Pharmacological Research 105 (2016) 186–197

Contents lists available at ScienceDirect

Pharmacological Research

journal homepage: www.elsevier.com/locate/yphrs

Evidence for an imbalance between tau *O*-GlcNAcylation and phosphorylation in the hippocampus of a mouse model of Alzheimer's disease

Eleonora Gatta[a,h], Tony Lefebvre[a], Silvana Gaetani[b], Marc Dos Santos[c], Jordan Marrocco[d,h,2], Anne-Marie Mir[a], Tommaso Cassano[e,*], Stefania Maccari[a,h], Ferdinando Nicoletti[b,f,1], Jérôme Mairesse[g,1,3]

[a] *Univ. Lille, CNRS, UMR 8576, UGSF, Unité de Glycobiologie Structurale et Fonctionnelle, 59000 Lille, France*
[b] *Dept. of Physiology and Pharmacology, Sapienza University of Rome, 00185 Rome, Italy*
[c] *INSERM, UMR-S 1130, Neuroscience Paris Seine, 75005 Paris, France*
[d] *Laboratory of Neuroendocrinology, The Rockefeller University, 10065 New York, NY, USA*
[e] *Dept. of Clinical and Experimental Medicine, University of Foggia, 71100 Foggia, Italy*
[f] *IRCCS Neuromed, 86077 Pozzilli, Italy*
[g] *Robert Debré Children Hospital INSERM U1141, 75019, France*
[h] *LIA France/Italy, International Associated Laboratory "Prenatal Stress and Neurodegenerative Diseases", Glycobiology of Stress-Related Diseases Team, UMR 8576, UGSF, Unité de Glycobiologie Structurale et Fonctionnelle, 59000 Lille, France; Neuromed, 86077 Pozzilli, Italy; Sapienza University of Rome, 00185 Rome, Italy*

ARTICLE INFO

Article history:
Received 7 April 2015
Received in revised form 6 January 2016
Accepted 6 January 2016
Available online 24 January 2016

Keywords:
O-GlcNAcylation
3xTg-AD mice
tau protein
hippocampus

ABSTRACT

Intracellular accumulation of hyperphosphorylated tau protein is linked to neuronal degeneration in Alzheimer's disease (AD). Mounting evidence suggests that tau phosphorylation and *O*-*N*-acetylglucosamine glycosylation (*O*-GlcNAcylation) are mutually exclusive post-translational modifications. *O*-GlcNAcylation depends on 3–5% of intracellular glucose that enters the hexosamine biosynthetic pathway. To our knowledge, the existence of an imbalance between tau phosphorylation and *O*-GlcNAcylation has not been reported in animal models of AD, as yet. Here, we used triple transgenic (3xTg-AD) mice at 12 months, an age at which hyperphosphorylated tau is already detected and associated with cognitive decline. In these mice, we showed that tau was hyperphosphorylated on both Ser396 and Thr205 in the hippocampus, and to a lower extent and exclusively on Thr205 in the frontal cortex. Tau *O*-GlcNAcylation, assessed in tau immunoprecipitates, was substantially reduced in the hippocampus of 3xTg-AD mice, with no changes in the frontal cortex or in the cerebellum. No changes in the expression of the three major enzymes involved in *O*-GlcNAcylation, i.e., glutamine fructose-6-phosphate amidotransferase, *O*-linked β-*N*-acetylglucosamine transferase, and *O*-GlcNAc hydrolase were found in the hippocampus of 3xTg-AD mice. These data demonstrate that an imbalance between tau phosphorylation and *O*-GlcNAcylation exists in AD mice, and strengthens the hypothesis that *O*-GlcNAcylation might be targeted by disease modifying drugs in AD.

* Corresponding author at: Department of Clinical and Experimental Medicine, University of Foggia, Viale Luigi Pinto 1, Foggia 71100, Italy. Fax: +39 0881 188 0432.
E-mail addresses: eleogatta@gmail.com (E. Gatta), tony.lefebvre@univ-lille1.fr (T. Lefebvre), silvana.gaetani@uniroma1.it (S. Gaetani), marc.dos_santos@etu.upmc.fr (M. Dos Santos), jmarrocco@mail.rockefeller.edu (J. Marrocco), tommaso.cassano@unifg.it, tommaso.cassano@gmail.com (T. Cassano), stefania.maccari@univ-lille1.fr (S. Maccari), ferdinandonicoletti@hotmail.com (F. Nicoletti), j_mairesse@hotmail.com (J. Mairesse).
[1] Co-last authors.
[2] Present address: Harold and Margaret Milliken Hatch Laboratory of Neuroendocrinology, The Rockefeller University, 1230 York Avenue, New York 10065, New York.
[3] Present address: Robert Debré Children Hospital INSERM U1141, 48 Boulevard Sérurier, 75019 Paris, France.

http://dx.doi.org/10.1016/j.phrs.2016.01.006
1043-6618/

1. Introduction

An impairment of brain glucose uptake is invariably associated with Alzheimer's disease (AD) and precedes by several decades the clinical onset of AD [46,41,10,38]. Because the defect in glucose uptake likely reflects a condition of central insulin/insulin-like growth factor resistance [47,17], AD has been considered as a localized form of diabetes ("type-3 diabetes") (reviewed by Ref. [11]. The defect in glucose consumption can make neurons vulnerable to damage via a number of mechanisms that include a general impairment of energetic metabolism and a defective hexosamine biosynthetic pathway (HBP)-dependent glycosylation. Three to five per cent of the glucose that enters the cell is utilized for HBP-dependent glycosylation. Glucose is converted into UDP-*N*-acetylglucosamine (UDP-GlcNAc), which acts as a GlcNAc donor in *O*-*N*-acetylglucosaminylation (*O*-GlcNAcylation) reactions. The enzyme *O*-linked β-*N*-acetylglucosamine transferase (OGT) transfers GlcNAc residues to protein substrates in all cellular compartments, whereas *O*-GlcNAc hydrolase (OGA) catalyzes the removal of the sugar (reviewed by [22].

Similarly to other cytoplasmic proteins [22,21], tau undergoes *O*-GlcNAcylation at specific serine (Ser) and threonine (Thr) residues that can also be phosphorylated, including Ser396 and Thr205 [51,48,24]. Tau *O*-GlcNAcylation and phosphorylation are mutually exclusive, suggesting that an impaired *O*-GlcNAcylation might cause tau hyperphosphorylation and aggregation [1,27,24,25,23,54,26]. If so, a reduction in tau *O*-GlcNAcylation might contribute to the pathophysiology of AD because hyperphosphorylated tau gives rise to neurofibrillary tangles, which are found in the AD brain and lie at the core of neurodegenerative processes associated with AD (reviewed by [43]). A recent study also suggests that changes in *O*-GlcNAcylation levels may also influence β-amyloid pathology, which is a second hallmark of the AD brain, in the presence of tau pathology [55]. Besides its putative relevance in the pathophysiology of AD, recent findings demonstrate the crucial involvement of *O*-GlcNAcylation in the modulation of synapses activity [49,12,50], especially in the hippocampus, a crucial structure for memory processes. This lays the groundwork for a link between hippocampus metabolic status, synaptic plasticity and cognitive performances through multiple proteins *O*-GlcNAcylation.

To our knowledge, the balance between tau *O*-GlcNAcylation and phosphorylation has never been studied in the brain of mice modeling AD. Here, we used the model of triple transgenic mice (3xTg-AD) developed by LaFerla and colleagues. This model harbors three mutant genes: presenilin-1 ($PS1_{M146V}$), Amyloid Precursor Protein (APP_{Swe}), and tau_{P301L} transgenes [36], and recapitulates all major hallmarks of AD, with an age-dependent central increase of both amyloid deposition and intracellular neurofibrillary tangles [37] associated with progressive cognitive decline. Cognitive decline is characterized by a progressive impairment of hippocampus-related spatial memory, which begins at 6–8 months of age [5,34] and is fully established at 12–14 months of age [8,9,16,2,14]. 3xTg-AD mice also show anxiety- and depressive-like behaviors, which are less age-dependent [5,9,34,14,42]. A particular feature that makes 3xTg-AD mice a valuable model for the study of *O*-GlcNAcylation is that these mice develop an age-related reduction in brain glucose uptake [35]. We now report that 12 month-old 3xTg-AD mice show a reduced tau *O*-GlcNAcylation associated with tau hyperphosphorylation in the hippocampus. Reduction of tau *O*-GlcNAcylation was region-specific and was not associated with changes in the expression and activity of enzymes that regulate protein *O*-GlcNAcylation.

2. Materials and methods

2.1. Animals

We used 12 month-old 3xTg-AD male mice harboring presenilin 1 ($PS1_{M146V}$), amyloid-precursor protein (APP_{Swe}), and tau_{P301L} mutations [36]. Age- and gender-matched *wild-type* (WT) animals were used as control. Animals were bread at the vivarium of the Puglia and Basilicata Experimental Zooprophylactic Institute (Foggia, Italy). Genotypes were confirmed by polymerase chain reaction (PCR) after tail biopsies [36]. Animals were housed at 22 °C with a 12 h light/dark cycle and food and water ad libitum.

2.2. Western blot analysis

Mice were killed by cervical dislocation. The hippocampus, frontal cortex and cerebellum were rapidly dissected and immediately stored at −80 °C. Tissues were homogenized at 4 °C in a lysis buffer containing 320 mM sucrose, 5 mM HEPES, pH 7.4, 500 mM NaF, 10% SDS, 80 mM streptozotocine, and phosphatase and protease inhibitors. The bicinchoninic acid (BCA) assay was used for the determination of protein concentrations. Homogenized tissues were resuspended in Laemmli reducing buffer, and 20 μg of proteins were separated by electrophoresis on Criterion TGX 4–15% precast SDS-polyacrylamide gels (BioRad), and transferred to nitrocellulose membranes (Bio-Rad). Transfer was performed at 4 °C in a buffer containing 35 mM Tris, 192 mM glycine, and 20% methanol.

