz, Alcohol hangover induces mitochondrial dysfunction and free radical production in mouse cerebellum, Neuroscience 304 (2015) 47–59.
[42] T. Kimura, D.J. Whitcomb, J. Jo, P. Regan, T. Piers, S. Heo, C. Brown, T. Hashikawa, M. Murayama, H. Seok, et al., Microtubule-associated protein tau is essential for long-term depression in the hippocampus, Philos. Trans. R. Soc. Lond. B Biol. Sci. 369 (2013) 20130144.
[43] C.A. Lasagna-Reeves, D.L. Castillo-Carranza, U. Sengupta, A.L. Clos, G.R. Jackson, R. Kayed, Tau oligomers impair memory and induce synaptic and mitochondrial dysfunction in wild-type mice, Mol. Neurodegener. 6 (2011) 39.
[44] J. Lemire, R. Mailloux, V.D. Appanna, Zinc toxicity alters mitochondrial metabolism and leads to decreased ATP production in hepatocytes, J. Appl. Toxicol. 28 (2008) 175–182.
[45] H. Liang, W.F. Ward, PGC-1alpha: a key regulator of energy metabolism, Adv. Physiol. Educ. 30 (2006) 145–151.
[46] M. Liesa, M. Palacin, A. Zorzano, Mitochondrial dynamics in mammalian health and disease, Physiol. Rev. 89 (2009) 799–845.
[47] C. Lismont, M. Nordgren, P.P. Van Veldhoven, M. Fransen, Redox interplay between mitochondria and peroxisomes, Front. Cell Dev. Biol. 3 (2015) 35.
[48] Y. Liu, A. Beyer, R. Aebersold, On the dependency of cellular protein levels on mRNA abundance, Cell 165 (2016) 535–550.
[49] S. Lopes, L. Teplytska, J. Vaz-Silva, C. Dioli, R. Trindade, M. Morais, C. Webhofer, G. Maccarrone, O.F.X. Almeida, C.W. Turck, et al., Tau deletion prevents stress-induced dendritic atrophy in prefrontal cortex: role of synaptic mitochondria,

Cereb. Cortex 27 (2017) 2580–2591.
[50] C.V. Ly, P. Verstreken, Mitochondria at the synapse, Neuroscientist 12 (2006) 291–299.
[51] E.M. Mandelkow, E. Mandelkow, Biochemistry and cell biology of tau protein in neurofibrillary degeneration, Cold Spring Harb. Perspect. Med. 2 (2012) a006247.
[52] N. Mnatsakanyan, G. Beutner, G.A. Porter, K.N. Alavian, E.A. Jonas, Physiological roles of the mitochondrial permeability transition pore, J. Bioenerg. Biomembr. 49 (2016) 13–25.
[53] A. Montagrin, C. Saiote, D. Schiller, The social hippocampus, Hippocampus (2017).
[54] M. Morris, P. Hamto, A. Adame, N. Devidze, E. Masliah, L. Mucke, Age-appropriate cognition and subtle dopamine-independent motor deficits in aged tau knockout mice, Neurobiol. Aging 34 (2013) 1523–1529.
[55] P.K. Mouli, G. Twig, O.S. Shirihai, Frequency and selectivity of mitochondrial fusion are key to its quality maintenance function, Biophys. J. 96 (2009) 3509–3518.
[56] A. Mudher, M. Colin, S. Dujardin, M. Medina, I. Dewachter, S.M. Alavi Naini, E.M. Mandelkow, E. Mandelkow, L. Buee, M. Goedert, et al., What is the evidence that tau pathology spreads through prion-like propagation? Acta Neuropathol. Commun. 5 (2017) 99.
[57] M.P. Murphy, How mitochondria produce reactive oxygen species, Biochem. J. 417 (2009) 1–13.
[58] M. Narasimhan, L. Mahimainathan, M.L. Rathinam, A.K. Riar, G.I. Henderson, Overexpression of Nrf2 protects cerebral cortical neurons from ethanol-induced apoptotic death, Mol. Pharmacol. 80 (2011) 988–999.
[59] T. Nguyen, P. Nioi, C.B. Pickett, The Nrf2-antioxidant response element signaling pathway and its activation by oxidative stress, J. Biol. Chem. 284 (2009) 13291–13295.
[60] H.M. Ni, J.A. Williams, W.X. Ding, Mitochondrial dynamics and mitochondrial quality control, Redox Biol. 4 (2015) 6–13.
[61] H. Otera, N. Ishihara, K. Mihara, New insights into the function and regulation of mitochondrial fission, Biochim. Biophys. Acta 1833 (2013) 1256–1268.
[62] L. Owen, S.I. Sunram-Lea, Metabolic agents that enhance ATP can improve cognitive functioning: a review of the evidence for glucose, oxygen, pyruvate, creatine, and L-carnitine, Nutrients 3 (2011) 735–755.
[63] S.P. Pallo, G.V. Johnson, Tau facilitates Abeta-induced loss of mitochondrial membrane potential independent of cytosolic calcium fluxes in mouse cortical neurons, Neurosci. Lett. 597 (2015) 32–37.
