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Beneficial effects of exercise in a transgenic mouse model of Alzheimer's disease-like Tau pathology

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ABSTRACT

Tau pathology is encountered in many neurodegenerative disorders known as tauopathies, including Alzheimer's disease. Physical activity is a lifestyle factor affecting processes crucial for memory and synaptic plasticity. Whether long-term voluntary exercise has an impact on Tau pathology and its pathophysiological consequences is currently unknown. To address this question, we investigated the effects of long-term voluntary exercise in the THY-Tau22 transgenic model of Alzheimer's disease-like Tau pathology, characterized by the progressive development of Tau pathology, cholinergic alterations and subsequent memory impairments. Three-month-old THY-Tau22 mice and wild-type littermates were assigned to standard housing or housing supplemented with a running wheel. After 9 months of exercise, mice were evaluated for memory performance and examined for hippocampal Tau pathology, cholinergic defects, inflammation and genes related to cholesterol metabolism. Exercise prevented memory alterations in THY-Tau22 mice. This was accompanied by a decrease in hippocampal Tau pathology and a prevention of the loss of expression of choline acetyltransferase within the medial septum. Whereas the expression of most cholesterol-related genes remained unchanged in the hippocampus of running THY-Tau22 mice, we observed a significant upregulation in mRNA levels of NPC1 and NPC2, genes involved in cholesterol trafficking from the lysosomes. Our data support the view that long-term voluntary physical exercise is an effective strategy capable of mitigating Tau pathology and its pathophysiological consequences.

Introduction

Tau proteins normally play an important role in microtubule polymerization and axonal transport (Buee et al., 2000; Sergeant et al., 2008). Tau pathology results from the intracellular aggregation of hyperphosphorylated and abnormally phosphorylated Tau proteins into filaments and is encountered in many neurodegenerative disorders known as Tauopathies (Sergeant et al., 2008). In Alzheimer's disease (AD), Tau pathology leads to neurofibrillary degeneration (NFD), which appears first in the entorhinal cortex and hippocampal formation and then reaches isocortical areas (Braak and Braak, 1991; Delacourte et al., 1999). Several studies have outlined the relationship between the spreading of NFD and cognitive deficits (Duyckaerts et al., 1997; Grober et al., 1999) and suggest that Tau pathology plays a key role in the pathological cascade leading to AD symptoms.

Several lifestyle-related factors such as diet or exercise might influence the onset of dementia and the risk of AD (Hillman et al., 2008; Pasinetti and Eberstein, 2008). In addition to improve overall health, attenuate depression (Dimeo et al., 2001) and mitigate cardiometabolic abnormalities, voluntary physical exercise could exert a beneficial effect on cognitive function (Cotman and Berchtold, 2007; Hillman et al., 2008). Epidemiological studies suggest that physical activity may prevent cognitive decline in the elderly, the occurrence of dementia and the risk of AD (Friedland et al., 2001; Landi et al., 2004; Laurin et al., 2001; Lautenschlager et al., 2008; Rolland et al., 2008; Scarmeas et al., 2009; Teri et al., 2003). In line, in rodents, voluntary exercise prevents cognitive decline during ageing (Samorajski et al., 1985). However, although Tau pathology is

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instrumental in the development of Tauopathies (Sergeant et al., 2008), whether voluntary exercise has an impact on Tau pathology and its pathophysiological consequences remains unknown. In the present study, we evaluated the effects of long-term voluntary exercise in the appropriate and reliable THY-Tau22 transgenic model exhibiting progressive and parallel development of Tau pathology and memory impairments in absence of motor deficits (Belarbi et al., 2009; Schindowski et al., 2006; Van der Jeugd et al., 2011).

Materials and methods

Animals

THY-Tau22 mice are characterized by the overexpression of human 4-repeat Tau mutated at sites G272V and P301S under the control of Thy1.2 promoter (Schindowski et al., 2006). Mice, of C57Bl6/J background, were housed in a pathogen-free facility (Techniplast cages 1284L), with ad libitum access to food and water and maintained on a 14 h light/10 h dark cycle. All protocols followed European animal welfare regulations and were approved by the local ethical committee (Approval n° AF 06/2010, 31/03/2010).

Experimental paradigm

Male THY-Tau22 ($n=28$) and wild-type (WT) littermate controls ($n=28$) were randomly assigned to standard housing or standard housing supplemented with a running wheel (12 cm diameter, Amiland, France) from 3 to 12 months of age. WT and THY-Tau22 mice experiencing long-term voluntary exercise are designated WT-RUN and THY-Tau22-RUN, respectively. At the end of the experiment, animals were evaluated for memory performance using the Y-maze test, weighed and sacrificed the following day.

