QUERY FORM

MP	
Manuscript ID	2016MP000522 [Art. Id: mp2017103]
Author	
Editor	
Publisher	

Journal: MP

Author :- The following queries have arisen during the editing of your manuscript. Please answer queries by making the requisite corrections at the appropriate positions in the text.

Query No.	Description	Author's Response
GQ	Author surnames have been highlighted - please check these carefully and indicate if the first name or surname have been marked up incorrectly. Please note that this will affect indexing of your article, such as in PubMed.	
Q1	Please provide the manufacturer's name and city name for Olympus FluoView FV1000, Germany.	
Q2	Please provide the manufacturer name and location details (city, state (if USA, Canada, Australia) and country) for Fiji Software.	
Q3	Please provide volume number. and page range in ref. 38.	
Q4	Please provide department name for all affiliations, if applicable.	

AOP

SPRINGER NATURE

Molecular Psychiatry (2017) 00, 1–10
© 2017 Macmillan Publishers Limited, part of Springer Nature. All rights reserved 1359-4184/17
www.nature.com/mp

IMMEDIATE COMMUNICATION

Tau-dependent suppression of adult neurogenesis in the stressed hippocampus

C Dioli[1,2], P Patrício[1,2], R Trindade[1,2], LG Pinto[1,2], J Silva[1,2], M Morais[1,2], E Ferreiro[3], S Borges[1,2], A Mateus-Pinheiro[1,2], AJ Rodrigues[1,2], N Sousa[1,2], JM Bessa[1,2], L Pinto[1,2] and I Sotiropoulos[1,2]

Stress, a well-known sculptor of brain plasticity, is shown to suppress hippocampal neurogenesis in the adult brain; yet, the underlying cellular mechanisms are poorly investigated. Previous studies have shown that chronic stress triggers hyperphosphorylation and accumulation of the cytoskeletal protein Tau, a process that may impair the cytoskeleton-regulating role (s) of this protein with impact on neuronal function. Here, we analyzed the role of Tau on stress-driven suppression of neurogenesis in the adult dentate gyrus (DG) using animals lacking Tau (Tau-knockout (Tau-KO)) and wild-type (WT) littermates. Unlike WTs, Tau-KO animals exposed to chronic stress did not exhibit reduction in DG proliferating cells, neuroblasts and newborn neurons; however, newborn astrocytes were similarly decreased in both Tau-KO and WT mice. In addition, chronic stress reduced phosphoinositide 3-kinase/mammalian target of rapamycin/glycogen synthase kinase-3β/β-catenin signaling in the DG, known to regulate cell survival and proliferation in WT but not in Tau-KO. These data establish Tau as a critical regulator of the cellular cascades underlying stress deficits on hippocampal neurogenesis in the adult brain.

Molecular Psychiatry (2017) **00,** 000–000. doi:10.1038/mp.2017.103

Uncorrected P

INTRODUCTION

Chronic stress and excessive glucocorticoid (GC) exposure are suggested to increase susceptibility to brain pathology as they are associated with neuroplastic deficits and impaired cognition, as well as mood disorders such as depression.[1–3] The hippocampus is a well-known target of chronic stress,[4] with previous studies demonstrating structural and functional changes in the hippocampal formation.[1,5,6] The dentate gyrus (DG) constitutes the anatomical and functional input of the hippocampus and it suffers from both stress-induced remodeling of the dendritic arbor of its granular cells and suppression of neurogenesis and gliogenesis.[7–10] However, it is mechanistically unclear how stress precipitates the suppression of cytogenesis in the adult DG as well as whether similar stress-driven pathways operate in the reduction of newborn neuronal and glial populations.

During the neurogenic process, newborn cells require a high degree of cytoskeletal plasticity, which allows them to divide, migrate and differentiate.[11] Through its different interacting proteins (e.g. microtubule (MT), actin, Fyn),[12] the Tau protein is involved in different cellular processes such as differentiation and morphogenesis of neurons including axonal growth,[11–15] synaptic plasticity[16] and regulation of *N*-methyl-D-aspartate receptors.[17] The function(s) and localization of Tau depend on its phosphorylation status, with hyperphosphorylation diminishing its MT-binding capacity.[14,18] Tau–MT interaction may also depend on the expression of different Tau isoforms; for example, Tau isoforms that contain three MT-binding domains (3R-Tau; present in neuroblasts) exhibit lower MT affinity, whereas four MT-binding domain Tau (4R-Tau), mainly found in mature neurons, binds with higher avidity to MTs. In pathological conditions, namely in Alzheimer's disease, both Tau hyperphosphorylation and abnormal 4R/3R-Tau ratio are related to neuronal atrophy and malfunction[17,19] accompanied by impaired neurogenesis in the hippocampus.[11,20–22] Herein, and in light of previous studies showing that chronic stress and GC evoke Tau hyperphosphorylation and accumulation, as well as cytoskeletal disturbances and neurostructural remodeling,[23–25] we explored the potential involvement of Tau on stress-driven deficits in cell fate, survival and differentiation in the adult hippocampal DG of animals lacking Tau (Tau-knockout (Tau-KO)) and wild-type (WT) littermates.

MATERIALS AND METHODS

Animals and experimental design

Six- to seven-month-old male Tau-KO animals[26] and their WT littermates (C57BL/6J background) were used in this study. For assessment of cell proliferation, half of the animals were injected with 5-bromo-2′-deoxyuridine (BrdU; 50 mg kg^{-1} per day) for 3 consecutive days before killing. For monitoring cell survival and differentiation, the rest of the animals were injected with BrdU (same injection scheme) 4 weeks before killing (Figure 1a and see also Supplementary Information).