[64] A. Pecorelli, S. Leoncini, C. De Felice, C. Signorini, C. Cerrone, G. Valacchi, L. Ciccoli, J. Hayek, Non-protein-bound iron and 4-hydroxynonenal protein adducts in classic autism, Brain Dev. 35 (2013) 146–154.
[65] M.J. Perez, D.P. Ponce, C. Osorio-Fuentealba, M.I. Behrens, R.A. Quintanilla, Mitochondrial bioenergetics is altered in fibroblasts from patients with sporadic alzheimer's disease, Front. Neurosci. 11 (2017) 553.
[66] M.J. Perez, R.A. Quintanilla, Development or disease: duality of the mitochondrial permeability transition pore, Dev. Biol. 426 (2017) 1–7.
[67] M.J. Perez, K. Vergara-Pulgar, C. Jara, F. Cabezas-Opazo, R.A. Quintanilla, Caspase-cleaved tau impairs mitochondrial dynamics in Alzheimer's disease, Mol. Neurobiol. (2017).
[68] M. Picard, B.S. McEwen, Mitochondria impact brain function and cognition, Proc. Natl. Acad. Sci. USA 111 (2014) 7–8.
[69] M. Poot, Y.Z. Zhang, J.A. Kramer, K.S. Wells, L.J. Jones, D.K. Hanzel, A.G. Lugade, V.L. Singer, R.P. Haugland, Analysis of mitochondrial morphology and function with novel fixable fluorescent stains, J. Histochem. Cytochem. 44 (1996) 1363–1372.
[70] R.A. Quintanilla, T.A. Matthews-Roberson, P.J. Dolan, G.V. Johnson, Caspase-cleaved tau expression induces mitochondrial dysfunction in immortalized cortical neurons: implications for the pathogenesis of Alzheimer disease, J. Biol. Chem. 284 (2009) 18754–18766.
[71] R.A. Quintanilla, R. von Bernhardi, J.A. Godoy, N.C. Inestrosa, G.V. Johnson, Phosphorylated tau potentiates Abeta-induced mitochondrial damage in mature neurons, Neurobiol. Dis. 71 (2014) 260–269.
[72] N. Raimundo, Mitochondrial pathology: stress signals from the energy factory, Trends Mol. Med. 20 (2014) 282–292.
[73] M. Rapoport, H.N. Dawson, L.I. Binder, M.P. Vitek, A. Ferreira, Tau is essential to beta -amyloid-induced neurotoxicity, Proc. Natl. Acad. Sci. USA 99 (2002) 6364–6369.
[74] S.M. Sarro, T.L. Unruh, J. Zuccolo, R. Sanyal, J.M. Luider, I.A. Auer-Grzesiak, A. Mansoor, J.P. Deans, Quantification of CD20 mRNA and protein levels in chronic lymphocytic leukemia suggests a post-transcriptional defect, Leuk. Res. 34 (2010) 1670–1673.
[75] A.C. Schinzel, O. Takeuchi, Z. Huang, J.K. Fisher, Z. Zhou, J. Rubens, C. Hetz, N.N. Danial, M.A. Moskowitz, S.J. Korsmeyer, Cyclophilin D is a component of mitochondrial permeability transition and mediates neuronal cell death after focal cerebral ischemia, Proc. Natl. Acad. Sci. USA 102 (2005) 12005–12010.
[76] M.L. Seibenhener, M.C. Wooten, Use of the open field maze to measure locomotor and anxiety-like behavior in mice, J. Vis. Exp. (2015) e52434.
[77] L.A. Sena, N.S. Chandel, Physiological roles of mitochondrial reactive oxygen species, Mol. Cell 48 (2012) 158–167.
[78] J.H. Son, J.H. Shim, K.H. Kim, J.Y. Ha, J.Y. Han, Neuronal autophagy and neurodegenerative diseases, Exp. Mol. Med. 44 (2012) 89–98.
[79] I. Sotiropoulos, M.C. Galas, J.M. Silva, E. Skoulakis, S. Wegmann, M.B. Maina, D. Blum, C.L. Sayas, E.M. Mandelkow, E. Mandelkow, et al., Atypical, non-standard functions of the microtubule associated Tau protein, Acta Neuropathol. Commun. 5 (2017) 91.
[80] J. St-Pierre, S. Drori, M. Uldry, J.M. Silvaggi, J. Rhee, S. Jager, C. Handschin, K. Zheng, J. Lin, W. Yang, et al., Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators, Cell 127 (2006) 397–408.
[81] D. Stewart, E. Killeen, R. Naquin, S. Alam, J. Alam, Degradation of transcription factor Nrf2 via the ubiquitin-proteasome pathway and stabilization by cadmium, J. Biol. Chem. 278 (2003) 2396–2402.
[82] X. Tang, L. Jiao, M. Zheng, Y. Yan, Q. Nie, T. Wu, X. Wan, G. Zhang, Y. Li, S. Wu, et al., Tau deficiency down-regulated transcription factor orthodenticle homeobox 2 expression in the dopaminergic neurons in ventral tegmental area and caused no obvious motor deficits in mice, Neuroscience 373 (2018) 52–59.
