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Genetic ablation of tau in postnatal neurons rescues decreased adult hippocampal neurogenesis in a tauopathy model

Sarah Houben[a], Karelle Leroy[a], Kunie Ando[a], Zehra Yilmaz[a], Cyprien Widomski[a], Luc Buée[b], Jean-Pierre Brion[a,*]

[a] Laboratory of Histology, Neuroanatomy and Neuropathology, UNI (ULB Neuroscience Institute), Faculty of Medicine, Université Libre de Bruxelles, 808, route de Lennik (Bldg G), B-1070 Brussels, Belgium
[b] INSERM, U1172. Université de Lille, Lille, France

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ABSTRACT

Impaired adult hippocampal neurogenesis has been reported as a feature of Alzheimer's disease and other tauopathies and might contribute to defects in learning and memory in these diseases. To assess the interference of tau pathology, a common key-lesion in these diseases, with adult hippocampal neurogenesis we analyzed adult neurogenesis in the hippocampal dentate gyrus in wild-type mice, Tg30 mice expressing a FTDP-17 mutant tau and the same Tg30 mice deficient for mouse tau (Tg30/tauKO). The volume of the granular layer, the number of granule cells and of neuronal precursors expressing the immature markers DCX or 3R-tau were analyzed in the dentate gyrus (DG) using unbiased stereological methods. The co-localization of neurogenic markers with the human mutant tau was also analyzed. We observed a significant reduction of the volume of the granular layer and of granule cells number in mutant tau Tg30 mice, but not in Tg30/tauKO mice. The number of neuronal precursors expressing the immature markers DCX or 3R-tau (the latter only expressed in wild-type and Tg30 mice) and the number of cells expressing the proliferation marker Ki-67 in the neurogenic subgranular zone of the DG was reduced in Tg30 but not in Tg30/tauKO mice. The density of phosphotau positive cells in the DG and the level of soluble human phosphotau was lower in Tg30/tauKO compared to Tg30 mice. The human mutant tau was expressed in mature granule cells in Tg30 and Tg30/tauKO mice but was not expressed in Sox2 positive neural stem cells and in DCX positive neuronal precursors/immature newborn neurons. These results demonstrate an impairment of adult hippocampal neurogenesis in a FTDP-17 mutant tau mice resulting from a decrease of proliferation affecting the pool of neuronal precursors. The mutant tau was not expressed in precursors cells in these mutant tau mice, suggesting that this neurogenic defect is cell non-autonomous. Interestingly, expression of endogenous wild-type tau in mature granule cells was necessary to observe this toxic effect of human mutant tau, since this impaired adult neurogenesis was rescued by lowering tau expression in Tg30/tauKO mice. These observations suggest that development of tau pathology in granule cells of the dentate gyrus is responsible for reduction of adult hippocampal neurogenesis also in human tauopathies by impairing proliferation of neuronal precursors, and that reduction of tau expression might be an approach to rescue this impairment.

Abbreviations: 3R tau, tau protein with 3 semi-repeated sequences in the microtubule binding domain; AD, Alzheimer's disease; APP, Amyloid protein precursor; CA3, Sector 3 of the Ammon' s horn in the hippocampus; DCX, Doublecortin; DG, Dentate gyrus; ECL, Enhanced chemiluminescence; FTDP-17, Frontotemporal dementia and Parkinsonism linked to chromosome 17; GFAP, Glial fibrillary acidic protein; GFP, Green fluorescent protein; PCR, polymerase chain reaction; PS1, Presenilin 1; RIPA, Radioimmunoprecipitation assay; SGZ, Subgranular zone; tauKO, tau gene inactivated (knock-out); TBS, Tris-buffered saline; YFP, yellow fluorescent protein

* Corresponding author at: Laboratory of Histology, Neuroanatomy and Neuropathology, ULB Neuroscience Institute, Université Libre de Bruxelles, 808 route de Lennik, 1070 Brussels, Belgium.
E-mail addresses: Sarah.Houben@ulb.ac.be (S. Houben), kleroy@ulb.ac.be (K. Leroy), kuniando@ulb.ac.be (K. Ando), Zehra.Yilmaz@ulb.ac.be (Z. Yilmaz), luc.buee@inserm.fr (L. Buée), jpbrion@ulb.ac.be (J.-P. Brion).

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1. Background

Two neurogenic areas containing neural stem cells are present in the adult mammalian brain: the subventricular zone lining the lateral ventricles that give rise to local interneurons in the olfactory bulb and the subgranular zone in the dentate gyrus (DG) of the hippocampus giving rise to new granule cell neurons (Altman and Das, 1965; Zhao et al., 2008). Newborn neurons migrating from the subgranular zone into the granular layer extend axons targeting CA3 pyramidal neurons through the mossy fiber pathway (Stanfield and Trice, 1988). These newly formed neurons integrate into circuits critical for hippocampal-dependent functions such as learning and memory, and anxiety-related behaviours (Revest et al., 2009). Hippocampal neurogenesis also occurs in the adult human brain (Eriksson et al., 1998) (Spalding et al., 2013). Despite evidence of hippocampal adult neurogenesis in humans (Boldrini et al., 2018; Kempermann et al., 2018), its robustness and level of decline during aging has been questioned (Knoth et al., 2010; Sorrells et al., 2018). Progressive memory loss is a characteristic clinical feature of AD and impairment of adult hippocampal neurogenesis is thought to play an important role in this process (Hollands et al., 2016; Lazarov and Marr, 2010; Lazarov et al., 2010; Mu and Gage, 2011). Evidence for reduced adult neurogenesis in other neurodegenerative diseases is also growing (Winner et al., 2011). Neurofibrillary tangles and senile plaques are key-neuropathological lesions of AD that develop in many brain areas during the progression of the disease, including in the hippocampus. Afferents pathways to the dentate gyrus are affected early by neurofibrillary tangles developing in the entorhinal cortex (Braak and Braak, 1991), and neurofibrillary tangles develop also in the granule cell layer (Braak and Braak, 1991; Dickson et al., 1986; Wakabayashi et al., 1997). This tau pathology in the DG might play a role in memory impairment during progression of tauopathies. The impact of tau pathology on adult hippocampal neurogenesis is still poorly understood in these human diseases. To address this question in a mammalian model, we analyzed and compared adult hippocampal neurogenesis in wild-type mice, in a mice model expressing a FTDP-17 mutant tau (Tg30), and in the same mice model deleted for endogenous tau and expressing this mutant human tau. Our results indicate that the postnatal expression of mutant tau in Tg30 mice is associated with a decrease of the pool of neuronal precursors, leading to a volume reduction of the DG and a reduction of granule cell number. The absence of mutant tau expression in neural stem cells and in neuronal precursors in the SGZ in Tg30 mice suggests that this impairment of adult hippocampal neurogenesis is cell non-autonomous, probably as a result of changes in the microenvironment of this neurogenic niche. This decrease in adult hippocampal neurogenesis in mutant tau mice was rescued in the same mouse line deleted for endogenous tau, suggesting that mutant tau effect is driven by endogenous tau and that a tau-lowering approach is a potential therapeutic avenue to rescue neurogenesis in tauopathies.

2. Material and methods

2.1. Animals

The generation and characterization of the mutant tau Tg30 transgenic line has been previously described (Leroy et al., 2007; Schindowski et al., 2006). These transgenic mice express a 1N4R human tau isoform mutated at positions G272 V and P301S, under control of a Thy-1.2 promoter. Only heterozygous Tg30 animals (n = 8) were used in this study, and littermates non-transgenic (named hereafter wild-type) mice were used as controls (n = 5). The Tg30/tauKO mice (n = 7) expressing only the human mutant tau G272 V/P301S and no endogenous mouse tau were described previously (Ando et al., 2011) and were generated by crossing with tauKO mice (Tucker et al., 2001). Only female mice were used for groups comparison. All mice were maintained into a C57BL/6JRj background. Mice were genotyped by PCR using previously described primers for human tau, murine tau and GFP on genomic DNA as reported (Ando et al., 2011). All mice were maintained on a 12 h light/dark cycle, with food and water ad libitum. All studies on animals were performed in compliance and following approval of the Ethical committee for the care and use of laboratory animals of the medical School of the Free University of Brussels.