Mice used for histological analyses ($n=6$ per group) were transcardially perfused with 0.9% NaCl and 4% paraformaldehyde in phosphate buffered saline (PBS) (0.1 mol/L PBS, pH 7.4) under deep anesthesia. Brains were removed, post-fixed for 24 h in 4% paraformaldehyde and cryoprotected in 20% sucrose in PBS. Serial free-floating coronal sections (40 μm thick) were cut on a freezing microtome (Leica), collected in a storage solution (PBS 0.1 M pH7.4; 33% ethylene glycol, 33% glycerol) and kept at −20 °C until use. The remaining mice were sacrificed by cervical dislocation and used for both BDNF ELISA and mRNA analysis by quantitative PCR ($n=7$–9/group). To that end, brains were rapidly removed, left and right hippocampi dissected out at 4 °C using a coronal acrylic slicer (Delta Microscopies, France) and stored at −80 °C until use.

Y-maze test

Mice were tested for hippocampus-dependent spatial memory using a two-trial Y-maze task (Sanderson et al., 2007). The arms of the maze were 22 cm long, 6.4 cm wide and 15 cm deep. The floor of the maze was covered with sawdust that was mixed after each trial in order to eliminate olfactory cues. Various extra-maze cues were placed on the surrounding walls. Experiments were conducted with an ambient light level of 6 lx. During the exposure phase, mice were assigned to two arms (the “start arm” and the “other arm”) that they were allowed to freely explore during 5 min, without access to the third arm of the maze (the “novel arm”) blocked by an opaque door. The assignment of arms was counterbalanced within each experimental group. Mice were then removed from the maze and returned to their home cage for 2 min. During the test phase, mice were placed at the end of the same “start arm” and allowed to freely explore all three arms during 1 min. The amount of time spent in each of the arms was recorded using EthovisionXT (Noldus, Netherlands). As previously showed by Sanderson et al. (2007), we have verified that in our experimental conditions, new arm preference was dependent on extra-maze cues (data not shown).

BDNF ELISA

BDNF levels were measured in extracts from frozen tissue using the Emax Immunoassay System (Promega, France) according to the modified extraction procedure published by Szapacs et al. (2004).

Tau and P-Tau ELISA

Samples used for BDNF ELISA were evaluated for soluble Total and P-Thr181 human Tau levels according to manufacturer's instructions (INNOTEST® hTau Ag and INNOTEST® PHOSPHO-TAU(181P), Innogenetics, Belgium).

Immunohistochemistry

Phosphorylated (AT8; pSer202/205) and abnormally phosphorylated (AT100; pSer212/pThr214) Tau species were immunolabeled as previously described (Schindowski et al., 2006) using AT8 (Thermo Scientific; 1:200) and AT100 (Thermo Scientific; 1:400) antibodies and the reaction was visualized with diaminobenzidine. Staining was semi-quantified as previously described (Green et al., 2006): photomicrographs were taken using a Leica digital camera, imported in ImageJ software (Scion) and converted to black and whites images. Threshold intensity was set and kept constant and the number of pixel, expressing staining density, was determined for both THY-Tau22 and THY-Tau22-RUN mice. Quantifications were performed blindly by at least two observers and averaged from six animals per group.

Immunofluorescence

Immunofluorescent labeling was performed using the following primary antibodies: AT100 (Thermo Scientific; 1:400), pS422 (Invitrogen; 1:1000), choline acetyltransferase (ChAT) (Chemicon; 1:1000), GFAP (Santa Cruz; 1:100) and NPC1 (Abcam; 1:250), and secondary antibodies coupled to Alexa 488 or 568 (Invitrogen). Sections were counterstained and mounted with Vectashield/DAPI (Vector). Images were acquired on an Apotome microscope (Imager Z1; Zeiss). The density of ChAT-positive cells in medial septum was determined as previously described (Belarbi et al., 2011) using ImageJ software by at least two observers blind to the experimental group and averaged from six animals per group.

mRNA extraction and quantitative real-time RT-PCR analysis

Total RNA was extracted from hippocampi and purified using the RNeasy Lipid Tissue Mini Kit (Qiagen, France). One microgram of total RNA was reverse-transcribed using the Applied Biosystems High-Capacity cDNA reverse transcription kit. Quantitative real-time RT-PCR analysis was performed on an Applied Biosystems Prism 7900 System using Power SYBR Green PCR Master Mix. The thermal cycler conditions were as follows: hold for 10 min at 95 °C, followed by 45 cycles of a two-step PCR consisting of a 95 °C step for 15 s followed by a 60 °C step for 25 s. Primer sequences are given in Supplementary Table 1. Cyclophilin A and actin were used as internal controls since their expression does not vary between experimental groups of mice and their amplification efficiency is comparable to the amplification efficiency of the target genes. Amplifications were carried out in triplicate and the relative expression of target genes was determined by the $\Delta\Delta C_T$ method.