Stress paradigm

Stressed mice were submitted to a 9-week protocol of chronic unpredictable stress consisting of four different stressors:

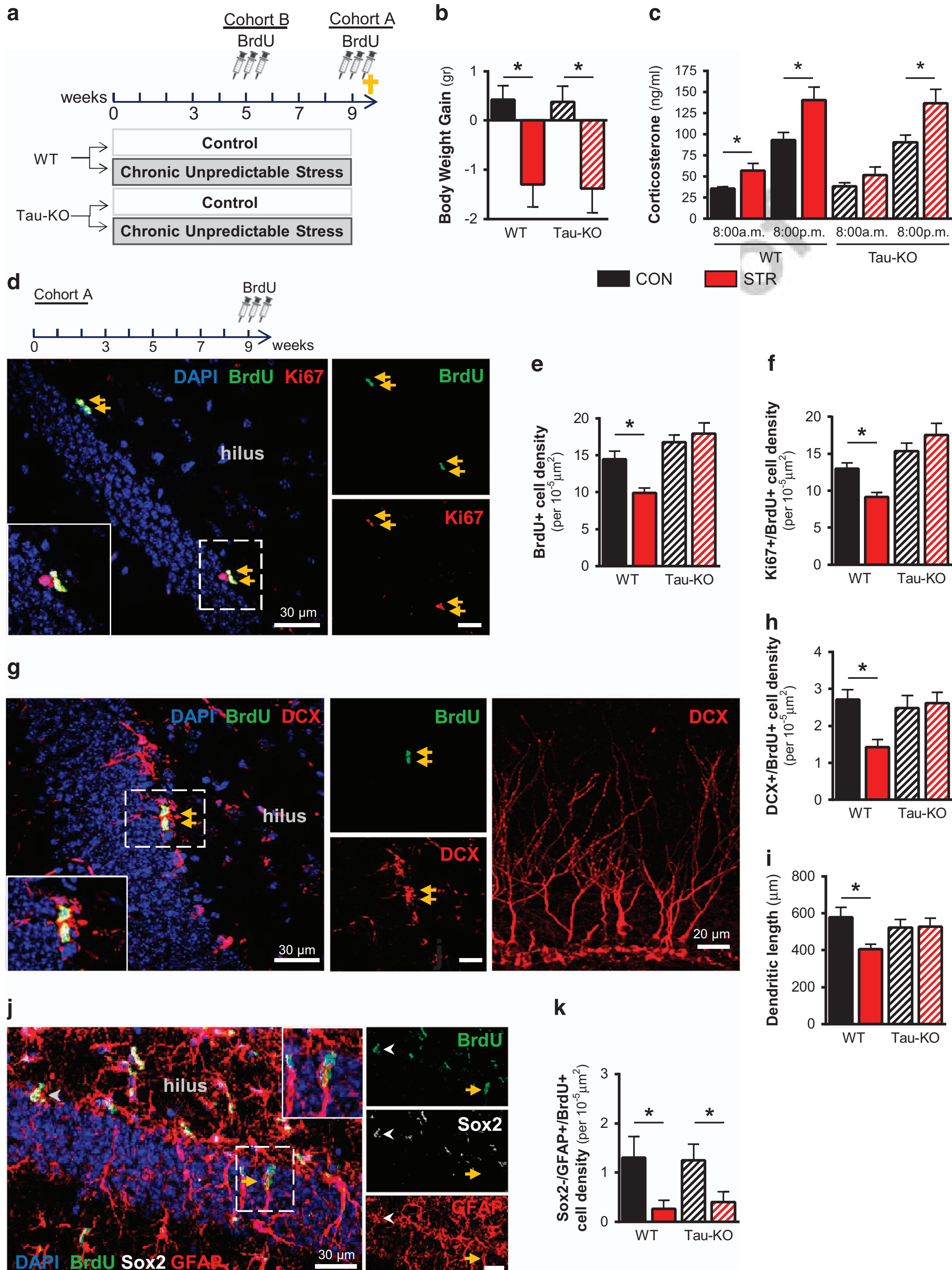
[1]Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Braga, Portugal; [2]ICVS/3B's-PT Government Associate Laboratory, Braga/Guimarães, Portugal; [3]Center for Neuroscience and Cell Biology (CNC), University of Coimbra, Coimbra, Portugal and [4]Institute for Interdisciplinary Research of the University of Coimbra (IIIUC), Coimbra, Portugal. Correspondence: Dr I Sotiropoulos, Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Campus de Gualtar, Braga 4710-057, Portugal.
E-mail: ioannis@med.uminho.pt
Received 23 May 2016; revised 21 March 2017; accepted 28 March 2017

Q4

restraint, vibrating platform, overcrowding and exposure to a hot air stream (one stressor per day for 3 h; 30 min for air stream). The time of the day and the order of stressors were randomly applied for each week as described previously.[24,27] Three experimental replicates for these studies were used (8–10 mice per group for each experimental replicate). For corticosterone (CORT) measurement, see Supplementary Information.

Immunofluorescent and western blot analysis

For double or triple immunofluorescent staining, coronal or vibratome sections of paraformaldehyde-perfused brains were used and analyzed by confocal microscope (Olympus FluoView FV1000, Germany) as previously described.[8,9] To assess cell densities, the corresponding DG areas were determined (Olympus BX51 microscope; Stereo Investigator Software, Microbrightfield, VT, USA). For three-dimensional dendritic reconstruction, confocal



© 2017 Macmillan Publishers Limited, part of Springer Nature.

images of doublecortin (DCX) staining were analyzed using the Fiji Software and simple neurite tracer plugin.[28] For western blot (WB) analysis, postnuclear protein extracts of macrodissected DG were analyzed as described previously.[24,25] For more information, see Supplementary Information.

Q2

Behavioral testing

In the Contextual Fear Discrimination Learning test, animals were placed in context A for 3 min and received a single 2-s foot shock at the end. The next day and for the following 8 days, animals were placed in two similar contexts for 3 min (the shock-associated context A and the similar, no-shock context B with 1 h difference), which are most likely recruiting the DG as described previously.[29,30] The discrimination ratio was calculated based on the formula: $\text{Freezing}_{\text{contA}} - \text{Freezing}_{\text{contB}}/\text{Freezing}_{\text{contA+B}}$.[30] In the Novel Object Recognition (NOR) test, animals were placed in the test arena, which contained two identical objects (10 min). The next day, animals were presented with a novel object (NO) and a familiar object (FO) (10 min); discrimination index was calculated based on the formula DI = (time in spent exploring NO − time in FO)/(time in NO+time in FO). In the Y-maze test, each animal was allowed to explore two arms of Y-maze apparatus for 10 min, and 1 h later, animal was placed in the Y-maze apparatus with access to all three arms (5 min). Animal behavior was analyzed with a video tracking system (Viewpoint, Champagne-au-Mont-d'or, France)—see also Supplementary Information.

Statistical analysis

Numerical data are presented as group means ± s.e.m. All data sets were subjected to two-way analysis of variance (ANOVA) before application of appropriate *post hoc* pair-wise comparisons (GraphPad Prism v.6.01; GraphPad Software, La Jolla, CA). Values of $P < 0.05$ were considered as significant differences.