We used the following primary antibodies: rabbit polyclonal anti-p-tauSer396 (1:5000; Santa Cruz Biotechnology, #sc-101815), rabbit polyclonal anti-p-tauThr205 (1:1000; Santa Cruz Biotechnology, #sc-101817), rabbit polyclonal anti-pan-tau (1:2000; Santa Cruz Biotechnology catalog #sc-5587), rabbit polyclonal anti-GFAT (1:300; Santa Cruz #sc-134894); mouse monoclonal anti-*O*-GlcNAc (1:2000, RL2; ThermoFisher Scientific; #MA1-072), rabbit polyclonal anti-OGT AL35 (1:2000; generously provided by G. W. Hart, Johns Hopkins University, Baltimore, MD, USA), chicken anti-OGA 345 (1:2000; generously provided by G. W. Hart) and mouse monoclonal anti-β-actin (dilution 1:80.000, Sigma–Aldrich). All primary antibodies were incubated overnight at 4 °C. Horseradish peroxidase (HRP)-conjugated secondary anti-mouse or anti-rabbit antibodies (purchased from GE Healthcare) were used at a dilution from 1:10000 to 1:20000 and were incubated for 1 h at room temperature. Enhancing chemiluminescence detection system (Pierce ECL, Thermo Scientific) was used for detection on autoradiography films (GE Healthcare). Signals obtained for tau, p-tau and β-Actin levels were quantified with GS-800 scanner (BioRad) associated to Quantity One software (BioRad).

Densitometric lane spetra profiles obtained for total *O*-GlcNAcylated proteins were generated by measuring the optical density (O.D.) of each line of pixel of a lane using a custom-built macro in ImageJ software.

2.3. Immunoprecipitation

Tissue was lysed in RIPA buffer (50 mM Tris–HCl, pH 8, 150 mM NaCl, 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS, 2 mM EDTA) containing phosphatase inhibitors, protease inhibitors and streptozotocin (80 mM). Protein concentrations were determined by the BCA assay. In order to avoid non specific binding, protein A sepharose beads (GE Healthcare) were first washed with RIPA buffer. After centrifugation, supernatant was discarded and protein A sepharose beads were incubated with protein extracts (0.5 mg per sample) for 2 h at 4 °C for pre-clearing. After centrifugation (4000 × *g*), extracts were incubated overnight with the pan-tau antibody (2 μg). Antibody-antigen complexes were incubated for 2 h with 30 μL of protein A beads before centrifugation (10 000 × *g*).

Samples were then resuspended in Laemmli reducing buffer before SDS-PAGE (see Western Blot Analysis section). The secondary antibody used for the detection of pan-tau and p-tau was an anti-rabbit True Blot® immunoglobulin (eBioscience), which limits the interference with the immunoprecipitating IgG heavy and light chains. The secondary antibody used for the detection of *O*-GlcNAc was a HRP-conjugated anti-mouse antibody (dilution 1:10.000, GE Healthcare).

2.4. OGT and OGA activity assays

Hippocampi of 3xTg-AD and wt mice were homogenized in buffer consisting of 50 mM Tris–Hcl (pH 7.4), 2 mM EDTA, 10 mM β-mercaptoethanol, 8,5% sucrose, phosphatase and protease inhibitors (Sigma–Aldrich®). Resulting homogenates were first centrifuged at 16 000 × *g* at 4 °C for 10 min. 2 volumes of 30% polyethylene glycol 8000 (in 25 mM HEPES containing 10 mM $MgCl_2$, pH 7,23) was added to supernatants. Samples were subsenquently centrifuged at at 16 000 × *g* at 4 °C for 10 min. The pellets obtained were resuspended in OGT assay buffer (25 mM HEPES, pH 7.5, 10 mM $MgCl_2$ and 1 mM EDTA). Extracts were then incubated 2 h at 24 °C with the addition of 0.4 μCi of UDP-[^{3}H]GlcNAc in the OGT assay buffer. After labeling, proteins were precipitated with trichloro-acetic acid (TCA) at a final concentration of 20%, and then recovered on glass microfiber filters (GF/A, Whatman), and intensively washed with 0.1% TCA under vacuum. The precipitates were rinsed with absolute ethanol and then counted on the filters in a liquid scintillation counter (Beckman). Each experiment was performed in triplicate.

OGA activities were determined in hippocampi homogenates using the method described in [30]. Briefly, hippocampi homogenates (100 μL/assay) were incubated 30 min at 37 °C in a buffer containing 50 mM sodium cacodylate (pH6.4), 2 mM p-nitro-phenyl-*N*-acetyl-β-D-glucosaminide, 0.3% bovine serum albumin, 2 mM β-mercaptoethanol, 2 mM EDTA, phosphatase and protease inhibitors (Sigma–Aldrich®). Reactions were stopped with the addition of 0.9 mL of 0.5 M sodium carbonate, and absorbance was measured at 400 nm. Each experiment was performed in triplicate.

2.5. Statistics

Statistical differences between groups were determined with Student's *t*-test. Correlations were performed using the Pearson's correlation analysis. Significance was set to $p < 0.05$.

3. Results

3.1. Enhanced tau phosphorylation in 3xTg-AD mice

We measured the levels of tau protein and its phosphorylated forms at Ser396 or Thr205, in the hippocampus, frontal cortex and cerebellum of 3xTg-AD mice and their WT counterparts.

In the hippocampus, 3xTg-AD mice showed >2 fold increase in total tau (pan-tau) protein levels ($t_{32} = 9.02$; $p < 0.001$; Fig. 1A), and also significant increases in p-tauSer396 ($t_{32} = 2.14$; $p < 0.05$; Fig. 1B) and p-tauThr205 ($t_{32} = 7.68$; $p < 0.001$; Fig. 1C), if levels were normalized by β-actin (Fig. 1A–C). Phosphorylated tau levels in 3xTg-AD mice were significantly reduced if normalized by the amount of pan-tau (p-tauSer396/pan-tau: $t_{32} = -6.73$; $p < 0.001$; p-tauThr205/pan-tau: $t_{32} = -3.11$; $p < 0.05$; Fig. 1D, E) because of the substantial accumulation of tau protein in the hippocampus of these mice. We believe that normalization of phosphorylated tau with respect to β-actin levels is more reliable for two reasons: (i) pan-tau levels incorporate different forms of phosphorylated tau that may be increased in 3xTg-AD mice; and (ii) phosphorylation may reduce the turnover rate of tau, thus increasing tau levels in brain tissue.

In the frontal cortex and cerebellum, 3xTg-AD mice also show significant increases in pan-tau protein levels ($t_8 = 3.661$; $p < 0.01$ and $t_8 = 4.516$; $p < 0.01$, respectively). However, this increase was much smaller then that found in the hippocampus (43% and 44% increase in the frontal cortex and cerebellum, respectively; Fig. 2A and F). In the frontal cortex of 3xTg-AD mice p-tauThr205 levels were significantly increased if normalized by β-actin ($t_8 = 3.152$; $p < 0.05$; Fig. 2C), but not if normalized by pan-tau (Fig. 2E). No significant changes were found in p-tauSer396 (Fig. 2B and D). In the cerebellum of 3xTg-AD mice, no significant changes in p-tau levels were found, although a trend to an increase in p-tauSer396 and p-tauThr205 was seen if levels were normalized by β-actin ($t_8 = 2.283$; $p = 0.052$ and $t_8 = 2.266$; $p = 0.053$, respectively) (Fig. 2G, H).

3.2. Overall protein O-GlcNAcylation is decreased in the hippocampus and cerebellum of 3xTg-AD mice

Using an anti-*O*-GlcNAc antibody, we first measured levels of all *O*-GlcNAcylated proteins with molecular size over 55 kDa. The optical density (O.D.) of all detectable bands of the immunoblots was measured. 3xTg-AD mice showed a significant reduction in the overall protein *O*-GlcNAcylation in the hippocampus ($t_8 = 6.367$; $p < 0.001$) and cerebellum ($t_8 = 3.634$; $p < 0.01$), but not in the frontal cortex ($t_8 = -0.051$; $p = 0.960$) (Fig. 2A–C). In the molecular weight range corresponding to the size of tau protein (60–70 kDa), a reduced *O*-GlcNAcylation was exclusively found in the hippocampus of 3xTg-AD mice ($t_8 = 2.678$; $p < 0.05$; see Fig. 3A). Interestingly, there was no change in the expression of GFAT, OGT, and OGA in any brain region (Fig. 4A–C), indicating that the reduction of *O*-GlcNAcylation found in the hippocampus and cerebellum of 3xTg-AD mice was not due to an altered expression of the rate-limiting enzyme of the HBP, nor of the enzyme regulating *O*-GlcNAcylation.

3.3. Reduced tau O-GlcNAcylation in the hippocampus of 3xTg-AD mice

To specifically examine whether *O*-GlcNAcylation of tau protein was altered in 3xTg-AD mice, we performed immunoprecipitation experiments with an anti-tau polyclonal antibody. We adopted this strategy because no anti-*O*-GlcNAc-tau antibodies are currently available.

We performed two experiments using different mice. Mice of experiment 1 were used for measurements of phosphorylated and *O*-GlcNAcylated tau in tau immunoprecipitates from the hippocampus, frontal cortex, and cerebellum. Mice of experiment 2 were used for measurements of *O*-GlcNAcylated tau exclusively in hippocampal tau immunoprecipitates.