2.2. Antibodies

The B19 antibody is a rabbit polyclonal antibody raised to adult bovine tau proteins, reacting with adult and foetal tau isoforms in bovine, rat, mouse and human nervous tissue in a phosphorylation-independent manner (Brion et al., 1991). The RD3 mouse monoclonal antibody is specific for three-repeat tau isoforms, respectively (de Silva et al., 2003). The AT8 antibody is a mouse monoclonal antibody (purchased from Innogenetics, Ghent, Belgium) specific for tau phosphorylated at Ser202 and Thr205 (AT8) (Goedert et al., 1995). The mouse monoclonal anti-murine tau JN-RF.5 mTau5 antibody was a generous gift from Dr. M Mercken (Ando et al., 2011; Sennvik et al., 2007). The mouse monoclonal PHF1 antibody reacting with tau phosphorylated at Ser396 and Ser404 (Otvos Jr. et al., 1994) and the mouse monoclonal MC1 antibody recognizing tau in a pathological conformation (Jicha et al., 1997) were a generous gift from Dr. P Davies. The BR21 rabbit polyclonal antibody is specific for human tau (Ando et al., 2011). The antibody to doublecortin (DCX) is a goat polyclonal antibody (C-18: sc-8066; Santa Cruz Biotechnology) reacting with mouse, rat and human DCX. The antibody to Sox2 is a goat polyclonal antibody (Santa Cruz Biotechnology; Y-17: sc-17,320). Other used antibodies were monoclonal rabbit antibodies to Ki-67 (SP6, Thermo Scientific) and to CD11b (EPR 1344, Abcam), mouse monoclonal antibodies to activated caspase 3 (AF835-SP, R&D), to α-tubulin (clone, DM1A, SIGMA) and to GFAP (G-A-5, Sigma).

2.3. Histological staining and immunohistochemistry

Mice were sacrificed by cervical dislocation and tissues were carefully dissected. Brain hemispheres were fixed in 10% (v/v) formalin solution during 24 h followed by 2 h of water wash before dehydration in alcohol for paraffin embedding. Tissue sections (15 µm thick) were stained with the Nissl method and haematoxylin for histological examination as previously described (Leroy et al., 2007). Tissue sections were also stained with the Gallyas silver-staining method to identify aggregated tau (Ando et al., 2011; Leroy et al., 2007). Immunohistochemistry with diaminobenzidine as chromogen and the avidin-biotin complex method (Vector Laboratories) or double immunofluorescence with Alexa Fluor 488 and 594 species-specific conjugated secondary antibodies (Molecular Probes) was performed on 15 µm coronal paraffin sections as described previously (Ando et al., 2011). Tissue sections were briefly incubated in 0.3% (v/v) H_2O_2 for 30 min to inhibit endogenous peroxidase and rinsed in water. Antigen retrieval was performed in citrate buffer in a pressure cooker for 20 min at 200 °C. Sections were then incubated with a blocking solution (normal serum 10% in TBS (Tris 0.01 M, NaCl 0.15 M, pH 7.4) for 1 h and incubated overnight at room temperature with the primary antibody. On the second day, sections were incubated with anti-mouse, anti-rabbit or anti-goat antibodies conjugated to biotin (Vector) followed by the avidin-biotin complex (Vector). Tissue sections were examined with Zeiss Axioplan I and Axiovert200M microscopes (Carl Zeiss GmbH, Jena, Germany) and digital images acquired using an AxiocamHRc camera (Carl Zeiss).

2.4. Preparation of tissue homogenates, SDS-PAGE and western blotting

After sacrifice by cervical dislocation and dissection, brain was snap frozen and kept at −80 °C until use. Tissue samples were homogenized with a glass/Teflon homogenizer at 4 °C in 10 volumes of a RIPA buffer

containing 50 mM Tris pH 7.4, 150 mM NaCl, 1% Nonidet P-40, 0.25% sodium deoxycholate, 1 mM phenylmethylsulphonyl fluoride, 25 µg/ml leupeptin, 25 µg/ml Pepstatin, 10 mM sodium pyrophosphate, 20 mM sodium fluoride, 1 mM sodium orthovanadate. The brain homogenates were centrifuged for 20 min at 20,000*g* and the supernatants (Sup1) were considered as RIPA soluble fraction. Samples were heated in Laemmli buffer at 100 °C for 5 min and applied to 10% Tris-Glycine SDS-polyacrylamide gel electrophoresis (PAGE) using mini PROTEAN III system according to the manufacturer's instructions (BioRad). For western blotting, the nitrocellulose membranes were blocked with 10% w/v semi-fat dry milk in Tris buffer saline (TBS 0.01 M Tris, 0.15 M NaCl, pH 7.4) and incubated with primary antibody for overnight. After several rinses, the membranes were incubated with anti-rabbit (#7074, Cell signalling) or anti-mouse (A6782, Sigma) secondary antibodies conjugated with horseradish peroxidase and developed with the ECL system (Pico, ThermoFisher Scientific). The chemiluminescent signal was captured using a Fusion SOLO 4S system (Vilber Lourmat) equipped with a DARQ-7 camera.

2.5. Cavalieri method for estimation of the volume of dentate gyrus

The volume of granule cell layer of the hippocampus was estimated using coronal sections (15 µm thickness) of the right hemispheres in 12 months old mice. These volumes estimations were performed with the Cavalieri estimator (Cavalieri, 1966) implemented in the Stereo-Investigator software (Version 9, MicroBrightField, Inc., Colchester, USA) on a motorized Zeiss AxioImagerM1 microscope, using coronal sections at 225 µm intervals to span the whole hippocampus. A sampling grid area of 110 µm × 110 µm and a counting frame area of 20 µm × 20 µm were used. Neuroanatomical delineations were made according to the mouse brain atlas of Paxinos (Paxinos and Franklin, 2001). Experiments were blindly performed.

2.6. Quantification of neuron number using design-based stereology

2.6.1. Total granular cells number

Stereological analyses were performed with the optical fractionator method as reported previously (Ando et al., 2011; Leroy et al., 2007). For the estimation of the neuron number in the dentate gyrus of the hippocampus in 12 months old mice, we used 10 coronal sections at 225 µm intervals for each animal. The counting was performed with a 100× oil objective with the StereoInvestigator software (Version 9, MicroBrightField, Inc., Colchester, USA) on a motorized Zeiss AxioImagerM1 microscope. Optical disector (Sampling grid area: 110 µm × 110 µm and counting frame area: 15 µm × 15 µm) were randomly and automatically distributed throughout the region of interest. Each neuron whose nucleolus came into focus within an optical dissector was counted. Estimation of the total number of cells was calculated using the number of counted neurons and the volume of dentate gyrus.

2.6.2. DCX, Tau 3R, and Ki-67 positive cells number

A stereological analysis with the optical fractionator method was also used to count on 10 coronal sections at 225 µm intervals, adjacent to those used for estimation of total granule cells numbers, the total number of DCX, 3R-tau and Ki-67 positive cells in the whole dentate gyrus using a sampling grid area of 60 µm × 60 µm and a counting frame area of 60 µm × 60 µm. All the surface of the dentate gyrus was however scanned with counting boxes to avoid important variations in cell counting when using a limited number of counting frames randomly distributed.

2.6.3. Glial cells and phosphotau positive cells

The density of GFAP positive cells in the DG was measured by counting the number of positive cells on sequential coronal sections of the dorsal hippocampus (3 non-adjacent sections/ mouse) and expressed as a density number after measurement of the surface of the DG using ImageJ. The density of phosphotau (PHF1, AT8, MC1) positive cells in the DG was measured similarly on sequential coronal sections of the dorsal hippocampus (2 non-adjacent sections/mouse).

2.7. Statistical analysis

Statistical analysis was performed using Prism 5 (GraphPad Software). Statistical comparisons were performed using one-way or two-way ANOVA followed by Bonferroni or Newman-Keuls post-tests for Multiple Comparisons or an unpaired Student t-test as noted in figure legends. Values of $P < .05$ were considered significant. Numbers of samples are indicated in the figure legends and histograms represent means ± SEM.