RESULTS

Stress-induced reduction of proliferating cells in the DG is blocked by Tau depletion

For monitoring the role of Tau in the modulation of the neurogenic alterations induced by chronic stress, we exposed animals lacking Tau protein (Tau-KO)[26] and their WT littermates to a chronic unpredictable stress paradigm for 9 weeks (see Figure 1a). To evaluate stress effects, we monitored body weight and serum CORT levels at the end of the stress protocol. Chronically stressed animals (STR) of both genotypes exhibited decreased weight gain compared with their non-stressed, control (CON) littermates ($p_{\text{wt}} = 0.006$ and $p_{\text{KO}} = 0.01$; two-way ANOVA *Stress* overall effect ($F_{1,76} = 21.7$, $P < 0.001$); Figure 1b). Furthermore, we found an overall effect of *Stress* on basal circulating CORT levels at the onset of the dark period (ZT12/2000 hours; two-way ANOVA, $F_{1,34} = 15.34$, $P = 0.0004$) (Figure 1c); *post hoc* analysis revealed a significant increase in CORT levels in both stressed WT ($P = 0.03$) and Tau-KO ($P = 0.04$) animals when compared with the corresponding CON (non-stressed) animals. Similarly, we found a *Stress* effect on CORT levels monitored at the beginning of the light phase (ZT00/0800 hours; $F_{1,34} = 6.797$, $P = 0.013$) (Figure 1c).

To evaluate alterations in the fast proliferating cells and the number of newly born cells in the subgranular zone of the DG, animals of cohort A were injected with BrdU (50 mg kg^{-1} per day) for three consecutive days before killing (Figures 1a and d). BrdU immunofluorescence analysis in the DG showed a clear interaction between *Stress* and *Genotype* (two-way ANOVA, $F_{1,32} = 6.538$, $P = 0.015$) in BrdU+ cells density (Figure 1d), with *post hoc* analysis revealing a significant reduction in stressed WT ($P = 0.007$) animals but not Tau-KO ($P = 0.91$) animals when compared with their corresponding CONs (Figure 1e). As BrdU+ cells in the DG remained in clusters of two to four cells, we also monitored the number of DG clusters; similarly to the cell density, two-way ANOVA statistical analysis showed a *Stress* × *Genotype* interaction in the number of clusters ($F_{1,108} = 7.628$, $P = 0.060$) with stressed WT exhibiting less clusters than stressed Tau-KO ($P = 0.02$; data not shown). In addition, we performed staining with the endogenous cell cycle marker Ki67 (Figure 1d) to analyze the pool of BrdU+ cells that are proliferative. Analysis of Ki67 and BrdU double-labeled cells showed a *StressxGenotype* interaction in cell density at the DG ($F_{1,26} = 9.498$, $P = 0.004$) with stressed WT animals showing a reduction when compared with CONs ($P = 0.02$). In contrast, stressed Tau-KO animals were not different from non-stressed (CON) Tau-KO ($P = 0.49$) (Figure 1f). No differences were found between CON animals of both genotypes ($P = 0.33$). Moreover, to analyze the total number of proliferating cells, the number of Ki67+ cells were monitored and similar results to Ki67+/BrdU+ double staining were found (Supplementary Figure 1). All the above findings suggest that the absence of Tau, while not affecting the DG proliferation under CON conditions, blocks the effect of chronic stress on cell proliferation in the adult hippocampal DG.

Tau ablation attenuates the stress-induced suppression of neurogenesis but not astrogenesis

We next evaluate the impact of stress on the DG neuroblasts measuring the BrdU-labeled cells that were costained with DCX, a cytoskeletal protein expressed in neuronal progenitors (Figure 1g, left panel). Two-way ANOVA revealed a *Stress* × *Genotype* interaction in the density of DCX/BrdU double-labeled cells at the DG ($F_{1,42} = 5.441$, $P = 0.024$). Further analysis showed that exposure to chronic stress reduced the DG density of DCX/BrdU neuroblasts in WT animals ($P = 0.04$); this was not found in the DG of Tau-KO animals where stressed and non-stressed mice exhibited similar levels of this cell sub-population ($P = 0.98$; Figure 1h). Furthermore, we also monitored dendritic length of DCX+ immature neurons in both WT and Tau-KO DGs (see Figure 1g, right panel). We found

Uncorrected Proof

Figure 1. Stress-induced suppression of proliferating cells and neuroblasts in the adult hippocampus is blocked by Tau ablation. (**a**) Schematic representation of experimental design where wild-type (WT) and Tau-knockout (Tau-KO) mice were exposed to 9 weeks of chronic stress; control (non-stressed; CON) and stressed (STR) animals of both genotypes were divided into two cohorts receiving 5-bromo-2′-deoxyuridine (BrdU) injections either immediately (Cohort A) or 4 weeks (Cohort B) before killing. (**b** and **c**) STR animals of both genotypes exhibited reduced body weight as compared with their corresponding (CON) counterparts. In addition, chronic stress resulted in elevated corticosterone (CORT) levels in both WT and Tau-KO animals measured at 2000 hours (peak of CORT secretion). (**d**) Representative micrographs of BrdU/Ki67 double-labeled cells in the hippocampal dentate gyrus (DG) of Cohort A animals (BrdU-injected before killing). (**e** and **f**) Exposure to chronic stress reduced the density of BrdU+ cells in DG of WT animals of Cohort A; this stress effect was not found in Tau-KO animals (**d** and **e**). Similarly, density of Ki67/BrdU+ cells was reduced in WT animals but not in Tau-KO animals (**d** and **f**). (**g–i**) Microphotographs of immunofluorescent staining of doublecortin (DCX)/BrdU+ cells (**i**) with chronic stress affecting the DCX/BrdU cell density (**h**) and dendritic length (**i**) only in WT animals. (**j** and **k**) Immunofluorescent staining of Sox2, GFAP and BrdU where Sox2−/GFAP+/BrdU+ (arrow) and Sox2+/GFAP+/BrdU+ (arrowhead) cells are shown. On the contrary to DCX+ cells, the density of Sox2−/GFAP+/BrdU+ cell population (**j**) was similarly decreased by stress in animals of both genotypes (**k**). All numerical data are shown as mean ± s.e.m. (*$P < 0.05$). GFAP, glial fibrillary acidic protein.

© 2017 Macmillan Publishers Limited, part of Springer Nature.

that chronic stress reduced dendritic length of immature WT neurons ($P = 0.03$) but not Tau-KO neurons ($P > 0.99$) (*Stress* × *Genotype* interaction $F_{1,55} = 4.23$, $P = 0.044$; Figure 1i). Importantly, this stress effect on immature neurons was found in the outer molecular layer (Supplementary Figure 2). We also quantified the pools of glial fibrillary acidic protein (GFAP)/BrdU double-labeled cells that were also Sox2+ (neural stem cells and progenitors) or Sox2 − (newborn astrocytes) (Figures 1j and k and Supplementary Figure 3). Chronic stress had a similar effect on both WT and Tau-KO DG ($P = 0.004$ and $P = 0.003$, respectively), with significant reductions in the number of Sox2 −/GFAP+/BrdU+ cells (main *Stress* effect, $F_{1,28} = 9{,}994$, $P = 0.003$; Figure 1j), while no difference was found among all groups in Sox2+/GFAP+/BrdU+ (Supplementary Figure 3).