In hippocampal immunoprecipitates from 3xTg-AD mice of experiment 1, pan-tau, p-tauSer396 and p-tauThr205 levels were significantly increased ($t_8 = 3.410$; $p < 0.01$; $t_8 = 3.188$; $p < 0.05$ and $t_8 = 4.428$; $p < 0.01$, respectively; Fig. 5A–C), whereas *O*-GlcNAcylated tau levels were reduced by >45% ($t_8 = -3.139$; $p < 0.05$; Fig. 5 D). When *O*-GlcNAcylated tau levels of experiment 1 data were normalized by p-tauSer396 or p-tauThr205 levels, the reduction of tau *O*-GlcNAcylation was even more prominent (>65%; $t_8 = -2.797$; $p < 0.05$ and $t_8 = -4.56$; $p < 0.01$, respectively) (Fig. 5E and F). There was a highly significant inverse correlation between p-tauSer396 and *O*-GlcNAcylated tau levels in the hippocampus (p-tauSer396 × *O*-GlcNAc, $r = -0.8819$; $p < 0.001$), whereas no correlation was found between p-tauThr205 and *O*-GlcNAcylated tau (Fig. 5G).

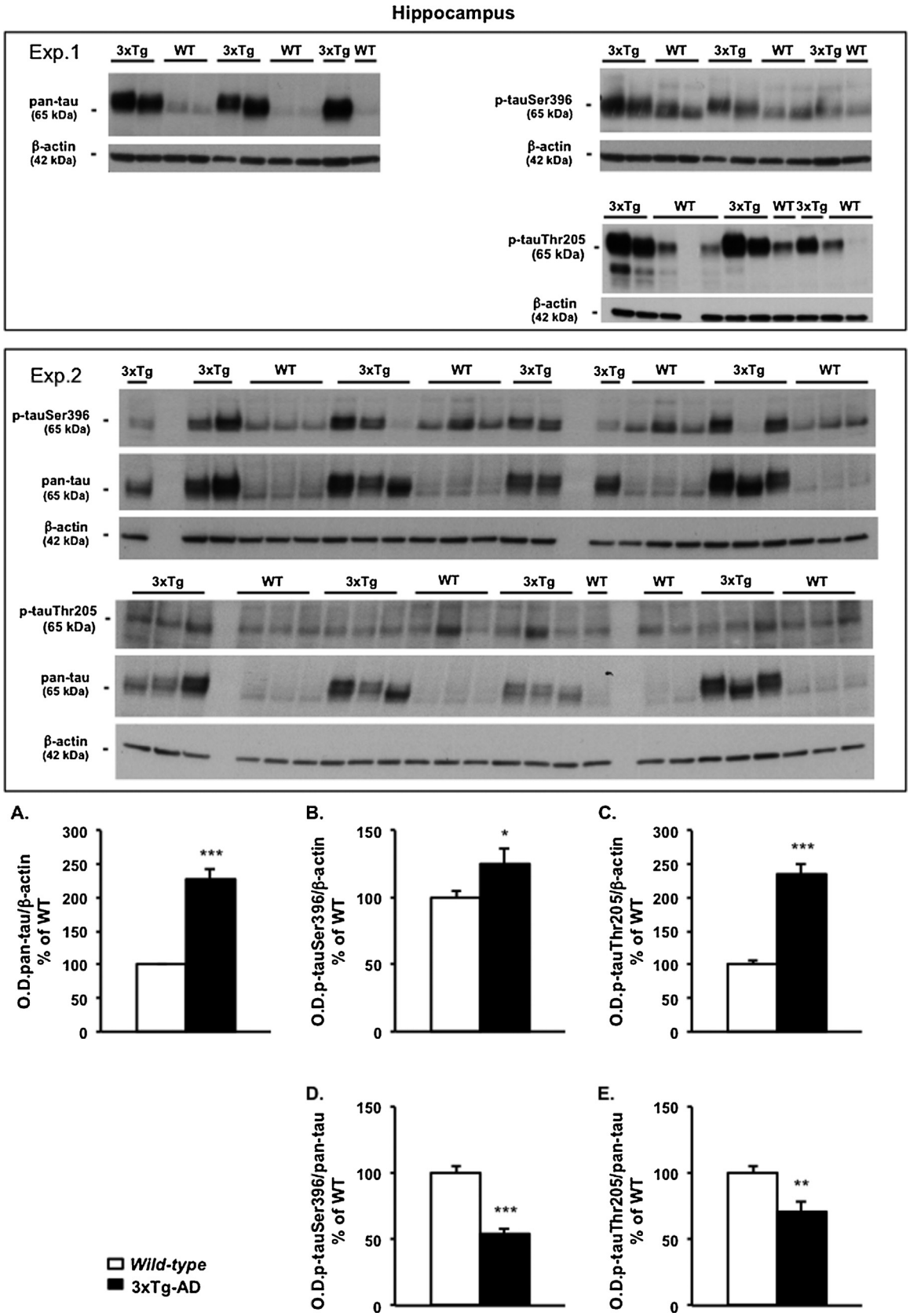

Fig. 1. Enhanced pan-tau and phosphorylated tau levels in the hippocampus of 3xTg-AD mice.
Pan-tau, p-tauSer396 and p-tauThr205 levels were measured by immunoblot in experiment 1 ($n = 5$ per group) and 2 ($n = 12$ per group), and values (means + S.E.M.) were expressed as percent of the respective groups of *wild-type* mice. Values were normalized by ß-actin (A–C) or pan-tau (D and E). $p < 0.05$ (*), $p < 0.01$ (**) or $p < 0.001$ (***) vs. the respective *wild-type* mice (Student's *t*-test). In experiment 2, pan-tau values were obtained from the same extracts run in two different immunloblots. Each single pan-tau value is the average of the two determinations in the two blots (one under p-tauSer396 and the other under p-tauThr205).

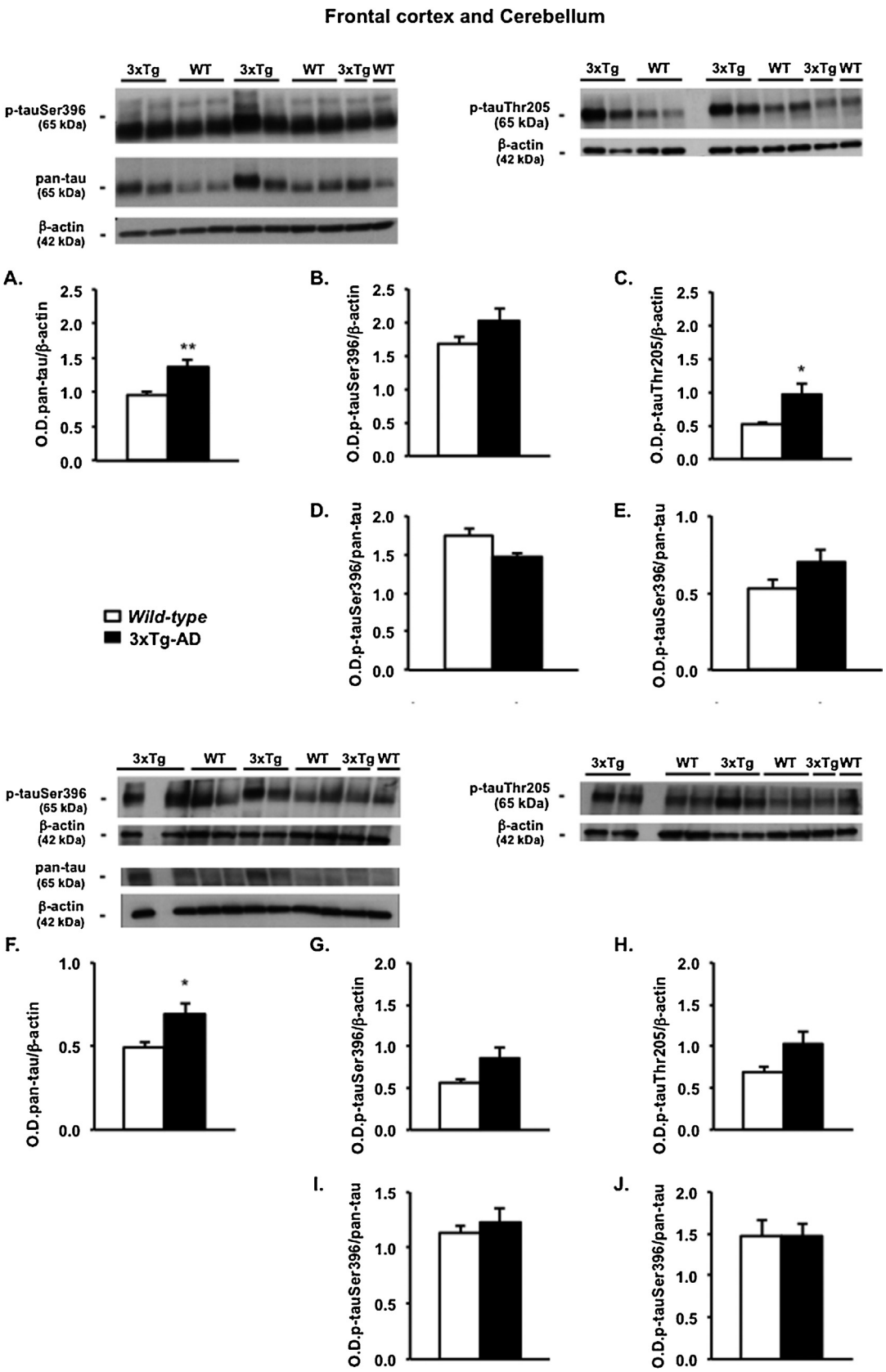

Fig. 2. Tau and phosphorylated-tau levels were in the frontal cortex and cerebellum of 3xTg-AD mice.
Values are means ± S.E.M. of 5 determinations per group and were normalized by ß-actin (A–C, F–H) or pan-tau (D, E, I, J). $p < 0.05$ (*), $p < 0.01$ (**) vs. the respective *wild-type* mice (Student's *t*-test).