3. Results

3.1. Expression of human mutant tau and 3R tau in the dentate gyrus

In Tg30 mice, human mutant tau expression is under control of the Thy-1.2 promoter, that drives expression in the brain starting in the postnatal period (Vidal et al., 1990). Human tau was not expressed at P0 (Fig. 1A) and at P7 (Fig. 1B) in the dentate gyrus. At P14, human tau was expressed in granule cells in the upper rows of the granule cell layer but not in the lower rows (Fig. 1C). At 3 months of age and later up to 12 months (Fig. 1D), human tau was expressed in the whole granule cell layer. The expression of tau protein isoforms is developmentally regulated (Francon et al., 1982), with 0N3R tau being the only species expressed in foetal brain (Brion et al., 1993; Goedert et al., 1989; Kosik et al., 1989); 0N3R tau expression decreases in postnatal brain with 4R tau being the major tau isoforms expressed in adult rodent brain (Brion et al., 1993; Kosik et al., 1989). In agreement with this developmental expression, the anti-3R tau antibody easily detected by western blotting the abundant 0N3R tau mouse tau isoform at P0 but not in the adult mouse brain (Supplementary Fig. 1). 3R tau is a marker of immature neurons and during adult neurogenesis, neuronal precursors express transiently the 0N3R foetal tau isoform (Bullmann et al., 2007; Llorens-Martin et al., 2012; McMillan et al., 2008). Accordingly, 3R tau positive cells were detected by immunohistochemistry in the granular and subgranular zones in wild-type and Tg30 mice (Fig. 5 and below for detailed analysis) but not in Tg30/tauKO mice since endogenous tau (including 3R-tau) is not expressed in the latter line.

3.2. Level of expression of human tau is similar in Tg30 and Tg30/tauKO mice but phosphotau level is reduced in Tg30/tauKO mice

By western blot analysis, total tau level in brain is increased about three times in Tg30 mice compared to wild-type mice and is increased about two times in Tg30/tauKO mice (Fig. 2A and D). The level of mouse tau was similar in WT and in Tg30 mice (Fig. 2B). As reported previously (Ando et al., 2011), Tg30/tauKO mice did not express any mouse tau (Fig. 2A) and the level of human tau was similar in Tg30 and Tg30/tauKO mice (Fig. 2C). These data indicate that the difference in total tau expression between Tg30 and Tg30/tauKO mice was due to the absence of mouse tau in Tg30/tauKO mice. The total level of soluble phosphotau (PHF1 positive), normalized to total tau, was decreased in Tg30/tauKO mice (Fig. 2G). We also assessed the levels of phosphorylated mouse tau (PHF1 positive lower bands, around 55 kDa) and of phosphorylated human tau (PHF1 positive lower bands, around 64 kDa), normalized to total mouse tau and total human tau, respectively. Phosphorylated mouse tau (PHF1 positive) was not significantly different in WT and Tg30 mice (Fig. 2E) whereas phosphorylated human tau (PHF1 positive) was decreased in Tg30/tauKO mice (Fig. 2F) compared to Tg30 mice.

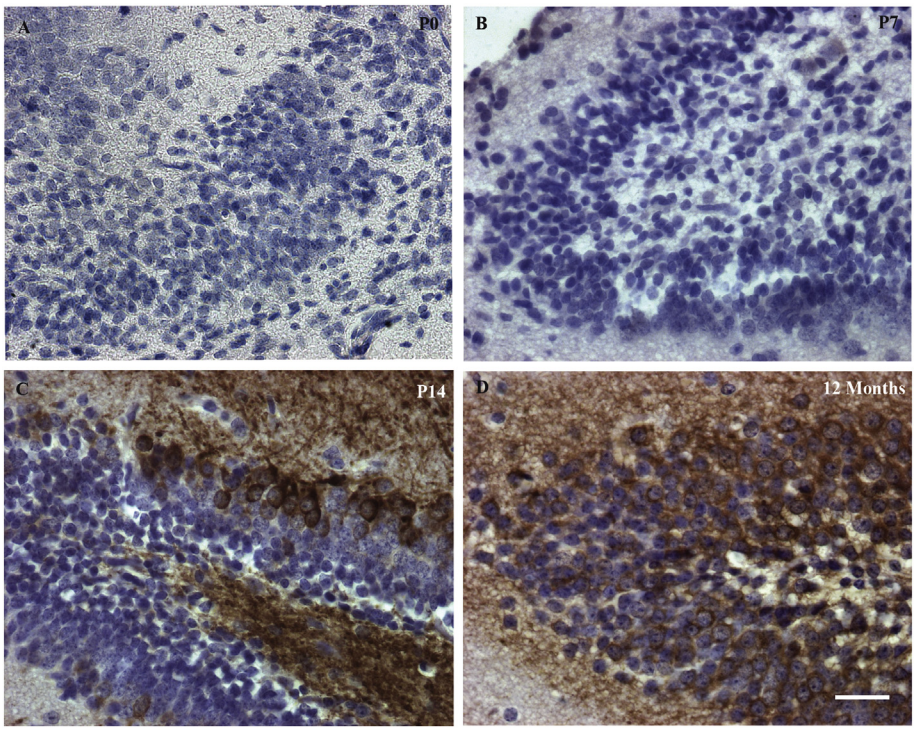

Fig. 1. Developmental expression of human transgenic mutant tau in the dentate gyrus of mutant tau Tg30 mice.
A–D: Immunolabelling with the BR21 anti-human tau antibody, at P0 (A), P7 (B), P14 (C), and at 12 months (D). Human tau expression is first detected in upper rows of granule cells at P14 and expressed in the whole granule cell layer at 12 months Haematoxylin counterstaining. Scale bar: 25 μm.

3.3. Accumulation of phosphotau in granule cells in the dentate gyrus in Tg30 and Tg30/tauKO mice

An advanced tau pathology is observed in several brain areas (hippocampal pyramidal layer, cortex, brainstem and spinal cord) in both Tg30 and Tg30/tauKO mice in 12-month-old mice (Ando et al., 2011; Leroy et al., 2007). We analyzed tau pathology in the DG of these mice after immunolabelling with the phosphotau antibodies AT8 and PHF and the MC1 antibody to conformationally altered tau (Fig. 3). Hyperphosphorylated tau (AT8 and PHF1 positive) and conformationally altered tau (MC1 positive) accumulated in the somatodendritic domain of some granule cells in both Tg30 (Fig. 3A, D, G) and Tg30/tauKO mice (Fig. 3B, E, F). The density of AT8, PHF1 and MC1 positive cells was lower in Tg30/tauKO mice than in Tg30 mice (Fig. 3C, F, I). Only very rare granule cells were stained with the Gallyas-silver staining method in Tg30 and Tg30/tauKO mice. Somatodendritic accumulation of phosphotau (AT8, PHF1) and conformationally altered tau (MC1) was never observed in granule cells in wild-type mice (Supplementary Fig. 2).

3.4. The volume of the granule cell layer and the number of granule cells are reduced in Tg30 mice but not in Tg30/tauKO mice

To assess if expression of the mutant human tau and absence of endogenous tau affected the number of granule cells in adult mice with advanced tau pathology, we first measured the volume of the total granule cell layer with the Cavalieri method in 12-month-old wild-type, Tg30 and Tg30/tauKO mice (Fig. 4A). The volume of the granule cell layer in Tg30 mice was significantly decreased compared to wild-type and Tg30/tauKO mice. We next estimated the total granule cell number in the same cohorts of mice with a stereological tool, the optical fractionator (Fig. 4B). The number of granule cells was significantly decreased in Tg30 mice compared to wild-type mice and Tg30/tauKO mice.

3.5. The number of DCX and 3R tau positive neuronal precursors is decreased in Tg30 mice but not in Tg30/tauKO mice

We next investigated if the pool of neuronal precursors cells and immature neurons was affected, using stereological estimation of the number of 3R tau (Fig. 5) and DCX (Fig. 6) positive cells in the subgranular and granular zone of the dentate gyrus in WT, Tg30 and Tg30/tauKO mice. 3R tau positive cells were observed in WT and Tg30 mice (Fig. 5A–D) but their number was significantly decreased in Tg30 mice compared to wild-type mice (Fig. 5E). As expected, 3R-tau was not detected in Tg30/tauKO mice. Expression of DCX is considered as a proxy marker of adult neurogenesis and DCX is highly expressed at P0 and easily detected by western blotting in brain homogenates but this expression strongly decreases in the adult brain (Supplement Fig. 1B). By immunohistochemistry, DCX positive cells were detected in the granular layer of the dentate gyrus in adult wild-type, Tg30 and Tg30/tauKO mice (Fig. 6A–F) but the number of DCX-positive cells was significantly decreased in Tg30 mice compared to wild-type and Tg30/tauKO mice (Fig. 6G). Altogether, these results indicated that the pool of neuronal precursors and immature neurons was reduced in Tg30 mice, and that absence of endogenous wild-type mouse tau increased the number of DCX positive cells in Tg30/tauKO mice.