Next, we monitored the effect of chronic stress on the generation and survival of adult born neurons using a different cohort of animals that were injected with BrdU (for three consecutive days) 4 weeks before killing (Figure 2a). Two-way ANOVA analysis of BrdU-stained cells showed a clear interaction between *Stress* and *Genotype* in BrdU+ cell density ($F_{1,26} = 4.366$, $P = 0.046$) with stressed WT exhibiting a reduction compared with their CONs ($P = 0.04$). Interestingly, stress had no effect on Tau-KO animals ($P = 0.81$) (Figure 2b). These findings suggest that chronic stress reduces the number of newly generated cells in the DG of adult WT animals, whereas Tau ablation blocks this effect of stress. To assess the phenotype of these BrdU-labeled cells, we performed double labeling of neuronal (NeuN) or GFAP and BrdU. Our statistical analysis showed a *Stress* × *Genotype* interaction on NeuN/BrdU+ cell density (two-way ANOVA; $F_{1,26} = 4.321$, $P = 0.047$), demonstrating that chronic stress reduced the number of newborn neurons in WT DG but not in Tau-KO DG ($P = 0.03$ and $P = 0.90$, respectively) (Figure 2c). Furthermore, analysis of Sox2 −/GFAP+/BrdU+ population in this cohort (see Figures 2d and e) exhibited an overall effect of Stress on cell density of this population ($F_{1,30} = 17{,}59$, $P < 0.0001$) with stress affecting both WT and Tau-KO animals ($P = 0.03$ and $P = 0.02$, respectively) (Figure 2d); no differences were found in Sox2+/GFAP+/BrdU+ cell sub-population (Supplementary Figure 4). Similar results were obtained when we used another marker to label neural stem cells, BLBP (Supplementary Figure 5). Altogether, these findings provide further support for a selective role of Tau in the stress-triggered reduction of neuroblasts and newly born neurons, but no astrocytes, in the adult DG.

Chronic stress triggers Tau hyperphosphorylation and alteration of Tau isoforms in adult DG

As previous studies have shown that chronic stress triggers Tau hyperphosphorylation and accumulation,[24] we next monitored the effect of stress on Tau using both immunofluorescence and WB analysis. As shown in Figure 3, levels of phosphorylated Tau at epitopes Ser396/404 (using PHF1 antibody) and Thr231 were increased in the DG of stressed WT ($P = 0.02$ and $P = 0.03$, respectively), whereas total Tau levels were not significantly altered by stress (Figures 3a, f and g). Measurement of 3R-Tau, characteristically expressed in early stages of neurogenesis (e.g. neuroblasts), showed that chronic stress significantly reduced its expression ($P = 0.02$), whereas levels of 4R-Tau, mainly expressed in mature neurons, were increased in WT DG ($P = 0.03$) (Figures 3a and g). Furthermore, in line with our WB analysis, we found that chronic stress increased the density of 4R-Tau+ cells ($P = 0.04$) as well as PHF1/BrdU+ cells ($P = 0.03$) in DG of WT animals (Figures 3i–l), which was also reflected in a significant increase in the percentage of PHF1+ cells among the BrdU DG sub-population in stressed animals ($P = 0.04$; data not shown).

Differential impact of stress on neuroplastic signaling and cognitive function in WT and Tau-KO animals

We next monitored the phosphoinositide 3-kinase/mammalian target of rapamycin/glycogen synthase kinase-3β/β-catenin (PI3K/mTOR/GSK3β/β-catenin) molecular pathway, known to regulate cell survival and proliferation in the adult DG[31,32] (Figures 3a–e). Molecular analysis of the DG revealed that chronic stress decreases PI3K protein levels in WTs ($P = 0.04$) while there is a tendency to increase it in Tau ($P = 0.07$) (two-way ANOVA, *Stress* × *Genotype* interaction; $F_{1,42} = 8.373$, $P = 0.006$) (Figures 3a and b). Furthermore, chronic stress decreases the levels of mTOR in WT ($P = 0.014$) but has no effect on Tau-KO DG ($P = 0.558$) (*Stress* × *Genotype* interaction; $F_{1,42} = 4.206$, $P = 0.046$; Figures 3a and c). In line, immunofluorescent analysis revealed a stress-driven reduction in density of mTOR/BrdU+ cell population of WT, but not Tau-KO, DG (*Stress* × *Genotype* interaction; $F_{1,30} = 4.223$, $P = 0.047$; Figures 3m and n). Furthermore, exposure to chronic stress increased levels of pTyr216-GSK3β in WTs ($P = 0.026$) followed by corresponding increased cytoplasmic levels of β-catenin in WTs only ($P = 0.026$) (*Stress* × *Genotype* interaction; $F_{1,42} = 7.016$ $P = 0.011$; Figures 3a and d). Note that these stress-evoked changes were not found in Tau-KO animals, suggesting that absence of Tau is involved in activation of GS3Kβ, as recently shown.[33] Altogether, the above findings indicate that chronic stress suppresses the PI3K-driven cellular cascade related to reduced mTOR levels and simultaneously increases the levels of active GSK3β; as a result, there is diminished cell proliferation and survival[32,34] (Figure 3h).

The hippocampus is a key brain structure regulating memory while animals with reduced DG neurogenesis exhibit deficits of hippocampus-dependent cognitive functions.[30,35] Thus, we monitored pattern separation as well as spatial and recognition memory using the Contextual Fear Discrimination Learning, Y-maze and NOR tests (Figure 4). Our findings show that exposure to chronic stress reduced the discrimination ratio in WT at days 7 and 8 (both $P < 0.05$; Figure 4b) reflecting stress-driven cognitive deficits in animals ability to distinguish between two similar contexts (context A and context B), recruiting the DG[29,30] (Figure 4; see also Supplementary Information). Note that there was no stress effect on Tau-KO animals (*StressxGenotype* interaction $F_{1,30} = 5.336$, $P = 0.027$; Figures 4a–c). In the Y-maze test (Figure 4d), we also found an interaction between *Stress* and *Genotype* on duration and frequency that animals visit the novel arm of the Y-maze apparatus (*dur* $F_{1,40} = 4.879$, $P = 0.033$; *frequ* $F_{1,40} = 4.985$, $P = 0.031$). Further analysis showed that chronic stress reduced the duration and frequency of novel arm visits in WT animals ($p_{dur} = 0{,}019$; $p_{frequ} = 0{,}010$) but not in Tau-KO animals (Figures 4e and f). The above findings were in agreement with the NOR results where stressed WT animals exhibited reduced discrimination index compared with their corresponding CONs ($P = 0.035$), whereas stressed and CON Tau-KOs exhibit no differences ($P = 0.861$) (two-way ANOVA *Stress* × *Genotype* interaction; $F_{1,70} = 6.122$, $P = 0.015$) (Figures 4g and h). Moreover, stressed WT animals but not Tau-KO animals exhibited increased anxiety levels as assessed by reduced time (WT $P = 0.003$; Tau-KO $P = 0.83$) that animals spend in the open arms of the elevated plus maze apparatus (two-way ANOVA *Stress* × *Genotype* interaction; $F_{1,44} = 6.682$, $P = 0.001$) (Figures 4i and j).