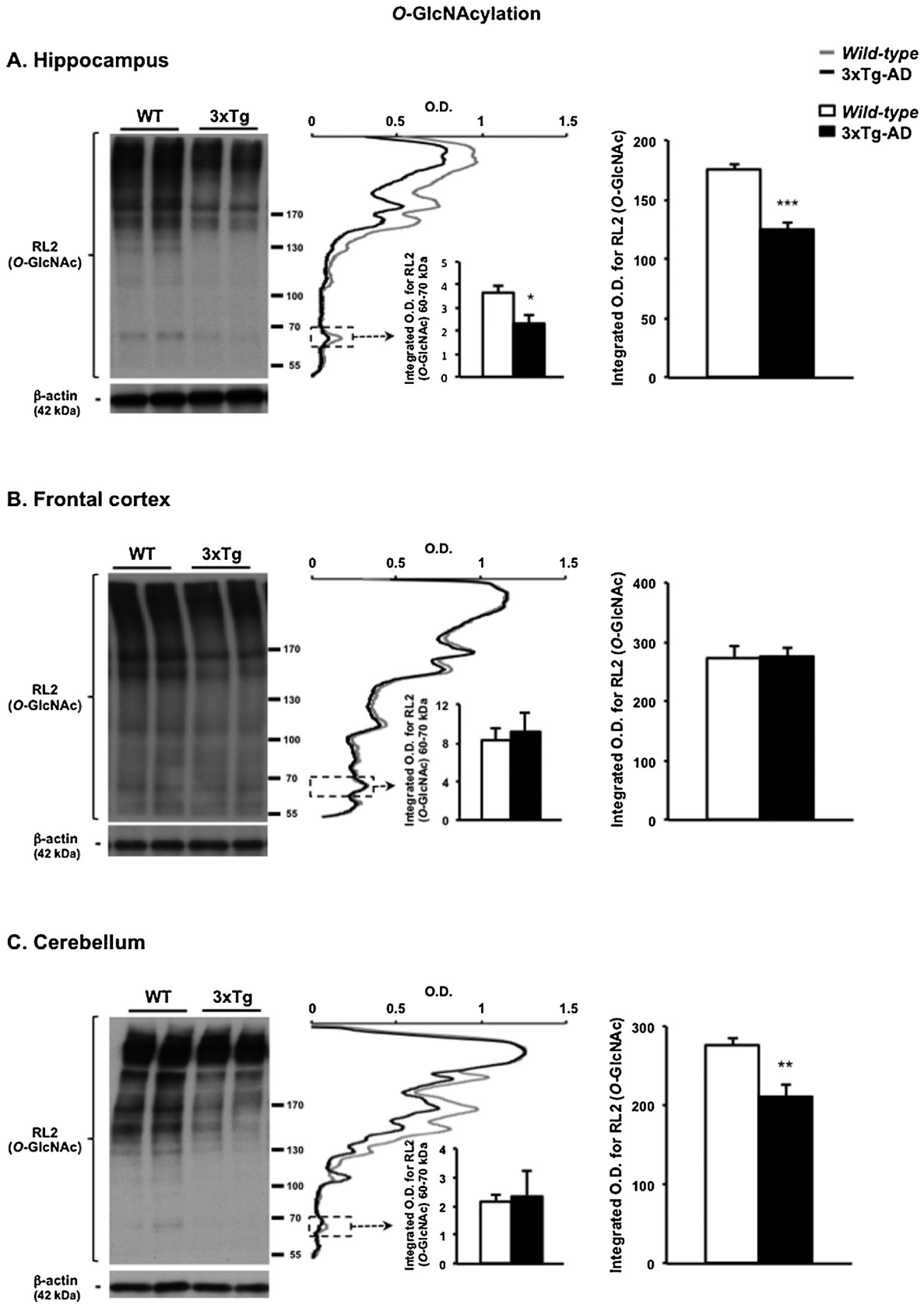

Fig. 3. Total *O*-GlcNAcylation levels are decreased in the hippocampus and cerebellum of 3xTg-AD mice.
Western Blot analysis was performed in the hippocampus (A), frontal cortex (B) and cerebellum (C) of *wild-type* (WT) and 3xTg-AD mice. *O*-GlcNAcylation representative immunoblots are shown. *O*-GlcNAcylation immunoblot signals obtained with an RL2 antibody are analyzed in a spectral mode with the optical density (O.D.) on β-actin ratio as a function of the molecular weight. Total signal as well as the 60–70 kDa bands are presented as histograms. Values are means ± S.E.M. of 5 determinations per group. $p < 0.05$ (*), $p < 0.01$ (**) or $p < 0.001$ (***) vs. the respective *wild-type* mice.

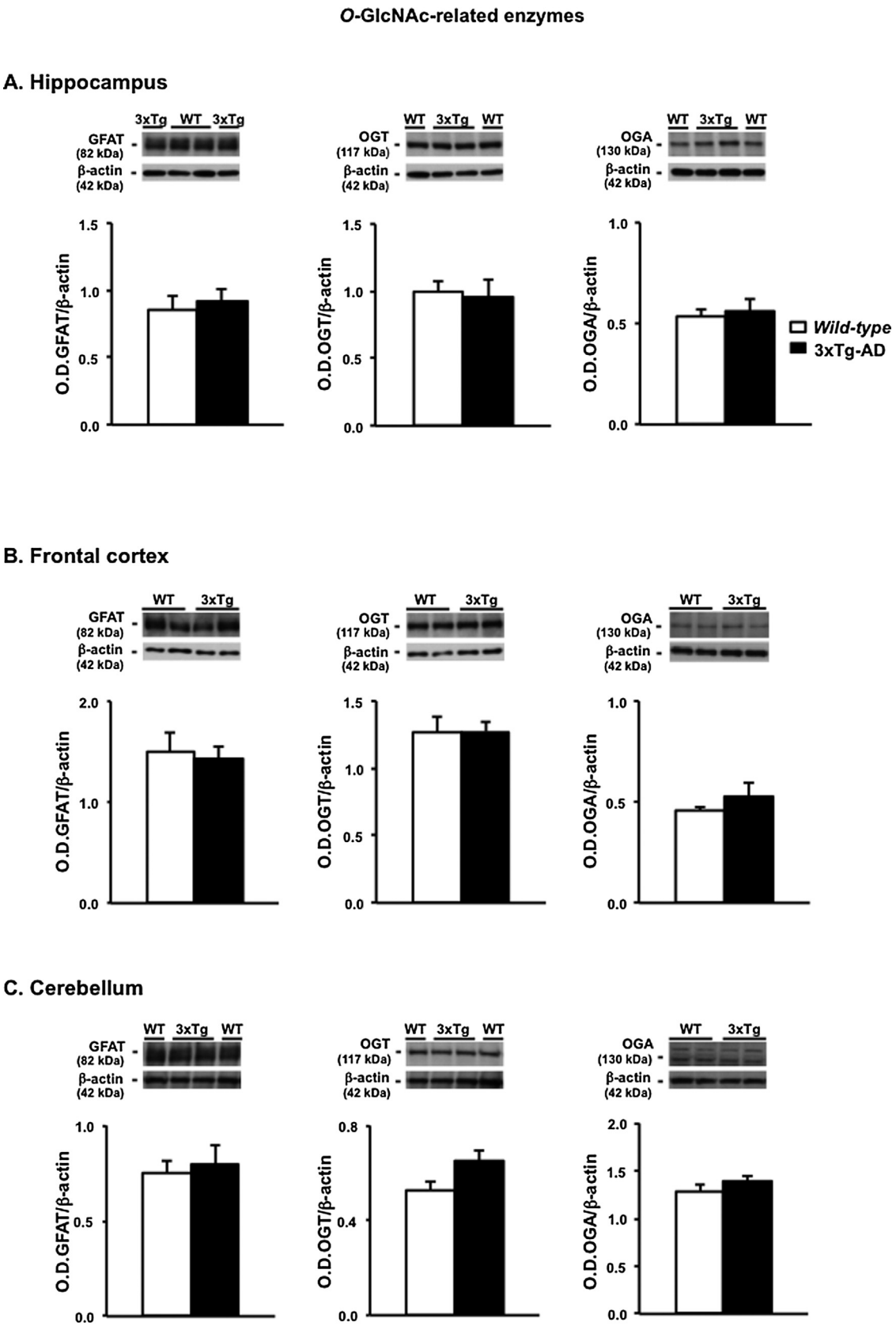

Fig. 4. Expression of *O*-GlcNAc-related enzyme is unchanged in 3xTg-AD mice.
Western Blot analysis was performed in the hippocampus (A), frontal cortex (B) and cerebellum (C) of *wild-type* (WT) and 3xTg-AD mice. Representative immunoblots are shown for glutamine fructose-6-phosphate amidotransferase (GFAT), *O*-linked β-*N*-acetylglucosamine transferase (OGT) and *O*-GlcNAc hydrolase (OGA). Values are means ± S.E.M. of 5 determinations per group.