3.6. The number of Ki-67 positive cells is decreased in Tg30 but not in Tg30/tauKO mice

To investigate mitotic activity in the dentate gyrus, we performed a stereological estimation of the number of Ki67 positive cells (identifying cells in all phases of the cell cycle, except G0) (Scholzen and Gerdes, 2000) in WT, Tg30 and Tg30/tauKO mice (Fig. 7). Positive cells with a nuclear KI-67 immunoreactivity were present in all genotypes (Fig. 7A–C). Compared to wild-type mice, the number of Ki67 positive cells was significantly reduced in Tg30 but was not significantly reduced in Tg30/tauKO mice. A trend (non-significant) for a higher number of Ki-67 positive cells in Tg30/tauKO mice compared to Tg30 mice was noted.

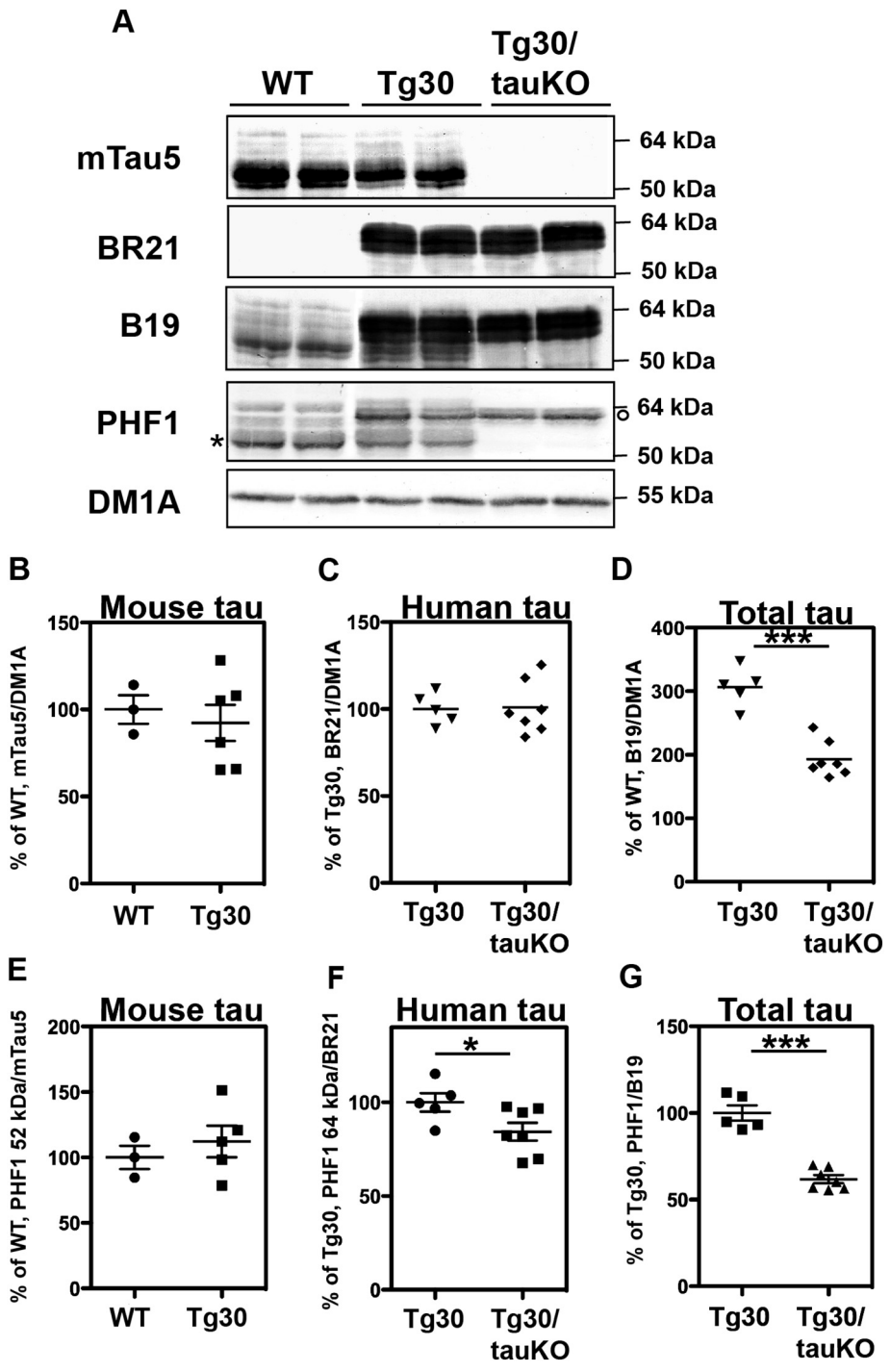

Fig. 2. The level of expression of human tau is similar in Tg30 and Tg30/tauKO mice but phosphotau level is reduced in Tg30/tauKO mice.
A: Western blot analysis of RIPA-soluble fractions of wild-type, Tg30 and Tg30/tauKO mouse brains at 12 months. Western blots were analyzed using the mTau5 antibody detecting only murine tau, the BR21 antibody specific for human tau, the B19 antibody detecting total tau (mouse and human). Hyperphosphorylated tau was detected using PHF1 antibody. α-tubulin was detected by DM1A antibody to control for protein loading.
B: The level of murine tau detected by mTau5 was not significantly different between wild-type and Tg30. For normalization, 100% was given to murine tau level in wild-type mouse brains.
C: The level of human tau detected by BR21 antibody was not significantly different in Tg30 versus Tg30/tauKO mouse brains.
D: The level of total tau was significantly decreased in Tg30/tauKO mouse brains compared to Tg30 brains.
E: The level of PHF1 positive murine tau (lower bands detected around 55 kDa, shown with an asterisk in A) normalized to mTau5 positive murine tau was not significantly different between wild-type and Tg30 brains.
F: The level of PHF1 positive human tau (lower bands detected around 64 kDa, shown with open circle in A) normalized to human tau was significantly different between Tg30 and Tg30/tauKO.
G: The level of total phosphorylated tau detected by PHF1 (55 to 64 kDa) was normalized to total tau. There was a significant reduction of hyperphosphorylated tau in Tg30/tauKO mouse brains. 100% was given to Tg30.
*: $p < 0.05$, ***: $p < .001$, by Student unpaired t-test. (WT n = 3, Tg30: n = 5; Tg30/tauKO: n = 7).

3.7. Absence of enhanced cell death in the dentate gyrus in Tg30 mice

Since decreased number of granule cells in Tg30 mice might also result from enhanced programmed cell death, we immunolabeled tissue sections with antibodies to activated caspase-3. Cells positive for activated caspase-3 were not detected in the dentate gyrus in Tg30 mice.

3.8. Absence of changes in density of GFAP positive cells in the DG in Tg30 and Tg30/tauKO mice

To assess if reduction in granule cell number and in neuronal precursors in Tg30 mice might be associated to changes in glial cells populations in the dentate gyrus, we analyzed this area after immunolabelling with anti-GFAP and anti-CD11b antibodies. The density of GFAP positive cells in the dentate gyrus was not different in WT, Tg30 and Tg30/tauKO mice (Supplementary Fig. 3). Activated CD11b positive microglial cells were rarely observed in the dentate gyrus and this was not different between the 3 different genotypes.