DISCUSSION

Prolonged exposure to stressful conditions and excessive GC levels is known to suppress hippocampal neurogenesis in adult brain.[4,6,8] While the suggested underlying mechanisms include GC-driven glutamate release and *N*-methyl-D-aspartate receptors signaling[36] and/or reduced levels of growth/neurotrophic factors,[37] little attention has been given to the importance of

Uncorrected Proof

© 2017 Macmillan Publishers Limited, part of Springer Nature.

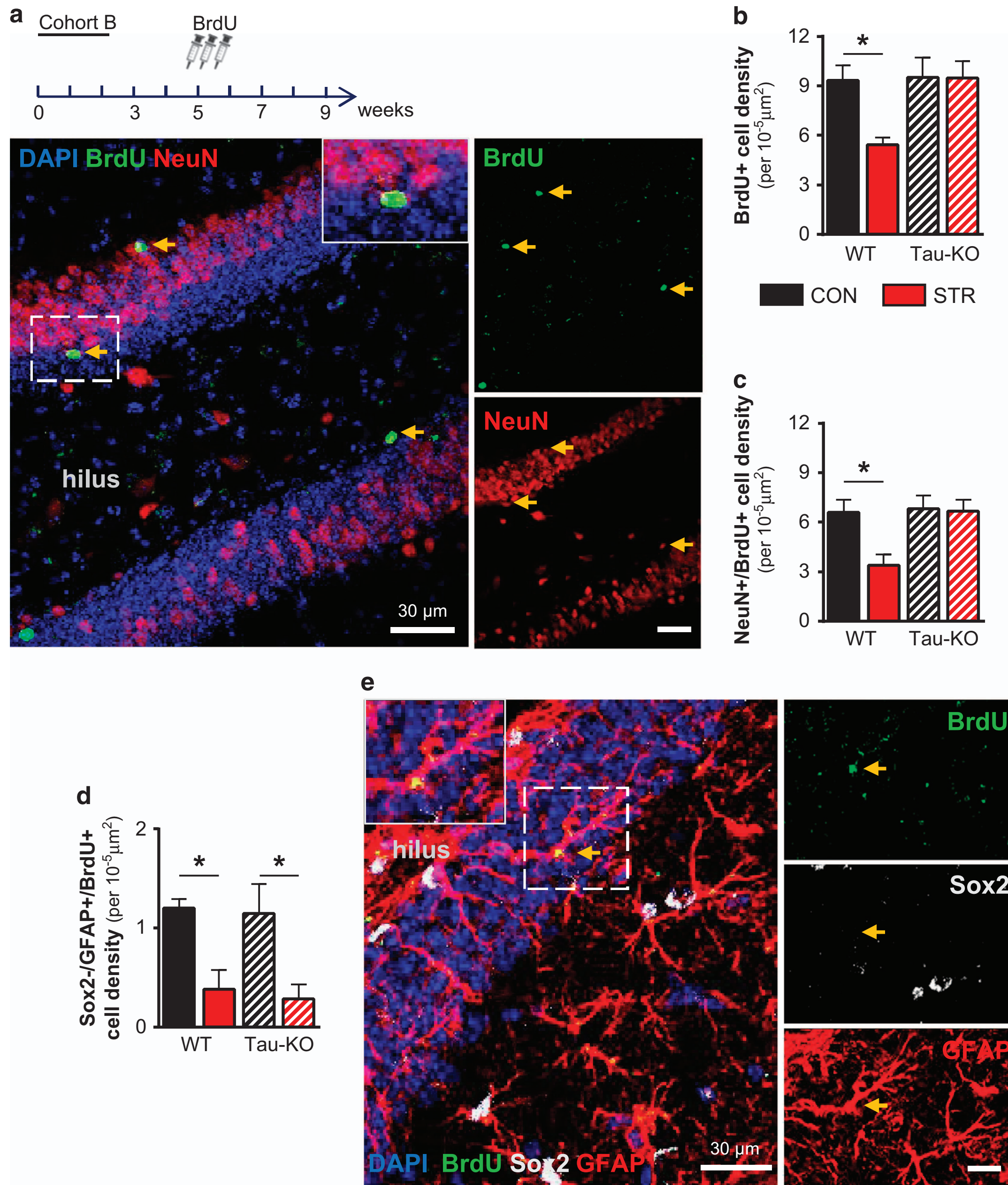


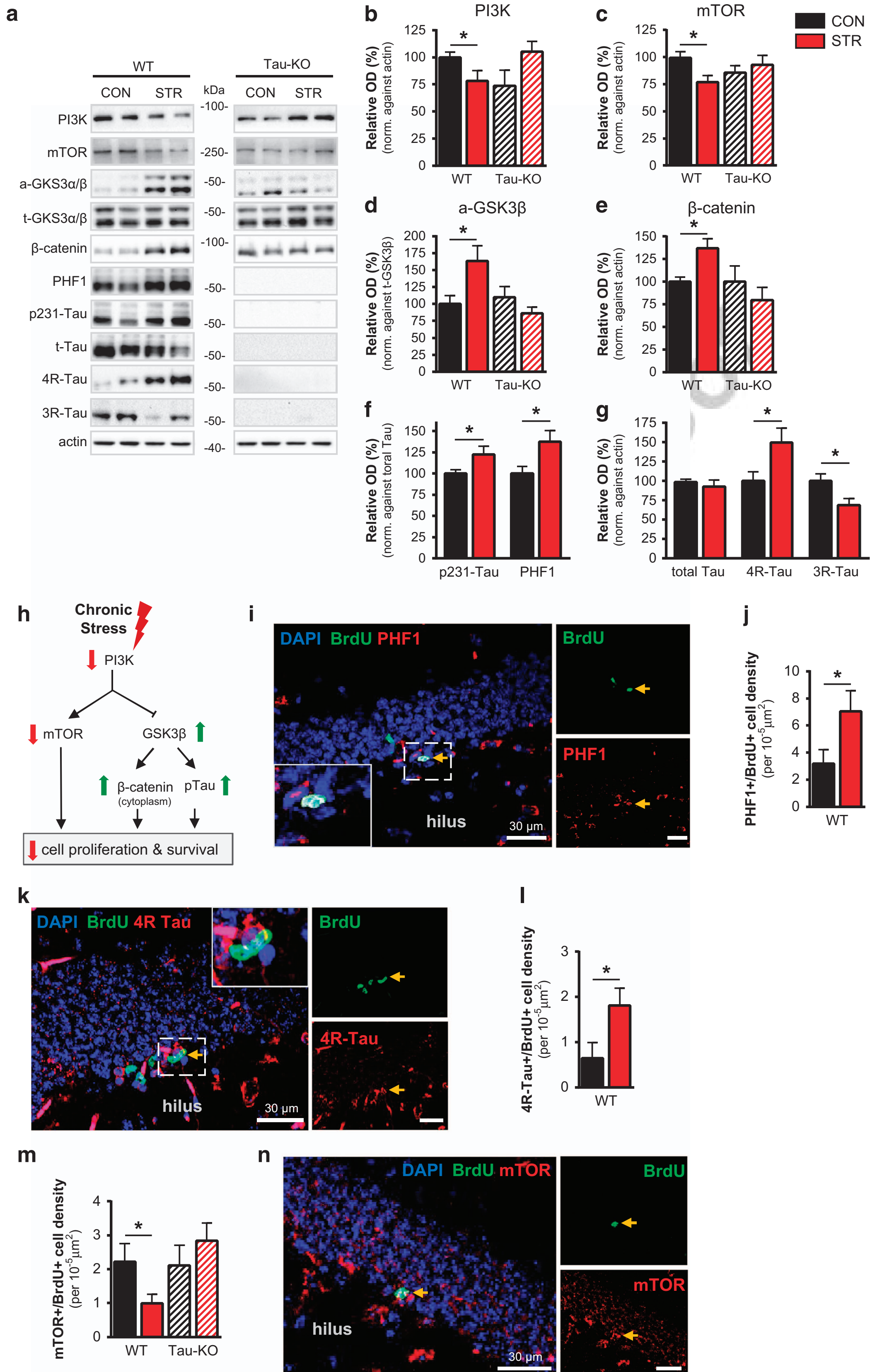
Figure 2. Absence of Tau attenuates the stress-driven neurogenic, but not astrogenic, deficits in adult dentate gyrus (DG). (**a–e**) DG analysis of animals injected with 5-bromo-2′-deoxyuridine (BrdU) 4 weeks before killing (Cohort **b**) with NeuN/BrdU (**a**) and GFAP/BrdU (**e**) double immunofluorescent staining. (**b**) Chronic stress evoked a decrease of BrdU+ cell density in WT DG but not in Tau-knockout (Tau-KO) DG. (**c**) Similarly, stress reduced the cell density of double-labeled NeuN/BrdU population in this cohort (reflecting newborn neurons) only in WT animals. (**d** and **e**) In contrast, chronic stress reduced the density of Sox2 − /GFAP+/BrdU+ cells (representing newborn astrocytes) in animals of both genotypes. All numerical data are shown as mean ± s.e.m (*$P < 0.05$). GFAP, glial fibrillary acidic protein; NeuN, neuronal.

cytoskeletal dynamics in newborn cells, which allows them to divide, migrate, differentiate and synaptically integrate into the pre-existent hippocampal network. Given that Tau has an important role in regulating neuronal architecture and function through its interaction with different cellular targets (e.g. MTs, F-actin, Fyn),[12] and the fact that stress and GC are known to trigger Tau hyperphosphorylation,[23,24] this study monitored the involvement of Tau in the suppression of hippocampal neurogenesis by chronic stress. We demonstrated that Tau ablation blocks the detrimental effect of chronic stress of proliferating cells, neuroblasts and newborn neurons, but not astrocytes, in the adult DG indicating a critical role for Tau in the stress-induced DG neurogenic impairment. These findings complement our recent demonstration that dendritic atrophy of (pre-existed) hippocampal and cortical neurons triggered by exposure to chronic stress was attenuated in animals lacking Tau.