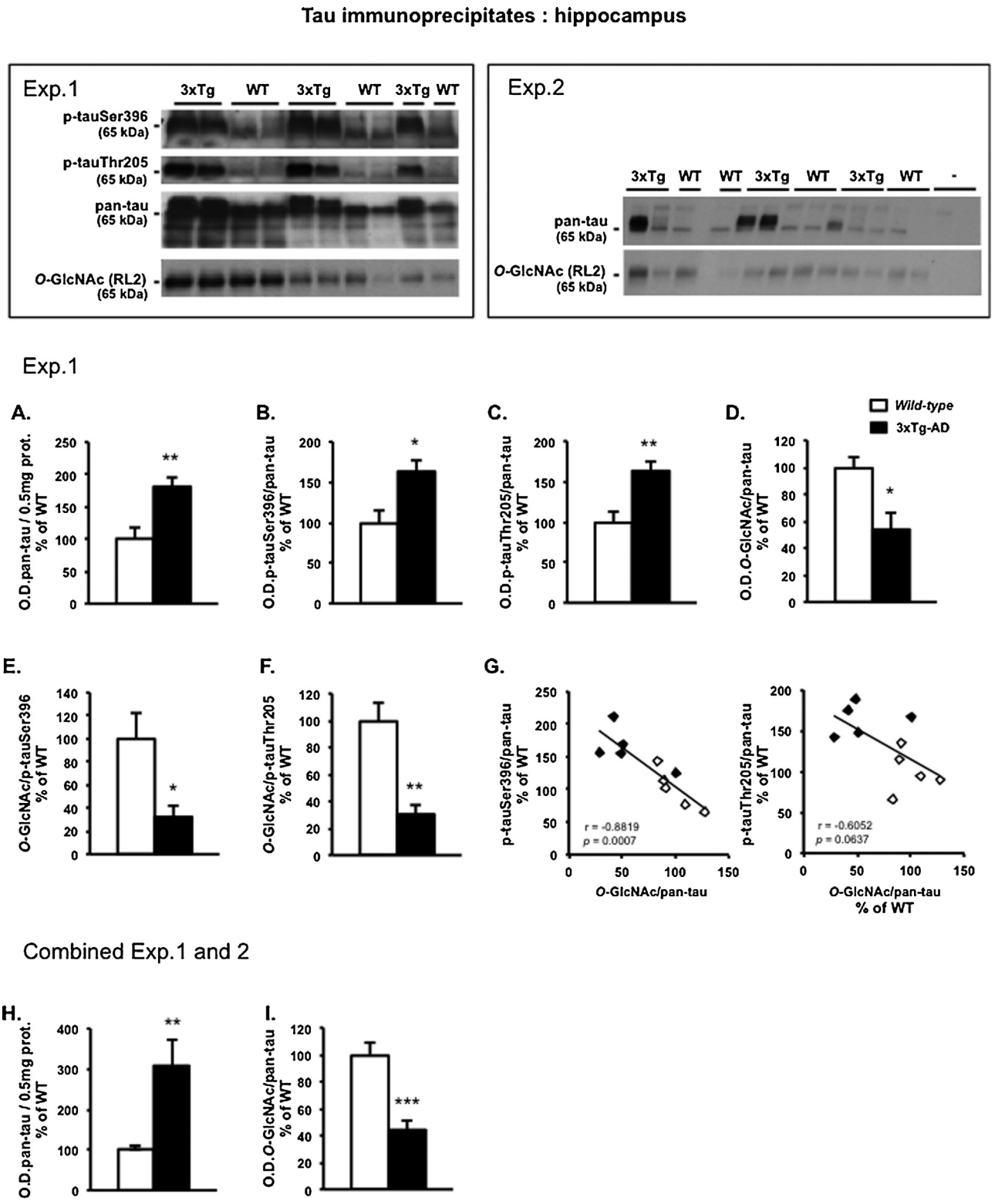

Fig. 5. Reduced *O*-GlcNAcylation of immunoprecipitated tau in the hippocampus of 3xTg-AD mice.
p-tauSer396 and p-tauThr205 were obtained exclusively from experiment 1 ($n = 5$ determinations per group) and normalized by the respective pan-tau values (B and C), whereas *O*-GlcNAc levels were obtained from experiments 1 (D) and combined experiments 1 and 2 ($n = 11$ determinations per group) and also normalized by the respective pan-tau levels (I). Normalization of *O*-GlcNAcylated tau by p-tauSer396 (E) or p-tauThr205 (F), as well as correlation analyses between GlcNAcylated tau × p-tauSer396 and *O* −GlcNAcylated tau × p-tauThr205 (G) were performed using exclusively values from experiment 1. Values in A–F, H and I are expressed as percent of *wild-type* mice and are means ± S.E.M. $p < 0.05$ (*), $p < 0.01$ (**) or $p < 0.001$ (***) vs. the respective *wild-type* mice (Student's *t*-test). The last two lanes of the blot in experiment 2 are from a pool of immunodepleted samples obtained for *wild-type* and 3xTg-AD mice.

Data of exepriments 1 and 2 were combined for the evaluation of tau *O*-GlcNAcylation with a stronger statistical power. Using this approach, the reduction of tau *O*-GlcNAcylation in 3xTgAD mice was fully confirmed with an extent even greater than that observed with data of experiment 1 alone (pan-tau: $t_{20} = 3.125$; $p < 0.01$; Fig. 5H; *O*-GlcNAcylated tau: $t_{20} = -5.144$; $p < 0.001$; Fig. 5I).

No significant changes in phosphorylated or *O*-GlcNAcylated tau were found in immunoprecipitates from the frontal cortex or the cerebellum of 3xTg-AD mice (Fig. 6). However, we found a direct correlation between p-tauThr205 and *O*-GlcNAcylated tau levels in frontal cortex (p-tauThr205 × *O*-GlcNAc, $r = 0.8103$; $p < 0.05$; Fig. 6G) and an inverse correlation between p-tauSer396 and *O*-GlcNAcylated tau levels in the cerebellum (p-tauSer396 × *O*-GlcNAcylated tau, $r = -0.6547$; $p < 0.05$).

3.4. OGT and OGA activities are not modified in the hippocampus of 3xTg-AD mice

The activity of enzymes regulating *O*-GlcNAcylation was assessed in hippocampus of 3xTg-AD and WT mice. OGT activity was unchanged in 3xTg-Ad mice ($t_7 = -0.418$; $p = 0.689$; Fig. 7A). OGA activity was not significantly enhanced in 3xTg-Ad mice mice ($t_{10} = 1.594$; $p = 0.142$; Fig. 7B).

4. Discussion

We used mice harboring human mutations of APP, PS1, and tau (3xTg-AD mice), which are considered as a putative mouse transgenic model of AD. We found a selective reduction of tau *O*-GlcNAcylation in the hippocampus of these mice, which was associated with the expected tau hyperphosphorylation [37]. To our knowledge, this is the first evidence that tau is hypo-*O*-GlcNAcylated in a mouse model of AD.

Although increasing evidence suggests that abnormalities of *O*-GlcNAcylation play a role in the pathophysiology of AD, studies on *O*-GlcNAcylation in the AD brain have produced contrasting results. [29] have found a reduced tau *O*-GlcNAcylation in brain tissue from AD patients, whereas increases in protein *O*-GlcNAcylation were found in two other reports [19,15]. There might be many explanations for the contrasting data obtained in the AD brain, including differences in the selected brain regions, sample preparation, type of anti-*O*-GlcNAc antibody, and, more importantly, the post-mortem interval before tissue sampling. It has been demonstrated that *O*-GlcNAcylation levels decline with post-mortem delay of brain tissue [28]. Here, the reduction of tau *O*-GlcNAcylation levels was only found in the hippocampus of 3xTg-AD mice, which harbor a tau mutation (tau_{P301L}), in addition to the APP and PS1 mutations. Steady-state levels of transgene-derived human tau protein are high in the hippocampus and cerebral cortex, and low in the cerebellum [36]. Hence, the evidence that the imbalance between tau *O*-GlcNAcylation and phosphorylation was observed in the hippocampus, but not in the cortex, indicates that expression of transgenic tau did not affect our results.

In 3xTg-AD mice, tau pathology appears first in the hippocampus, where it is already visible at 12 months of age, and later spreads to the cortex [36,32]. Thus, the selective reduction in tau *O*-GlcNAcylation found in the hippocampus of 12-month-old 3xTg-AD mice is in line with the pattern of tau pathology in these mice. Several mechanisms may account for the reduction of tau *O*-GlcNAcylation in the hippocampus of 3xTg-AD mice, such as a primary phosphorylation of tau, a defective expression/activity of HBP enzymes, and a reduction in glucose uptake in neurons. These three hypotheses are not mutually exclusive. An increased phosphorylation might be directly linked to the reduction in tau *O*-GlcNAcylation because, in tau immunoprecipitates, glycosylation was reduced only in the region in which phosphorylation was enhanced, i.e., in the hippocampus. However, the general defect of protein *O*-GlcNAcylation found in the hippocampus suggests that a reduced *O*-GlcNAcylation might be a primary event, facilitating tau phosphorylation on hypoglycosylated Ser or Thr residues. Expression of the HBP rate-limiting enzyme, GFAT, as well as the expression of enzymes regulating *O*-GlcNAcylation, i.e., OGT, and OGA, did not change in the hippocampus of 3xTg-AD mice, suggesting that the pathway was not constitutively defective in these mice. In addition, the activity of OGT and OGA was not modified in these animals. Hence, a primary defect in glucose uptake might be responsible for the reduced *O*-GlcNAcylation. Accordingly, [13] have found a reduction in brain glucose uptake associated with a decreased expression of the type-3 neuronal glucose transporter (GLUT-3) in 12-month-old 3xTg-AD mice. We were intrigued from the finding that tau *O*-GlcNAcylation and phosphorylation was unchanged in cerebellar immunoprecipitates in spite of the reduction in protein *O*-GlcNAcylation observed in cerebellar homogenates of 3xTg-AD mice. This suggests that an overall reduction in *O*-GlcNAcylation might contribute to, but is not sufficient for, tau hyperphosphorylation in 3xTg-AD mice. It is likely that the balance between tau *O*-GlcNAcylation and phosphorylation is regulated by multiple mechanisms that include the intracellular availability of UDP-GlcNAc and the region-specific activation of protein kinases that phosphorylate tau, such as glycogensynthase kinase-3β (GSK-3β) and type-5 cyclin-dependent kinase [20,4]. Perhaps it is only in the hippocampus that these different mechanisms converge in 12-month-old 3xTg-AD mice.