3.9. Human mutant tau is not expressed in SOX2, DCX and 3R tau-positive precursors

To investigate if decrease in number of neuronal precursors and immature neurons in Tg30 mice might be associated to expression of human mutant tau in these cells, we first performed a double immunofluorescent labelling with the anti-human tau and anti-DCX

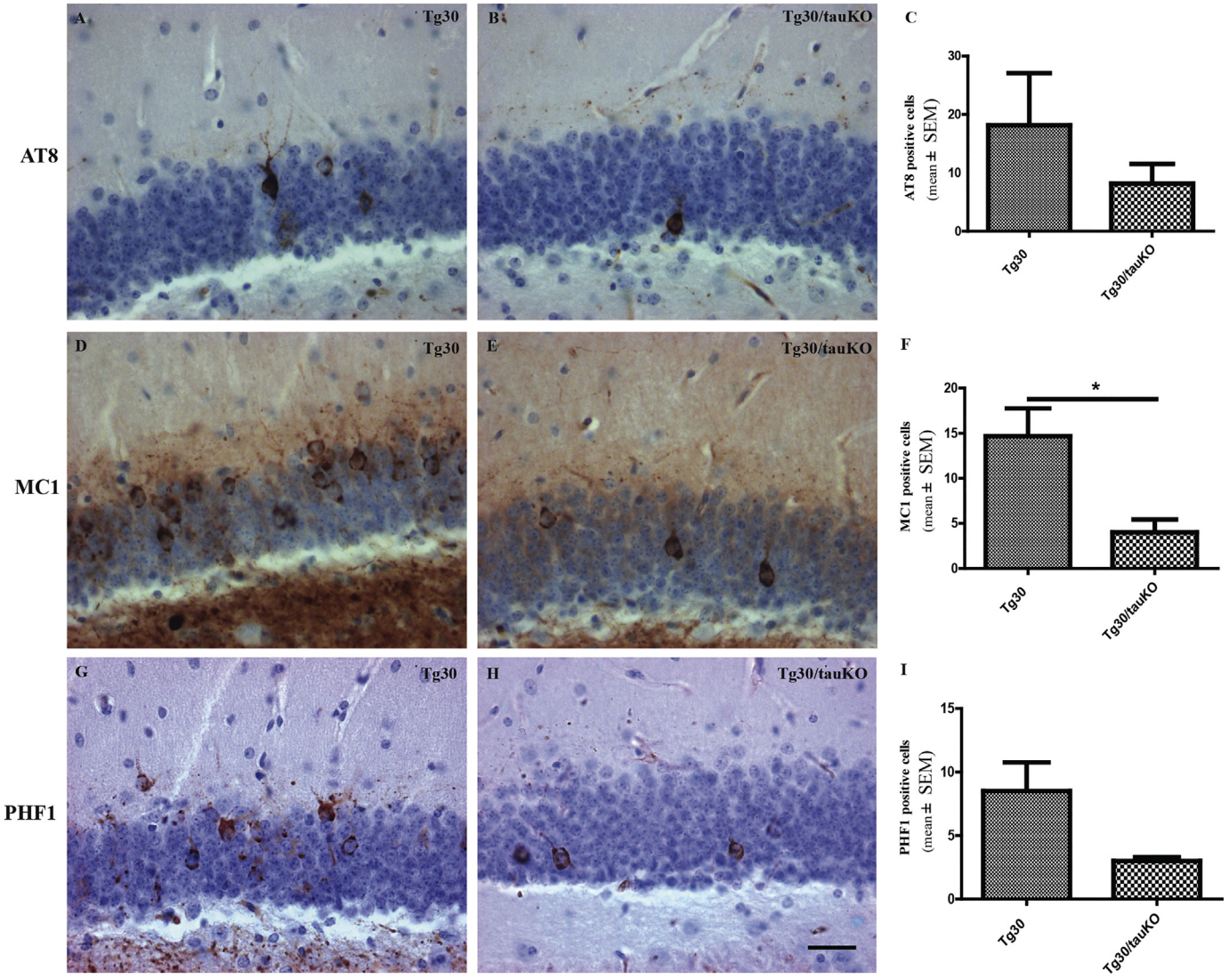

Fig. 3. Tau pathology in the dentate gyrus is higher in Tg30 than in Tg30/tauKO mice.
Distribution of phosphotau positive cells in the dentate gyrus of 12-month-old Tg30 (A, D, G) and Tg30/tauKO (B, E, H) mice.
A–C: Immunolabelling with the AT8 tau antibody in Tg30 (A) and Tg30/tauKO mice (B), and density estimation of AT8 positive cells (C).
D–F: Immunolabelling with the MC1 tau antibody in Tg30 (D) and Tg30/tauKO (E) mice and density estimation of MC1 positive cells (F).
G–I: Immunolabelling with the PHF1 tau antibody in Tg30 (G) and Tg30/tauKO (H) mice and density estimation of PHF1 positive cells (I).
Phosphorylated tau (AT8, PHF1) and conformationally abnormal tau (MC1) accumulated in the somatodendritic domain of some granule cells in both Tg30 and Tg30/tauKO mice, but their density is higher in Tg30 than in Tg30/tauKO mice.
Haematoxylin counterstaining. *: $p < .05$, by Student unpaired t-test (Tg30: n = 3; Tg30/tauKO: n = 3).
Scale bar: 25 μm.

antibodies (Fig. 8D–F). As described above (Fig. 1), human tau was expressed in the whole granule cell layer (Fig. 8D) but was not expressed in DCX positive cells (Fig. 8E and F). Double immunolabelling with the anti-human tau and anti-3R tau antibodies also showed absence of expression of human tau in 3R tau positive cells. We finally investigated the expression of human tau in neural stem cells by double immunofluorescence with the anti-human tau and the anti-Sox2 antibodies (Fig. 8A–C). Human tau (Fig. 8A) was not expressed in Sox2 positive cells (Fig. 8B and C).

4. Discussion

Using unbiased stereological methods, we demonstrate in this study a decrease of total granule cell number in the DG and a defect of adult hippocampal neurogenesis in the Tg30 transgenic tauopathy mouse model expressing a human FTDP-17 mutant tau, and a rescue of this neurogenesis defect after deletion of endogenous wild-type tau in the same model (Tg30/tauKO mice). Adult neurogenesis in the DG includes proliferation, survival or death, and differentiation of neuronal progenitors into mature granule cells. During adult neurogenesis, neuronal precursors express transiently the 0N3R foetal tau isoform (Bullmann et al., 2007; Llorens-Martin et al., 2012; McMillan et al., 2008). This 0N3R tau isoform and the immature marker doublecortin (DCX) are co-expressed in the same population of cells in the SGZ. Our results suggest that a reduction of the pool of these neuronal precursors positive for 3R-tau and DCX and giving rise to new granule cells is responsible for a decrease of adult neurogenesis in the DG of Tg30 mice. This reduction in the pool of neuronal progenitors in Tg30 mice was associated to a reduction of the pool of proliferating cells in the SGZ, as assessed by Ki-67 labelling. This suggests that the reduction of adult neurogenesis in Tg30 mice results at least in part from a reduction of proliferation of precursor cells.

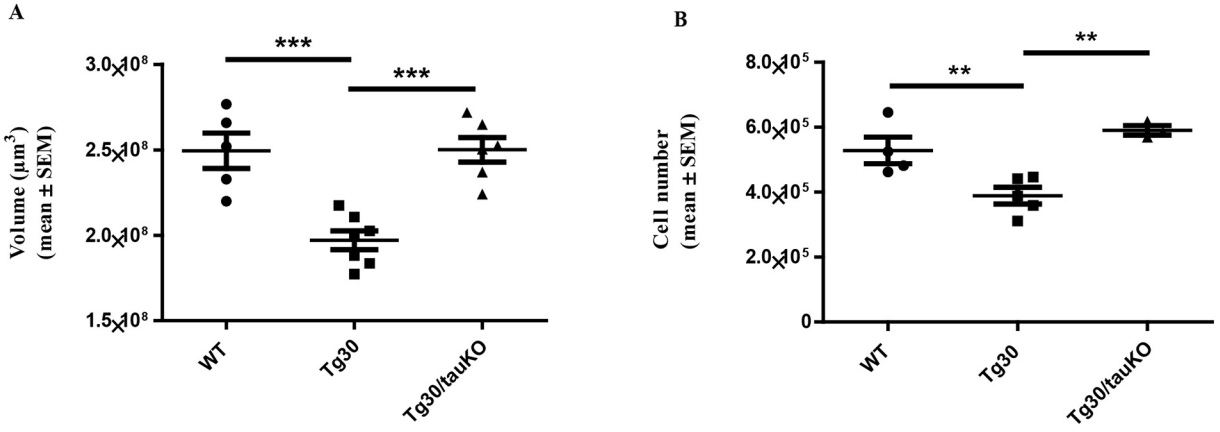

Fig. 4. Tg30 mice, but not Tg30/tauKO mice, have a reduced DG volume and a reduction of granule cell number in the DG.
A: Volume estimation of the dentate gyrus with the Cavalieri method in wild-type (WT), Tg30 and Tg30/tauKO 12-month-old mice. This volume is significantly decreased in Tg30 mice compared to other genotypes and is not affected in Tg30/tauKO mice (WT: n = 5; Tg30: n = 8 Tg30/tauKO: n = 6).
B: Stereological estimation of total granule cell number in 12-month-old WT, Tg30 and Tg30/tauKO mice in the whole DG. Tg30 mice, but not Tg30/tauKO mice, have a lower granule cell number compared to WT mice (WT: n = 4; Tg30: n = 5; Tg30/tauKO: n = 3).
** $p < .01$; *** $p < .001$ by one-way ANOVA and Newman-Keuls post-tests.