[38,39] Altogether, these findings highlight Tau protein as a key mediator of the deleterious effects of stress on hippocampal plasticity and function, related to both dendritic remodeling of (old) neurons and on the genesis of newly born neurons in adult brain. Interestingly, the absence of Tau did not block the suppressive role of chronic stress on the astrocytic population. This finding is likely related to the differential expression of Tau in these cell population (Tau is mainly expressed in neurons, with low Tau levels being found in

© 2017 Macmillan Publishers Limited, part of Springer Nature.

astrocytes[40]), but it may also suggest that different cellular cascades operate in the stress-triggered reduction of newborn neuronal and glia populations.

Tau protein is described to regulate cytoskeletal assembly and dynamics participating in different cytoskeleton-involving cellular process including axonal branching, neuronal polarity, axonal



© 2017 Macmillan Publishers Limited, part of Springer Nature.

transport and intracellular trafficking.[12,14,15] However, adult Tau-KO animals do not display behavioral, neurostructural or axonal abnormalities[26,41] Accordingly, all different DG sub-populations (proliferating cells, neuroblasts, newborn astrocytes and neurons) monitored in this study were not altered in Tau-KO under CON (non-stressed) conditions when compared with their WT littermates.[11,42] The absence of Tau-KO phenotype may reflect the recruitment of compensatory mechanisms during brain development, for example, through altered expression of other cytoskeletal proteins.[26,43] In addition, it is reported that Tau-KO cells exhibit a transient delay in their dendritic maturation,[42] which may explain why, in adult brain neurons, no severe neurostructural or proteomic differences were found.[38,39] However, accumulating evidence suggests that Tau protein may be necessary for the manifestation of changes of brain plasticity in adult brain (related to both dendritic plasticity and neurogenesis) driven by different intrinsic and extrinsic conditions such as Aβ[44] and hibernation,[45,46] as well as acute and chronic stress.[39,42]

Similarly to brain development,[47,48] the expression of 3R- and 4R-Tau isoforms is shifted during adult neurogenesis.[11]