The reduction of *O*-GlcNAcylation we have found in the hippocampus of 12-month-old 3xTg-AD mice may have a deep impact on the pathological phenotype of these mice contributing to tau aggregation, synaptic dysfunction, behavioral abnormalities, and neurodegeneration [52]. Of note, *O*-GlcNAcylation may modulate mechanisms of activity-dependent synaptic plasticity, e.g., long-term potentiation (LTP) and long-term-depression (LTD) in the hippocampus by regulating the expression and activity of AMPA receptors and synapsin I [49,12,45,50]. Whether, and to what extent, the reduced protein *O*-GlcNAcylation has any role on the impairment of hippocampal synaptic plasticity and spatial memory observed in old 3xTg-AD mice [36,5,9,8,16,34,2,14] is an interesting question that warrants further investigation. Although a correlation between tau hyperphosphorylation and cognitive decline in 3xTg-AD mice has not been demonstrated so far [7,37,39,3], a reduced tau *O*-GlcNAcylation leading to tau hyperphosphorylation might contribute to the impairment of activity-dependent synaptic plasticity associated with AD. Accordingly, tau phosphorylation at Serine 396 is required for hippocampal LTD [40], and tau hyperphosphorylation at epitopes recognized by PHF-1 (Ser 396/404) and AT8 (Ser199/202-Thr205) is enhanced in the hippocampus following LTD induction [33]. In addition, activation of tau-phosphorylating enzyme, GSK-3β, [31,44] and tau phosphorylation [44] are required for the impairment of hippocampal LTP caused by β-amyloid$_{1-42}$ ($A\beta_{1-42}$). Thus, a reduced tau *O*-GlcNAcylation might be a primary mechanism in the chain of events favoring synaptic weakening over synaptic potentiation in response to extracellular aggregates of $A\beta_{1-42}$. It will be interesting to examine whether drugs that enhance *O*-GlcNAcylation, such as the OGA inhibitor, Thiamet G, slow the progression of AD-related pathology and the impairment of synaptic plasticity underlying cognitive dysfunction in 3xTg-AD mice, and whether these drugs improve cognitive dysfunction in 3xTg-AD mice. Interestingly, systemic administration of Thiamet G, has been found to reduce tau pathology, slow neurodegeneration, and prolong survival in mouse models of tauopathies [53,6,18], and to prevent cognitive decline and amyloid deposits in the bigenic tau/APP mutant mice [55].

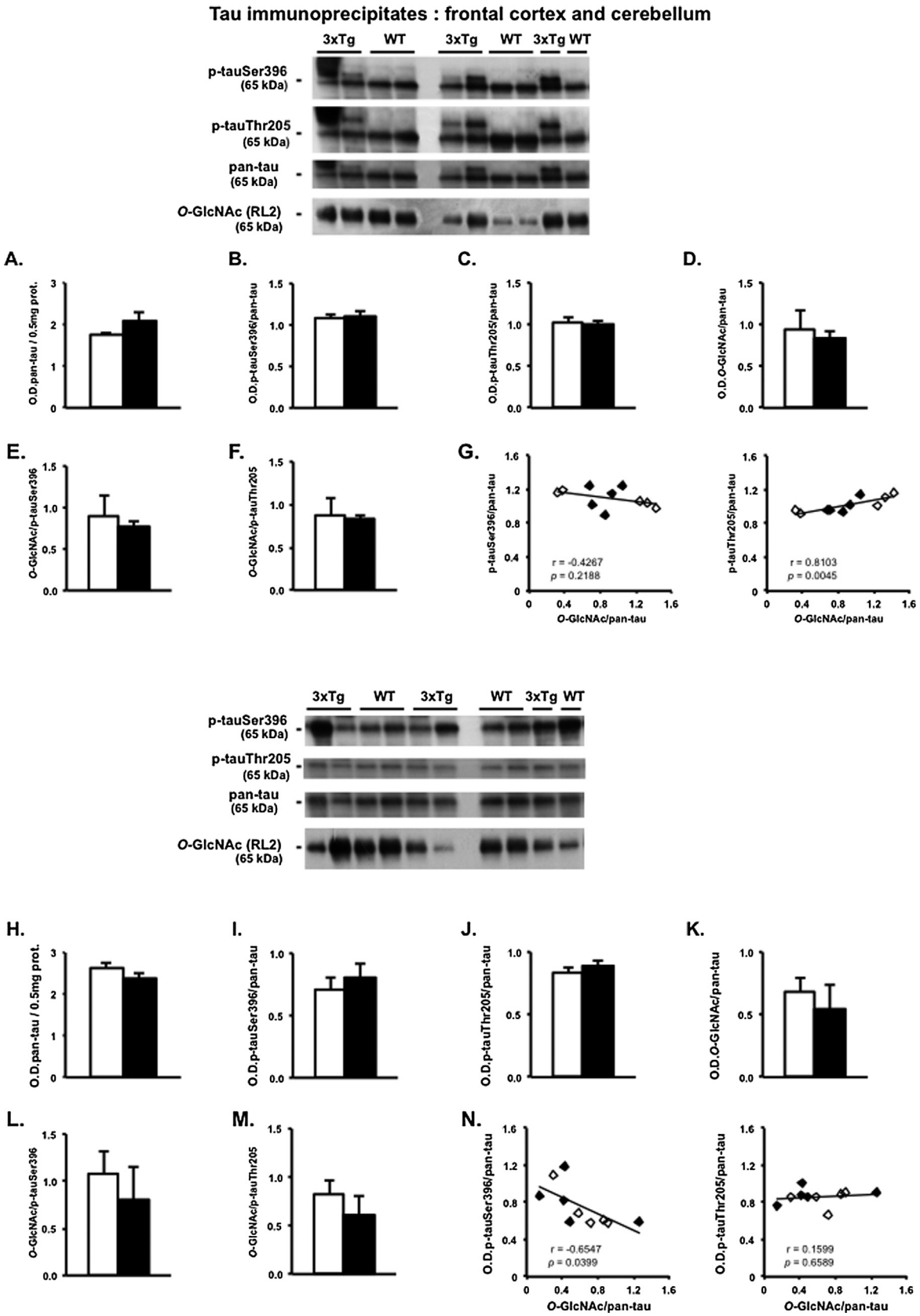

Fig. 6. *O*-GlcNAcylation of tau immunoprecipitated is unchanged in thefrontal cortex and cerebellum of 3xTg-AD mice.
Western Blot analysis was performed in the frontal cortex and cerebellum of *wild-type* (WT) and 3xTg-AD mice. Immunoblots are shown for pan-tau, p-tauSer396, p-tauThr205, and *O*-GlcNAc. Histograms obtained for pan-tau (A, H), p-tauSer396 (B, I), p-tauThr205 (C, J), and *O*-GlcNAc (D, K), as well as ratio *O*-GlcNAc/p-tauSer396 (E, L) and *O*-GlcNAc/p-tauThr205 (F, M). Correlation found for *O*-GlcNAcylated tau x p-tauSer396 and *O*-GlcNAcylated tau × p-tauThr205 are shown (G, N). Values are means ± S.E.M. of 5 determinations per group.

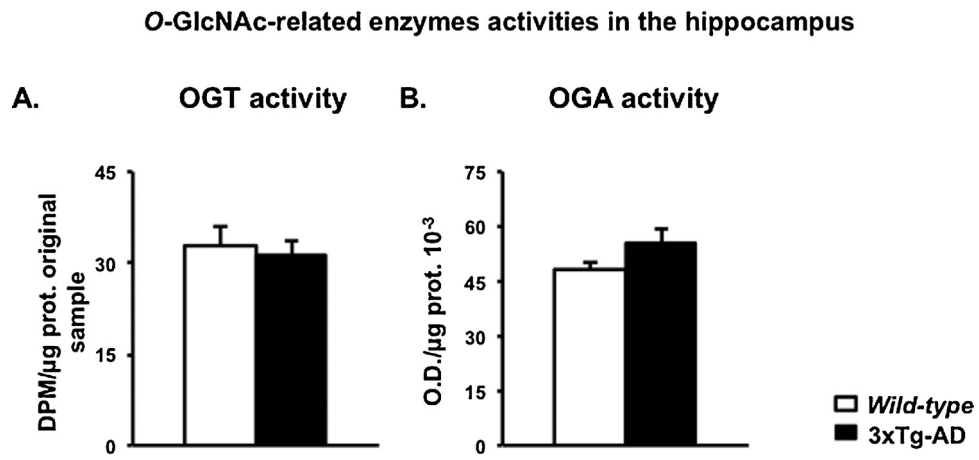

Fig. 7. OGT and OGA activities are not modified in the hippocampus of 3xTg-AD mice.
Enzymatic activity was assessed in the hippocampus of *wild-type* (WT) and 3xTg-AD mice. OGT activity (A) was determined using a labeling with UDP-[^{3}H]GlcNAc. The ratio between DPM and the amount of protein of the original sample (µg) is presented. Values are means ± SEM of 5 determinations for 3xTg-AD mice and 3 for *wild-type* animals. OGA activity (B) was determined toward *p*-nitro-phenyl-*N*-acetyl-β-D-glucosaminide as a substrate. The ratio between optical density (O.D.) and amount of protein of the original sample (µg) is presented. Values are means ± SEM of 6 determinations per group.

5. Conclusions

Our data demonstrate that an imbalance between tau phosphorylation and *O*-GlcNAcylation exists specifically in the hippocampus of 3xTg-AD mice. This defect in proteins *O*-GlcNAcylation, including tau, found in the hippocampus might be an upstream causative factor contributing to cognitive decline. This strengthens the hypothesis that *O*-GlcNAcylation might be targeted by disease modifying drugs in AD.

Conflict of interest

Authors declare that there are no conflicts of interest.

Acknowledgments

The study was supported by University of Lille 1, Sapienza University of Rome (Frame Agreement signed on February 15th, 2007), by CNRS in the framework of the International Associated Laboratory—Prenatal Stress and Neurodegenerative Diseases (LIA-PSND), co-directed by S. Maccari and F. Nicoletti. E. Gatta was supported by Fondation de France and the Ministry of French Education; Marc Dos Santos was supported by the Ministry of French Education; Dr. J. Mairesse was supported by the "Fondation pour la Recherche Médicale" and then by the "Agence Nationale de la Recherche" (ANR), France.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phrs.2016.01.006.