A potential toxicity associated to the expression of this mutant tau in neural stem cells and neuronal precursors could not be directly responsible for their decreased number since we could not demonstrate the expression of human mutant tau in Sox2, 3R tau or in DCX-positive cells. Expression of mutant tau in Tg30 mice is under the control of the Thy-1.2 promoter, that drives expression in the brain (Vidal et al., 1990) under tight developmental control. The expression appears during early postnatal development and represents a terminal differentiation marker in the nervous system (Morris, 1985). In mice expressing yellow fluorescent protein (YFP) under the direction of a Thy-1 promoter, YFP expression was detected only in mature neurons, and YFP was not expressed in undifferentiated neurospheres containing neural stem cells (Corti et al., 2006), supporting our observation of absence of mutant tau expression under control of Thy-1 promoter in neural stem cells. Altogether, this suggest that the depletion of neuronal precursors in Tg30 mice is a cell non-autonomous pathological process, not directly depending of the expression of the human mutant tau in these cells. Finally, the reduction of granule cell number in Tg30 did not result from an increased apoptosis that was not observed, in agreement with a previous study in THY-tau 22 mice expressing the same human mutant tau also in the hippocampus (Schindowski et al., 2008). We hypothesize that modifications of the microenvironment of the SGZ in Tg30 mice rather play a role in this depletion of neuronal precursors. The microenvironment of SGZ constitutes a neurogenic niche with specific molecular and cellular factors permissive for and modulators of adult neurogenesis (Horgusluoglu et al., 2017). Glial cells, mature neurons, the vasculature and neuronal connections in the neurogenic niche of the SGZ play a role in the differentiation of neural progenitor cells. Neuroinflammation and proinflammatory cytokines affects adversely hippocampal neurogenesis, but we did not detect significant change in activated microglial cells density in the DG, that was similar in WT, Tg30 and Tg30/tauKO mice. Wnt signalling regulates hippocampal adult neurogenesis and Wnt proteins are produced by hippocampal astrocytes (Lie et al., 2005). The density of GFAP positive cells was not different between WT, Tg30 and Tg30/tauKO mice suggesting that the global contribution of astroglial cells to the microenvironment of the neurogenic niche is similar in these genotypes but variations in subpopulations of astroglial cells between these genotypes are not excluded since GFAP positive cells comprise stem cells, immature astrocytes and mature astrocytes (Gotz et al., 2015). Highly aggregated tau (as detected by Gallyas-silver staining) was not significantly detected in granule cells, suggesting that formation of highly aggregated tau species cannot account for decreased adult neurogenesis in Tg30 mice. Mature granule cells in the DG of Tg30 mice however widely expressed the human mutant tau protein, and some of them also had high content of phosphorylated tau and conformationally altered tau, whereas levels of murine PHF1 positive tau was not different between WT and Tg30 mice. Expression of this pathological human tau species, although not in neuronal precursors, might affect pathways regulating final steps of adult neurogenesis in the neurogenic niche of the SGZ: e.g. it is conceivable that extracellular release of abnormal tau from mature granule cells (Yamada et al., 2014) and its diffusion into the neurogenic niche in the SGZ could affect this microenvironment resulting in decreased adult neurogenesis.

A reduction of adult neurogenesis has been reported in the genomic-based htau mouse model, expressing wild-type human tau, and suggested to be due to a cell-autonomous mechanism (Komuro et al., 2015). Since transgenic tau was expressed in neuronal precursors in this htau model, and not in the Tg30 model that we investigated, mechanisms of reduced adult neurogenesis might be different in the two models. A decrease of the volume, a reduction of granule cell number and a reduction of neuronal precursors in the ventral DG was also reported in a previous study of a mutant 2N4R tau (G272 V, P301L, R406W) mouse model (Llorens-Martin et al., 2011). The study of adult neurogenesis in APP or PS1 transgenic mice has yielded conflicting results (Lazarov et al., 2010) although ablation of neurogenesis in APP/PS1 mice induces cognitive deficits (Hollands et al., 2017). Impairment of adult neurogenesis in the DG has been reported in the 3xTg-AD mouse model (Rodriguez et al., 2008), but the simultaneous expression of mutants APP, PS1 and tau in this model makes it complex to analyze the relative contribution of each transgenic protein on impairment of adult neurogenesis.

The Tg30/tauKO mouse represents a model expressing only human mutant 4R tau in postnatal neurons in a murine tau-null background. Contrary to Tg30 mice, Tg30/tauKO mice did not show a decrease in volume of the DG and in the granule cell number. They also exhibited an increased number of DCX positive precursors in the SGZ. These data imply that absence of endogenous tau favoured proliferation and/or survival of neuronal precursors. In a previous study in a tauKOKI mouse strain expressing a wild-type 2N4R human tau in a murine tau-null background, an increase of hippocampal volume and of neuronal number and of DCX positive cells in the DG, compared to non-transgenic, was also observed (Sennvik et al., 2007). The level of expression of human mutant tau was similar in Tg30 and Tg30/tauKO mice (Ando et al., 2011), excluding difference in human mutant tau expression as responsible for the observed differential effects on adult neurogenesis