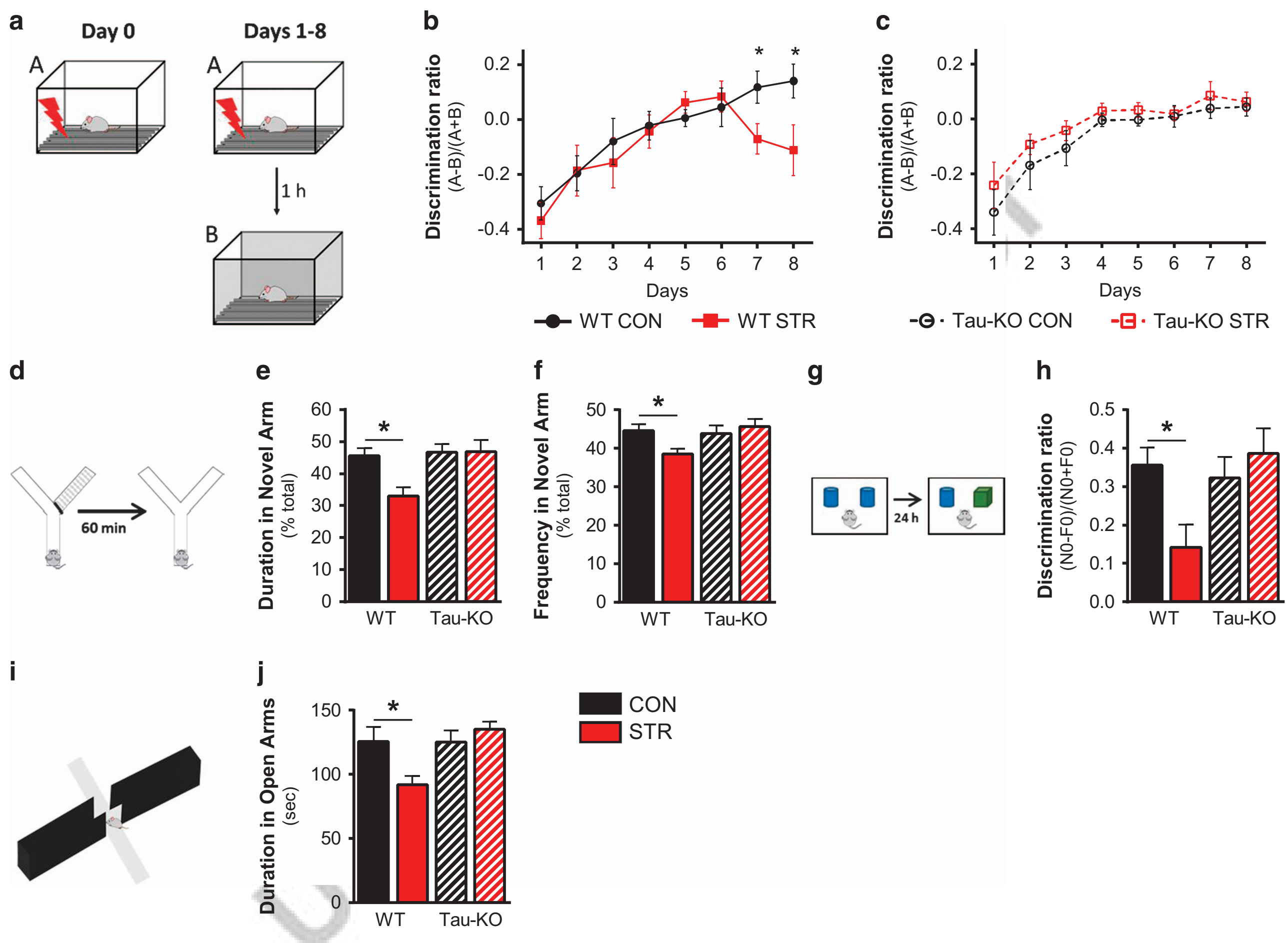


Figure 4. Cognitive deficits evoked by stress are attenuated in Tau-knockout (Tau-KO) animals. (**a**) Experimental design of the test discrimination between two similar contexts, the shock-associated context A and the shock-free context B. (**b** and **c**) Chronic stress decreased the discrimination ratio in days 7 and 8 in wild-type (WT) animals but not Tau-KO animals. In the Y-maze test (**d**), stressed WT animals exhibited reduced duration (**e**) and frequency (**f**) of visits in novel arm of apparatus as compared with control (CON) WTs; this reduction was not observed in Tau-KO animals. (**g** and **h**) Similarly, discrimination index in the Novel Object Recognition (NOR) test (**g**) was reduced by stress in WT animals but not Tau-KO animals (**h**), suggesting a significant resilience of Tau-KO animals against cognition-impairing effect of stress. (**i** and **j**) Chronic stress also reduced the time that WT animals but not Tau-KO animals spent in open arms of elevated plus maze (EPM) apparatus indicating anxious behavior. All numerical data are shown as mean ± s.e.m. (*$P < 0.05$).

Figure 3. Chronic stress suppresses neuroplastic signaling in adult dentate gyrus (DG) in a Tau-dependent manner. Representative blots (**a**) and quantification (**b**–**g**) of the cellular cascades (**h**) analyzed in DG of wild-type (WT) and Tau-knockout (Tau-KO) animals. Chronic stress reduces the expression of phosphoinositide 3-kinase (PI3K) (**b**) and mammalian target of rapamycin (mTOR) (**c**) protein levels followed by increased levels of (active) pTyr216-GSK3β (**d**) and β-catenin (cytoplasm) (**e**) in DG of WT animals but not in Tau-KO animals. In addition, phosphorylation levels of Tau at Thr231 and Ser396/404 (PHF1) epitopes are increased by chronic stress in WT DG (**f**). Furthermore, while it did not affect total Tau protein levels, exposure to chronic stress increased the protein levels of 4R-Tau isoforms, whereas decreased 3R-Tau levels in WT DG (**g**). (**i**–**n**) Representative microphotographs and quantification of PHF1/BrdU+ (**i** and **j**), 4R-Tau/BrdU+ (**k** and **l**) and mTOR/BrdU+ (**m** and **n**) cells in DG showing the impact of chronic stress in line with findings of above western blot (WB) analysis. All numerical data are shown as mean ± s.e.m. (*$P < 0.05$).

© 2017 Macmillan Publishers Limited, part of Springer Nature.