References

[1] C.S. Arnold, G.V. Johnson, R.N. Cole, D.L. Dong, M. Lee, G.W. Hart, The microtubule-associated protein tau is extensively modified with O-linked *N*-acetylglucosamine, J. Biol. Chem. 271 (1996) 28741–28744.
[2] D. Arsenault, C. Julien, C. Tremblay, F. Calon, DHA improves cognition and prevents dysfunction of entorhinal cortex neurons in 3xTg-AD mice, PLoS One 6 (2011), http://dx.doi.org/10.1371/journal.pone.0017397, e17397.
[3] D. Baglietto-Vargas, M. Kitazawa, E.J. Le, T. Estrada-Hernandez, C.J. Rodriguez-Ortiz, R. Medeiros, K.N. Green, F.M. LaFerla, Endogenous murine tau promotes neurofibrillary tangles in 3xTg-AD mice without affecting cognition, Neurobiol. Dis. 62 (2014) 407––415, http://dx.doi.org/10.1016/j.nbd.2013.10.019.
[4] K. Baumann, E.M. Mandelkow, J. Biernat, H. Piwnica-Worms, E. Mandelkow, Abnormal Alzheimer-like phosphorylation of tau-protein by cyclin-dependent kinases cdk2 and cdk5, FEBS Lett. 336 (1993) 417–424.
[5] L.M. Billings, S. Oddo, K.N. Green, J.L. McGaugh, F.M. LaFerla, Intraneuronal Abeta causes the onset of early Alzheimer's disease-related cognitive deficits in transgenic mice, Neuron 45 (2005) 675–688, http://dx.doi.org/10.1016/j.neuron.2005.01.040.
[6] P. Borghgraef, C. Menuet, C. Theunis, J.V. Louis, H. Devijver, H. Maurin, C. Smet-Nocca, G. Lippens, G. Hilaire, H. Gijsen, D. Moechars, F. Van Leuven, Increasing brain protein *O*-GlcNAc-ylation mitigates breathing defects and mortality of Tau.P301L mice, PLoS One 8 (2013), http://dx.doi.org/10.1371/journal.pone.0084442, e84442.
[7] A. Caccamo, S. Oddo, L.X. Tran, F.M. LaFerla, Lithium reduces tau phosphorylation but not A beta or working memory deficits in a transgenic model with both plaques and tangles, Am. J. Pathol. 170 (5) (2007) 1669–1675.
[8] J.C. Carroll, E.R. Rosario, L. Chang, F.Z. Stanczyk, S. Oddo, F.M. LaFerla, C.J. Pike, Progesterone and estrogen regulate Alzheimer-like neuropathology in female 3xTg-AD mice, J. Neurosci. 27 (2007) 13357–13365, http://dx.doi.org/10.1523/JNEUROSCI.2718-07.2007.
[9] L.K. Clinton, L.M. Billings, K.N. Green, A. Caccamo, J. Ngo, S. Oddo, J.L. McGaugh, F.M. LaFerla, Age-dependent sexual dimorphism in cognition and stress response in the 3xTg-AD mice, Neurobiol. Dis. 28 (2007) 76––82, http://dx.doi.org/10.1016/j.nbd.2007.06.013.
[10] S. Cunnane, S. Nugent, M. Roy, A. Courchesne-Loyer, E. Croteau, S. Tremblay, A. Castellano, F. Pifferi, C. Bocti, N. Paquet, H. Begdouri, M. Bentourkia, E. Turcotte, M. Allard, P. Barberger-Gateau, T. Fulop, S.I. Rapoport, Brain fuel metabolism, aging, and Alzheimer's disease, Nutrition 27 (2011) 3–20, http://dx.doi.org/10.1016/j.nut.2010.07.021.
[11] S.M. De la Monte, M. Tong, Brain metabolic dysfunction at the core of Alzheimer's disease, Biochem. Pharmacol. 88 (2014) 548–559, http://dx.doi.org/10.1016/j.bcp.2013.12.012.
[12] N. Din, I. Ahmad, I. Ul Haq, S. Elahi, D.C. Hoessli, A.R. Shakoori, The function of GluR1 and GluR2 in cerebellar and hippocampal LTP and LTD is regulated by interplay of phosphorylation and *O*-GlcNAc modification, J. Cell. Biochem. 109 (2010) 585––597, http://dx.doi.org/10.1002/jcb.22436.
[13] F. Ding, J. Yao, J.R. Rettberg, S. Chen, R.D. Brinton, Early decline in glucose transport and metabolism precedes shift to ketogenic system in female aging and Alzheimer's mouse brain: implication for bioenergetic intervention, PLoS One 8 (2013), http://dx.doi.org/10.1371/journal.pone.0079977, e79977.
[14] M. Filali, R. Lalonde, P. Theriault, C. Julien, F. Calon, E. Planel, Cognitive and non-cognitive behaviors in the triple transgenic mouse model of Alzheimer's disease expressing mutated APP, PS1, and Mapt (3xTg-AD), Behav. Brain Res. 234 (2012) 334––342, http://dx.doi.org/10.1016/j.bbr.2012.07.004.
[15] S. Förster, A.S. Welleford, J.C. Triplett, R. Sultana, B. Schmitz, D.A. Butterfield, Increased *O*-GlcNAc levels correlate with decreased *O*-GlcNAcase levels in Alzheimer disease brain, Biochim. Biophys. Acta 1842 (2014) 1333––1339, http://dx.doi.org/10.1016/j.bbadis.2014.05.014.
[16] L. Giménez-Llort, G. Blázquez, T. Cañete, B. Johansson, S. Oddo, A. Tobeña, F.M. LaFerla, A. Fernández-Teruel, Modeling behavioral and neuronal symptoms of Alzheimer's disease in mice: a role for intraneuronal amyloid, Neurosci. Biobehav. Rev. 31 (2007) 125––147, http://dx.doi.org/10.1016/j.neubiorev.2006.07.007.