[83] C. Tapia-Rojas, F. Aranguiz, L. Varela-Nallar, N.C. Inestrosa, Voluntary running attenuates memory loss, decreases neuropathological changes and induces neurogenesis in a mouse model of alzheimer's disease, Brain Pathol. 26 (2016) 62–74.
[84] C. Tapia-Rojas, F.J. Carvajal, R.G. Mira, C. Arce, J.M. Lerma-Cabrera, J.A. Orellana, W. Cerpa, R.A. Quintanilla, Adolescent binge alcohol exposure affects the brain function through mitochondrial impairment, Mol. Neurobiol. 55 (2018) 4473–4491.
[85] C. Tapia-Rojas, N.C. Inestrosa, Wnt signaling loss accelerates the appearance of neuropathological hallmarks of Alzheimer's disease in J20-APP transgenic and wild-type mice, J. Neurochem. 144 (2018) 443–465.
[86] C. Tapia-Rojas, C.B. Lindsay, C. Montecinos-Oliva, M.S. Arrazola, R.M. Retamales, D. Bunout, S. Hirsch, N.C. Inestrosa, Is L-methionine a trigger factor for Alzheimer's-like neurodegeneration?: changes in Abeta oligomers, tau phosphorylation, synaptic proteins, Wnt signaling and behavioral impairment in wild-type mice, Mol. Neurodegener. 10 (2015) 62.
[87] C. Teyssier, H. Ma, R. Emter, A. Kralli, M.R. Stallcup, Activation of nuclear receptor coactivator PGC-1alpha by arginine methylation, Genes Dev. 19 (2005) 1466–1473.
[88] P. Therond, Oxidative stress and damages to biomolecules (lipids, proteins, DNA), Ann. Pharm. Fr. 64 (2006) 383–389.
[89] J.F. Turrens, Mitochondrial formation of reactive oxygen species, J. Physiol. 552 (2003) 335–344.
[90] A.M. van der Bliek, Q. Shen, S. Kawajiri, Mechanisms of mitochondrial fission and fusion, Cold Spring Harb. Perspect. Biol. (2013) 5.
[91] J.Y. Vargas, M. Fuenzalida, N.C. Inestrosa, In vivo activation of Wnt signaling pathway enhances cognitive function of adult mice and reverses cognitive deficits in an alzheimer's disease model, J. Neurosci. 34 (2014) 2191–2202.
[92] P. Verma, A. Singh, D.N. Nthenge-Ngumbau, U. Rajamma, S. Sinha, K. Mukhopadhyay, K.P. Mohanakumar, Attention deficit-hyperactivity disorder suffers from mitochondrial dysfunction, BBA Clin. 6 (2016) 153–158.
[93] M. Vos, E. Lauwers, P. Verstreken, Synaptic mitochondria in synaptic transmission and organization of vesicle pools in health and disease, Front. Synaptic Neurosci. 2 (2010) 139.
[94] K.A. Vossel, J.C. Xu, V. Fomenko, T. Miyamoto, E. Suberbielle, J.A. Knox, K. Ho, D.H. Kim, G.Q. Yu, L. Mucke, Tau reduction prevents Abeta-induced axonal transport deficits by blocking activation of GSK3beta, J. Cell Biol. 209 (2015) 419–433.
[95] X. Wang, B. Su, H.G. Lee, X. Li, G. Perry, M.A. Smith, X. Zhu, Impaired balance of mitochondrial fission and fusion in Alzheimer's disease, J. Neurosci. 29 (2009) 9090–9103.
[96] Y. Wang, M. Martinez-Vicente, U. Kruger, S. Kaushik, E. Wong, E.M. Mandelkow, A.M. Cuervo, E. Mandelkow, Tau fragmentation, aggregation and clearance: the dual role of lysosomal processing, Hum. Mol. Genet. 18 (2009) 4153–4170.
[97] P. Wareski, A. Vaarmann, V. Choubey, D. Safiulina, J. Liiv, M. Kuum, A. Kaasik, PGC-1{alpha} and PGC-1{beta} regulate mitochondrial density in neurons, J. Biol. Chem. 284 (2009) 21379–21385.
[98] S. Wegmann, E.A. Maury, M.J. Kirk, L. Saqran, A. Roe, S.L. DeVos, S. Nicholls, Z. Fan, S. Takeda, O. Cagsal-Getkin, et al., Removing endogenous tau does not prevent tau propagation yet reduces its neurotoxicity, EMBO J. 34 (2015) 3028–3041.
[99] B. Westermann, Bioenergetic role of mitochondrial fusion and fission, Biochim. Biophys. Acta 1817 (2012) 1833–1838.
[100] M. Yang, J.L. Silverman, J.N. Crawley, Automated three-chambered social approach task for mice, Curr. Protoc. Neurosci. (2011) 26 (Chapter 8, Unit 8).
[101] Y.C. Zuo, H.L. Li, N.X. Xiong, J.Y. Shen, Y.Z. Huang, P. Fu, H.Y. Zhao, Overexpression of tau rescues Nogo-66-Induced neurite outgrowth inhibition in vitro, Neurosci. Bull. 32 (2016) 577–584.