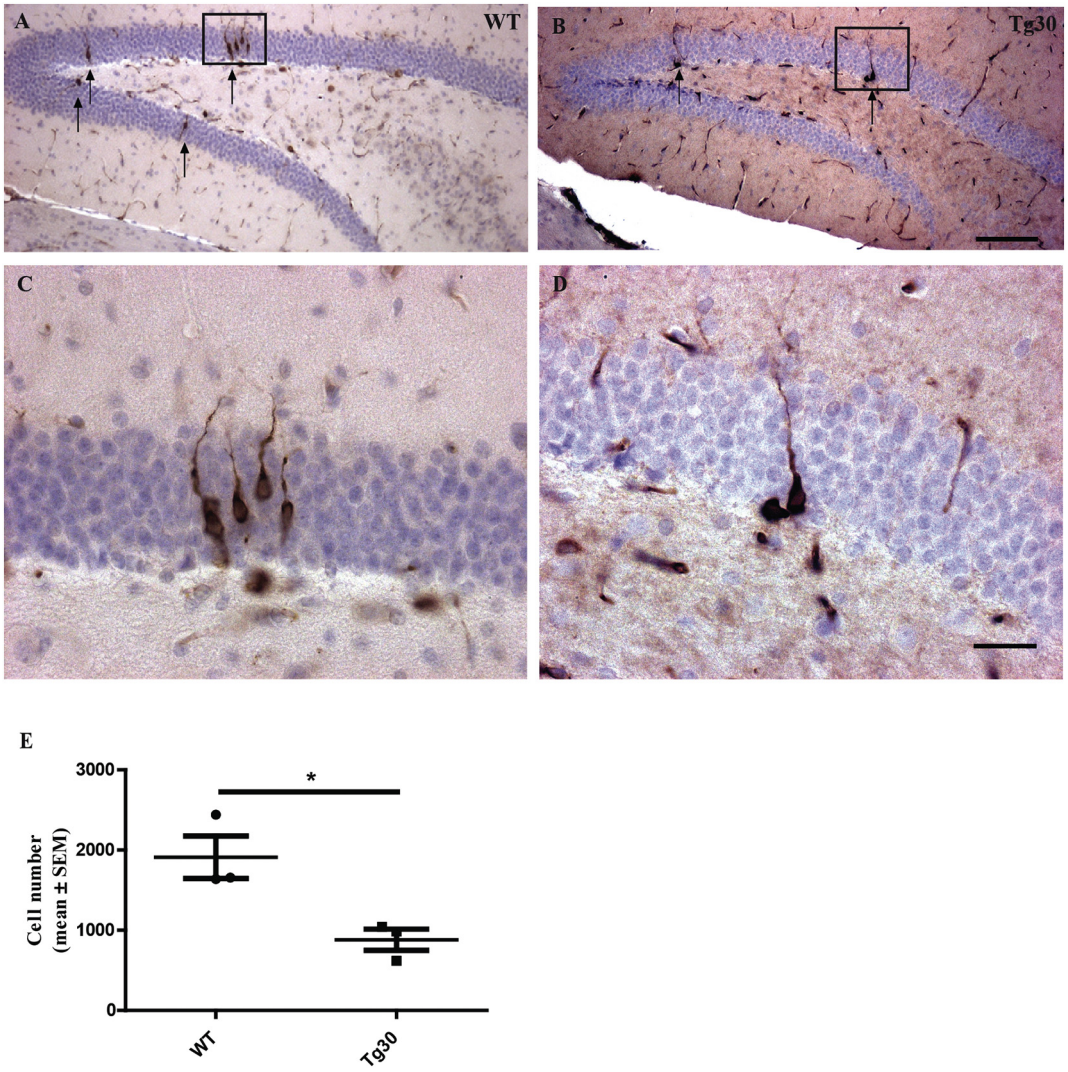

Fig. 5. Tg30 mice have a reduced number of 3R tau-positive neuronal precursors in the DG.
A–D: Distribution of 3R tau-positive cells in the dentate gyrus in WT (A) and in Tg30 (B) mice. 3R tau-positive cells in the insets in (A) and (B) are shown at higher magnification in (C) and (D), respectively.
E: Stereological determination of the total number of 3R tau-positive cells in the granule cell layer in the whole DG. 3R tau-positive cells are less numerous in Tg30 compared to WT mice. Haematoxylin counterstaining. * $p < .05$, by Student unpaired t-test. (WT: n = 3; Tg30: n = 3).
Scale bars: A, B: 100 µm; C, D: 25 µm.

between these two lines. The level of soluble human phosphotau was lower in Tg30/tauKO mice compared to Tg30 mice and the density of hyperphosphorylated and conformationally altered tau positive cells in the granular layer was decreased in Tg30/tauKO mice. These results suggest that reduction of human phosphotau in Tg30/tauKO mice might be responsible for the rescuing effect on adult neurogenesis. An intriguing difference between Tg30 and Tg30/tauKO mice is that expression of wild-type, endogenous 3R tau in neuronal precursors in Tg30 mice appears necessary to observe the neurogenic defect when mutant tau is expressed in more mature granule cells. We hypothesize that 3R tau might also be a key effector of a pathological signalling involving abnormal human phosphotau expressed, and possibly secreted, by mature granule cells in these models. Stressful conditions suppress adult hippocampal neurogenesis (Leuner and Gould, 2010) and Tg30 mice develop with aging a severe motor phenotype and a drastic muscle atrophy (Leroy et al., 2007) that can be considered as a disease state involving a chronic stress, a condition that might thus play also a role in the defect in adult neurogenesis observed in Tg30 mice. Interestingly, absence of tau expression in tauKO mice was found to be protective against adult hippocampal neurogenesis deficits in animals submitted to stressful conditions (Dioli et al., 2017; Pallas-Bazarra et al., 2016). The partial rescue of adult hippocampal neurogenesis in Tg30/tauKO mice, that also develop a motor deficit (Ando et al., 2011) might thus also be related to this neuroprotective effect of absence of tau expression against chronic stress. The Wnt/ß-catenin pathway regulates hippocampal neurogenesis by supporting proliferation of neural progenitor cells, by inactivation of Glycogen synthase kinase 3 (GSK-3) and stabilization of ß-catenin (Shimizu et al., 2008). Active GSK-3ß (phosphorylated at Tyr216) is associated to tau lesions in Tg30 mice (Leroy et al., 2007) and GSK-3ß inhibition arrested the formation of tau lesions in Tg30 mice (Leroy et al., 2010). Active GSK-3ß is increased in the DG of stressed WT mice, but not in the DG of stressed tauKO mice (Dioli et al., 2017), supporting a role for a differential activity of GSK-3ß between Tg30 and Tg30/tauKO mice leading to an improved neurogenesis in the latter. Phosphotau epitopes recognized by PHF1 and AT8 antibodies are GSK-3 dependent (Hanger et al., 2009) and the lower level of PHF1 positive phosphotau in Tg30/tauKO mice compared to Tg30 mice supports also this hypothesis.

Genetic reduction of endogenous tau in P301L mutant tau mice (Wegmann et al., 2015) and reduction of tau by anti-sense

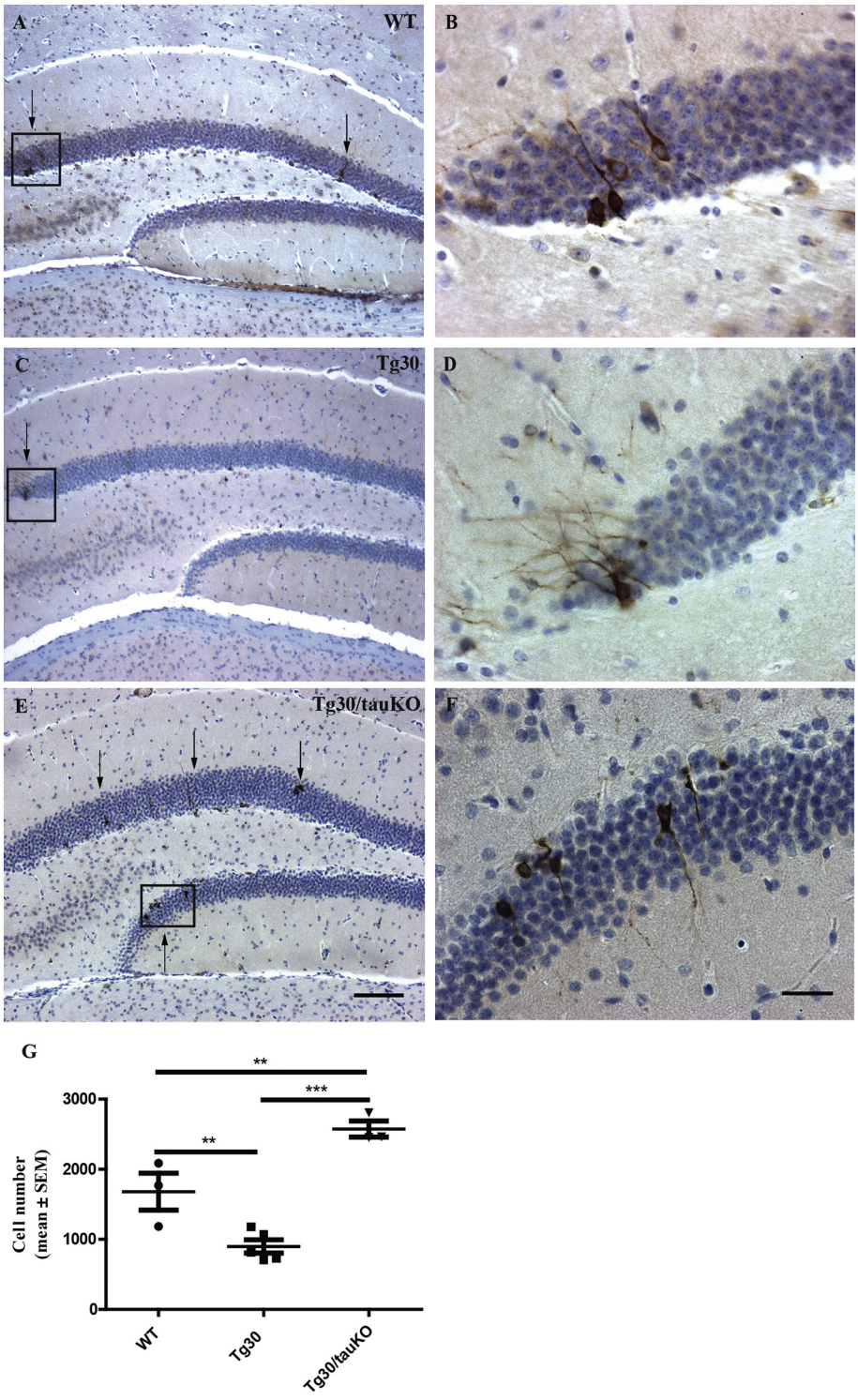

Fig. 6. Tg30 mice, but not Tg30/tauKO mice, have a reduced number of DCX-positive neuronal precursors in the DG.
A–F: Distribution of DCX-positive cells in the dentate gyrus in WT (A), Tg30 (C) and Tg30/tauKO (E) mice. DCX-positive cells in insets in (A, C, E) are shown at higher magnification in (B, D, F), respectively.
G: Stereological determination of the total number of DCX-positive cells in the granule cell layer in the whole DG. DCX-positive cells are less numerous in Tg30 compared to WT and Tg30/tauKO mice. Haematoxylin counterstaining. **p < .001; ***p < .001, by one-way ANOVA and Newman-Keuls post-test (WT: n = 3; Tg30: n = 5; Tg30/tauKO: n = 3).
Scale bars: A, C, E: 100 µm; B, D, F: 25 µm.

oligonucleotides in P301S mutant tau mice (DeVos et al., 2017) has previously been observed to reduce neurotoxicity, e.g. neuronal loss, in these models. Genetic reduction of tau also improved a deficient phenotype in mice expressing mutant APP (Roberson et al., 2007) or mutants APP and PS1 (Leroy et al., 2012) and developing amyloid deposits. Among others, deletion of tau prevented against postsynaptic Aß toxicity (Ittner et al., 2010), Aß-induced axonal transport defect (Vossel et al., 2010) and protected against seizures (Devos et al., 2013), neuroinflammation (Maphis et al., 2015), traumatic brain injury (Cheng et al., 2014) and stroke-associated excitotoxicity (Bi et al., 2017). Our results further support a protective role of tau reduction for improving adult neurogenesis defects and strengthens the interest of tau-lowering therapy in tauopathies.