[17] M.L. Giuffrida, F. Caraci, P. De Bona, G. Pappalardo, F. Nicoletti, E. Rizzarelli, A. Copani, The monomer state of beta-amyloid: where the Alzheimer's disease protein meets physiology, Rev. Neurosci. 21 (2010) 83–93.
[18] D.L. Graham, A.J. Gray, J.A. Joyce, D. Yu, J. OMoore, G.A. Carlson, M.S. Shearman, T.L. Dellovade, H. Hering, Increased *O*-GlcNAcylation reduces pathological tau without affecting its normal phosphorylation in a mouse model of tauopathy, Neuropharmacology 79 (2014) 307––313, http://dx.doi.org/10.1016/j.neuropharm.2013.11.025.
[19] L.S. Griffith, B. Schmitz, *O*-linked *N*-acetylglucosamine is upregulated in Alzheimer brains, Biochem. Biophys. Res. Commun. 213 (1995) 424––431, http://dx.doi.org/10.1006/bbrc.1995.2149.
[20] D.P. Hanger, K. Hughes, J.R. Woodgett, J.P. Brion, B.H. Anderton, Glycogen synthase kinase-3 induces Alzheimer's disease-like phosphorylation of tau: generation of paired helical filament epitopes and neuronal localisation of the kinase, Neurosci. Lett. 147 (1992) 58–62.
[21] J.A. Hanover, M.W. Krause, D.C. Love, Bittersweet memories: linking metabolism to epigenetics through *O*-GlcNAcylation, Nat. Rev. Mol. Cell Biol. 13 (2012) 312–321, http://dx.doi.org/10.1038/nrm3334.
[22] G.W. Hart, C. Slawson, G. Ramirez-Correa, O. Lagerlof, Cross talk between *O*-GlcNAcylation and phosphorylation: roles in signaling, transcription, and chronic disease, Annu. Rev. Biochem. 80 (2011) 825–858, http://dx.doi.org/10.1146/annurev-biochem-060608-102511.
[23] M.J. Kang, C. Kim, H. Jeong, B.-K. Cho, A.L. Ryou, D. Hwang, I. Mook-Jung, E.C. Yi, Synapsin-1 and tau reciprocal *O*-GlcNAcylation and phosphorylation sites in mouse brain synaptosomes, Exp. Mol. Med. 45 (2013) e29, http://dx.doi.org/10.1038/emm.2013.56.
[24] T. Lefebvre, S. Ferreira, L. Dupont-Wallois, T. Bussière, M.-J. Dupire, A. Delacourte, J.-C. Michalski, M.-L. Caillet-Boudin, Evidence of a balance between phosphorylation and *O*-GlcNAc glycosylation of Tau proteins—a role in nuclear localization, Biochim. Biophys. Acta 1619 (2003) 167–176.
[25] T. Lefebvre, C. Guinez, V. Dehennaut, O. Beseme-Dekeyser, W. Morelle, J.-C. Michalski, Does *O*-GlcNAc play a role in neurodegenerative diseases? Expert Rev. Proteomics 2 (2005) 265–275, http://dx.doi.org/10.1586/14789450.2.2.265.
[26] S. Lim, M.M. Haque, G. Nam, N. Ryoo, H. Rhim, Y.K. Kim, Monitoring of intracellular tau aggregation regulated by OGA/OGT inhibitors, Int. J. Mol. Sci. 16 (9) (2015) 20212–20224.
[27] F. Liu, T. Zaidi, K. Iqbal, I. Grundke-Iqbal, R.K. Merkle, C.X. Gong, Role of glycosylation in hyperphosphorylation of tau in Alzheimer's disease, FEBS Lett. 512 (2002) 101–106.
[28] F. Liu, K. Iqbal, I. Grundke-Iqbal, G.W. Hart, C.-X. Gong, *O*-GlcNAcylation regulates phosphorylation of tau: a mechanism involved in Alzheimer's disease, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 10804––10809, http://dx.doi.org/10.1073/pnas.0400348101.
[29] F. Liu, J. Shi, H. Tanimukai, J. Gu, J. Gu, I. Grundke-Iqbal, K. Iqbal, C.-X. Gong, Reduced *O*-GlcNAcylation links lower brain glucose metabolism and tau pathology in Alzheimer's disease, Brain 132 (2009) 1820–1832, http://dx.doi.org/10.1093/brain/awp099.
[30] Y. Liu, X. Li, Y. Yu, J. Shi, Z. Liang, X. Run, Y. Li, C.L. Dai, I. Grundke-Iqbal, K. Iqbal, F. Liu, C.-X. Gong, Developmental regulation of protein *O*-GlcNAcylation, *O*-GlcNAc transferase, and *O*-GlcNAcase in mammalian brain, PLoS One 7 (8) (2012) e43724, http://dx.doi.org/10.1371/journal.pone.0043724.
[31] J. Jo, D.J. Whitcomb, K.M. Olsen, T.L. Kerrigan, S.C. Lo, G. Bru-Mercier, B. Dickinson, S. Scullion, M. Sheng, G. Collingridge, K. Cho, Aβ(1-42) inhibition of LTP is mediated by a signaling pathway involving caspase-3, Akt1 and GSK-3β, Nat. Neurosci. 14 (5) (2011) 545––547, http://dx.doi.org/10.1038/nn.2785.
[32] M.A. Mastrangelo, W.J. Bowers, Detailed immunohistochemical characterization of temporal and spatial progression of Alzheimer's disease-related pathologies in male triple-transgenic mice, BMC Neurosci. 9 (2008) 81, http://dx.doi.org/10.1186/1471-2202-9-81.
[33] S. Mondragón-Rodríguez, E. Trillaud-Doppia, A. Dudilot, C. Bourgeois, M. Lauzon, N. Leclerc, J. Boehm, Interaction of endogenous tau protein with synaptic proteins is regulated by *N*-methyl-*D*-aspartate receptor-dependent tau phosphorylation, J. Biol. Chem. 287 (38) (2012) 32040–32053, http://dx.doi.org/10.1074/jbc.M112.401240.
[34] R.L. Nelson, Z. Guo, V.M. Halagappa, M. Pearson, A.J. Gray, Y. Matsuoka, M. Brown, R.F. Clark, M.P. Mattson, Prophylactic treatment with paroxetine ameliorates behavioral deficits and retards the development of amyloid and tau pathologies in 3xTgAD mice, Exp. Neurol. 205 (2007) 166–176, http://dx.doi.org/10.1016/j.expneurol.2007.01.037.
[35] R.M. Nicholson, Y. Kusne, L.A. Nowak, F.M. LaFerla, E.M. Reiman, J. Valla, Regional cerebral glucose uptake in the 3xTG model of Alzheimer's disease highlights common regional vulnerability across AD mouse models, Brain Res. 1347 (2010) 179––185, http://dx.doi.org/10.1016/j.brainres.2010.05.084.
[36] S. Oddo, A. Caccamo, J.D. Shepherd, M.P. Murphy, T.E. Golde, R. Kayed, R. Metherate, M.P. Mattson, Y. Akbari, F.M. LaFerla, Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction, Neuron 39 (2003) 409–421.
[37] S. Oddo, A. Caccamo, D. Cheng, B. Jouleh, R. Torp, F.M. LaFerla, Genetically augmenting tau levels does not modulate the onset or progression of Abeta pathology in transgenic mice, J. Neurochem. 102 (4) (2007) 1053––1063.
[38] R. Ossenkoppele, N. Tolboom, J.C. Foster-Dingley, S.F. Adriaanse, R. Boellaard, M. Yaqub, A.D. Windhorst, F. Barkhof, A.A. Lammertsma, P. Scheltens, W.M. van der Flier, B.N.M. van Berckel, Longitudinal imaging of Alzheimer pathology using [11C]PIB, [18F]FDDNP and [18F]FDG PET, Eur. J. Nucl. Med. Mol. Imaging 39 (2012) 990–1000, http://dx.doi.org/10.1007/s00259-012-2102-3.
[39] A. Parachikova, V. Vasilevko, D.H. Cribbs, F.M. LaFerla, K.N. Green, Reductions in amyloid-beta-derived neuroinflammation, with minocycline, restore cognition but do not significantly affect tau hyperphosphorylation, J. Alzheimers Dis. 21 (2) (2010) 527––542, http://dx.doi.org/10.3233/JAD-2010-100204.
[40] P. Regan, T. Piers, J.H. Yi, D.H. Kim, S. Huh, S.J. Park, J.H. Ryu, D.J. Whitcomb, K. Cho, Tau phosphorylation at serine 396 residue is required for hippocampal LTD, J. Neurosci. 35 (12) (2015) 4804–4812, http://dx.doi.org/10.1523/JNEUROSCI.2842-14.2015.
[41] E.M. Reiman, R.J. Caselli, L.S. Yun, K. Chen, D. Bandy, S. Minoshima, S.N. Thibodeau, D. Osborne, Preclinical evidence of Alzheimer's disease in persons homozygous for the epsilon 4 allele for apolipoprotein E, N. Engl. J. Med. 334 (1996) 752–758, http://dx.doi.org/10.1056/NEJM199603213341202.
[42] A. Romano, L. Pace, B. Tempesta, A.M. Lavecchia, T. Macheda, G. Bedse, A. Petrella, C. Cifani, G. Serviddio, G. Vendemiale, S. Gaetani, T. Cassano, Depressive-like behavior is paired to monoaminergic alteration in a murine model of Alzheimer's disease, Int. J. Neuropsychopharmacol. 18 (2014), http://dx.doi.org/10.1093/ijnp/pyu020.
[43] D.J. Selkoe, Alzheimer's disease: genes proteins, and therapy, Physiol. Rev. 81 (2001) 741–766.
[44] O.A. Shipton, J.R. Leitz, J. Dworzak, C.E. Acton, E.M. Tunbridge, F. Denk, H.N. Dawson, M.P. Vitek, R. Wade-Martins, O. Paulsen, M. Vargas-Caballero, Tau protein is required for amyloid {beta}-induced impairment of hippocampal long-term potentiation, J. Neurosci. 31 (5) (2011) 1688–1692, http://dx.doi.org/10.1523/JNEUROSCI.2610-10.2011.
[45] Y. Skorobogatko, A. Landicho, R.J. Chalkley, A.V. Kossenkov, G. Gallo, K. Vosseller, *O*-linked β-*N*-acetylglucosamine (*O*-GlcNAc) site thr-87 regulates synapsin I localization to synapses and size of the reserve pool of synaptic vesicles, J. Biol. Chem. 289 (2014) 3602––3612, http://dx.doi.org/10.1074/jbc.M113.512814.
[46] G.W. Small, J.C. Mazziotta, M.T. Collins, L.R. Baxter, M.E. Phelps, M.A. Mandelkern, A. Kaplan, A. La Rue, C.F. Adamson, L. Chang, Apolipoprotein E type 4 allele and cerebral glucose metabolism in relatives at risk for familial Alzheimer disease, JAMA 273 (1995) 942–947.
[47] E. Steen, B.M. Terry, E.J. Rivera, J.L. Cannon, T.R. Neely, R. Tavares, X.J. Xu, J.R. Wands, S.M. de la Monte, Impaired insulin and insulin-like growth factor expression and signaling mechanisms in Alzheimer's disease—is this type 3 diabetes? J. Alzheimers Dis. 7 (2005) 63–80.
[48] M. Takahashi, Y. Tsujioka, T. Yamada, Y. Tsuboi, H. Okada, T. Yamamoto, Z. Liposits, Glycosylation of microtubule-associated protein tau in Alzheimer's disease brain, Acta Neuropathol. 97 (1999) 635–641.
[49] M.K. Tallent, N. Varghis, Y. Skorobogatko, L. Hernandez-Cuebas, K. Whelan, D.J. Vocadlo, K. Vosseller, In vivo modulation of *O*-GlcNAc levels regulates hippocampal synaptic plasticity through interplay with phosphorylation, J. Biol. Chem. 284 (2009) 174–181, http://dx.doi.org/10.1074/jbc.M807431200.
[50] E.W. Taylor, K. Wang, A.R. Nelson, T.M. Bredemann, K.B. Fraser, S.M. Clinton, R. Puckett, R.B. Marchase, J.C. Chatham, L.L. McMahon, O-GlcNAcylation of AMPA receptor GluA2 is associated with a novel form of long-term depression at hippocampal synapses, J. Neurosci. 34 (2014) 10–21, http://dx.doi.org/10.1523/JNEUROSCI.4761-12.2014.
[51] J.Z. Wang, I. Grundke-Iqbal, K. Iqbal, Glycosylation of microtubule-associated protein tau: an abnormal posttranslational modification in Alzheimer's disease, Nat. Med. 2 (1996) 871––875.
[52] S.J. Webster, A.D. Bachstetter, P.T. Nelson, F.A. Schmitt, L.J. Van Eldik, Using mice to model Alzheimer's dementia: an overview of the clinical disease and the preclinical behavioral changes in 10 mouse models, Front. Genet. 5 (88) (2014), http://dx.doi.org/10.3389/fgene.2014.00088.
[53] S.A. Yuzwa, X. Shan, M.S. Macauley, T. Clark, Y. Skorobogatko, K. Vosseller, D.J. Vocadlo, Increasing O-GlcNAc slows neurodegeneration and stabilizes tau against aggregation, Nat. Chem. Biol. 8 (2012) 393––399, http://dx.doi.org/10.1038/nchembio.797.
[54] S.A. Yuzwa, A.H. Cheung, M. Okon, L.P. McIntosh, D.J. Vocadlo, *O*-GlcNAc modification of tau directly inhibits its aggregation without perturbing the conformational properties of tau monomers, J. Mol. Biol. 426 (2014) 1736–1752, http://dx.doi.org/10.1016/j.jmb.2014.01.004.
[55] S.A. Yuzwa, X. Shan, B.A. Jones, G. Zhao, M.L. Woodward, X. Li, Y. Zhu, E.J. McEachern, M.A. Silverman, N.V. Watson, C.-X. Gong, D.J. Vocadlo, Pharmacological inhibition of *O*-GlcNAcase (OGA) prevents cognitive decline and amyloid plaque formation in bigenic tau/APP mutant mice, Mol. Neurodegener. 9 (2014) 42, http://dx.doi.org/10.1186/1750-1326-9-42.