5. Conclusions

Altogether, our results indicate that postnatal expression of a human FTDP-17 mutant tau in mature granule cells of the DG decreases adult hippocampal neurogenesis and leads to a reduction of adult granule cell pool and DG atrophy. Absence of expression of endogenous wild-type tau in mature granule cells in the same model rescues this decreased adult hippocampal neurogenesis. These in vivo models will be useful for

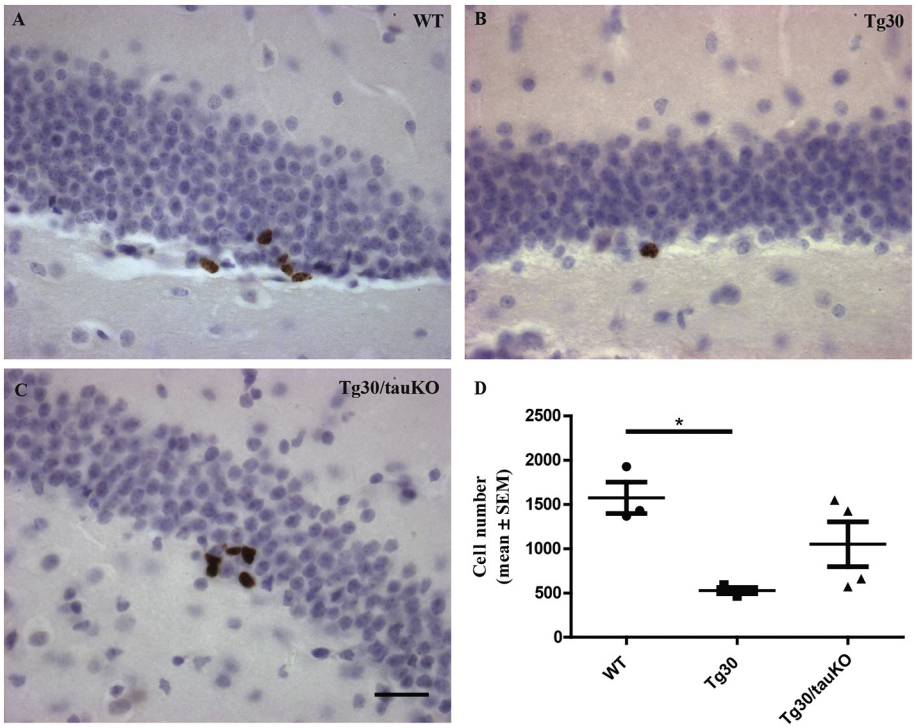

Fig. 7. Tg30 mice, but not Tg30/tauKO mice, have a reduced number of Ki67-positive dividing cells in the DG.
A–C: Distribution of Ki67-positive cells in the dentate gyrus in WT (A), Tg30 (B) and Tg30/tauKO (C) mice. D: Stereological determination of the total number of Ki67-positive cells in the granule cell layer in the whole DG. The number of Ki67-positive cells is significantly reduced in Tg30 compared to WT mice but is not reduced in Tg30/tauKO compared to WT mice. * $p < .05$, by one-way ANOVA and Newman-Keuls post-tests (WT: n = 3; Tg30: n = 3; Tg30/tauKO: n = 4).
Scale bar: 25 μm.

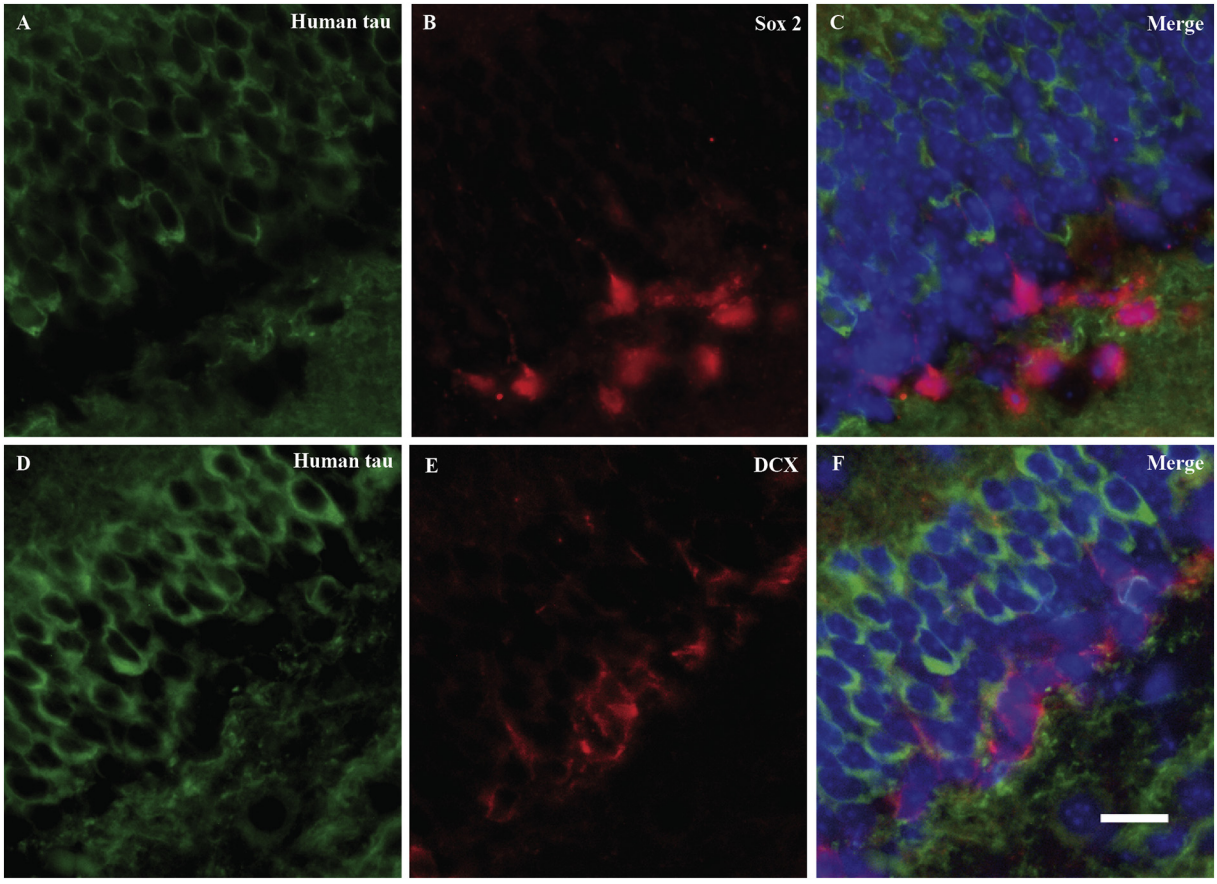

Fig. 8. Absence of expression of human mutant tau in neural stem cells and in neuronal precursors in DG in Tg30 mice.
A–C: Double immunofluorescent labelling with the anti-human tau antibody (A) and the anti- anti-SOX2 antibodies (B). The SOX-2 positive stem cells do not express the human transgenic tau (merging in C and DAPI counterstaining).
D–F Double immunofluorescent labeling with the anti-human tau antibody (D) and the anti-DCX antibodies (E). The DCX positive neuronal precursors do not express the human transgenic tau (merging in F and DAPI counterstaining).
Scale bar: 25 μm.

the understanding of impairment of adult neurogenesis in human tauopathies and for investigating the interest of reducing expression of tau to rescue this impairment.

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