Specifically, 3R-Tau isoforms, which bind with less avidity to MTs, are present in neuroblasts, which is viewed as relevant to endow these cells with the necessary cytoskeleton plasticity.[11] On the other hand, 4R-Tau isoforms, which exhibit higher affinity to MTs, are mainly expressed in mature neurons offering the necessary stability for the establishment/maintenance of dendritic/axonal structures of the newly integrated neuron. Our findings show that chronic stress increases the 4R/3R-Tau ratio in DG without affecting total Tau levels. Increased expression of 4R-Tau (without change of total Tau) is recently shown to lead to increased Tau phosphorylation and brain pathology.[49] Another study based on human pluripotent stem cell-derived neurons from patients with *tau* mutation relates premature developmental expression of 4R-Tau, changes in 4R/3R-Tau and hyperphosphorylation status with abnormal, earlier neuronal maturation.[50] Moreover, imbalance of 4R/3R-Tau and the consequent cytoskeletal disturbances are causally related to neuronal pathology in Tau pathologies such as Alzheimer's disease where impaired adult neurogenesis are also found[20,22,51] (but also see Boekhoorn *et al*.[52]). Importantly, genetically induced expression of 4R-Tau is shown to suppress proliferation in cells[53] and mouse hippocampus,[54] leading to downregulation of transcripts involved in cellular growth and proliferation. Given that alterations in the tight CON of Tau phosphorylation and isoform specificity can result in aberrations of proliferation,[55,56] our findings showing that chronic stress also increases Tau phosphorylation in DG of WT animals in epitopes Thr231 and Ser396/404 (the later detected by PHF1 antibody) are of particular relevance. Previous studies already suggested that increased PHF1-detected Tau phosphorylation in neuroblasts and newly differentiating neurons may underlie, at least in part, the impaired DG proliferation and neuronal maturation in prepathological stages of Alzheimer's disease Tg animals.[21] Combined with 4R/3R imbalance, the stress-driven increase in phosphorylation of these epitopes, shown to decrease MT affinity of Tau,[57] could impair the complex and tight regulation of Tau diminishing the cellular CON over the cytoskeletal dynamics and network essential for proliferating cells and neuroblasts.[14] Based on the current findings suggesting stress-driven disturbances on critical and interdependent parameters of Tau dynamics (isoforms and phosphorylation), future studies should monitor the impact of chronic stress on DG of animals with conditional deletion of specific Tau isoforms to clarify further the interplay and individual contribution of altered expression of Tau isoform, as well as phosphorylation in stress-driven neurogenic deficits.

The above Tau phosphoepitopes are targets of the kinase GSK3β,[57,58] whose active form (phosphorylated at Tyr216) is also increased in hippocampal DG of stressed WT animals but not of stressed Tau-KO animals. GSK3β is an essential component of the Wnt signaling pathways, with activation of PI3K inhibiting GSK3β and, in turn, triggering the translocation of β-catenin from the cytoplasm to the nucleus promoting proliferation and survival of progenitor cells.[31,34,59] Our findings suggest that chronic stress suppresses the above pathway in WT animals, which certainly helps to understand the stress-driven reduction of WT hippocampal neurogenesis. In line with the recently reported blockage of GSK3β activation in Aβ-driven Alzheimer's disease cellular model by Tau ablation,[33] our findings show that exposure to chronic stress revealed a Tau-dependent activation of GSK3 and reduction of the levels of mTOR in DG; a recent work shows that GSK3 also suppresses mTOR signaling in the hippocampus,[60] whereas reduced levels or inhibition of mTOR reduces DG neurogenesis.[32] While the mechanistic understanding of Tau role on GSK3β activation is under intensive investigation, the current findings are in agreement with previous experimental studies showing that chronic stress and/or GC activate GSK3β,[23,24] as well as a suppression of mTOR signaling in the hippocampus.[61–63] Importantly, human studies report deficits in mTOR signaling in PFC of patients of depression,[64] a disease state related to chronic stress and reduced neurogenesis.[1,2] A possible way through which mTOR impacts on neurogenesis may include the regulation of the spatial and local CON of Tau mRNA translation promoting axonal formation[65] as mTOR inhibition is known to reduce the expression levels of Tau protein and blocks neuronal polarity in the developing neuron.[65] While other cellular pathways cannot be excluded (for a review see Egeland *et al*.[66]), this study provides novel evidence that Tau could serve as an essential protein involved in the neurogenic deficits evoked by chronic stress in the adult hippocampus. This is in line with a very recent study showing that forced swim test, an acute stressor, caused Tau-dependent neurogenic deficits.[42] As differences of stress duration and severity may exhibit differential impact of brain connectome and function,[67] future studies should clarify whether the cellular role of Tau is similar under acute and chronic stress conditions and also monitor the potential beneficial role of blockage of GC receptors under stress[23,66,68] as both stress-induced Tau hyperphosphorylation and suppressed neurogenesis can be, at least partly, mimicked by GC administration.[24,69] Overall, our current findings add novel mechanistic insights about Tau involvement into the cellular cascades that convey the pathogenic role of chronic stress in brain plasticity and open new pathways for strategies designed to mitigate its deleterious effects.

Uncorrected Proof

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

We thank Dr Peter Davies (Albert Einstein College, NY, USA) for PHF1 antibody. IS, AJR and LGP are holders of Portuguese Foundation for Science and Technology (FCT) Investigator grants (IF/01799/2013, IF/00883/2013, IF/01079/2014, respectively). This work was funded by FCT research grants 'PTDC/SAU-NMC/113934/2009' (IS), the Portuguese North Regional Operational Program (ON.2) under the National Strategic Reference Framework (QREN), through the European Regional Development Fund (FEDER) as well as the Project Estratégico co-funded by FCT (PEst-C/SAU/LA0026-/2013) and the European Regional Development Fund COMPETE (FCOMP-01-0124-FEDER-037298).

REFERENCES

1 Sousa N, Almeida OF. Disconnection and reconnection: the morphological basis of (mal)adaptation to stress. *Trends Neurosci* 2012; **35**: 742–751.
2 de Kloet ER, Joels M, Holsboer F. Stress and the brain: from adaptation to disease. *Nat Rev Neurosci* 2005; **6**: 463–475.
3 McEwen BS, Bowles NP, Gray JD, Hill MN, Hunter RG, Karatsoreos IN *et al*. Mechanisms of stress in the brain. *Nat Neurosci* 2015; **18**: 1353–1363.
4 Leuner B, Gould E. Structural plasticity and hippocampal function. *Annu Rev Psychol* 2010; **61**: C111–C113.
5 Magarinos AM, McEwen BS. Stress-induced atrophy of apical dendrites of hippocampal CA3c neurons: involvement of glucocorticoid secretion and excitatory amino acid receptors. *Neuroscience* 1995; **69**: 89–98.
6 Lucassen PJ, Pruessner J, Sousa N, Almeida OF, Van Dam AM, Rajkowska G *et al*. Neuropathology of stress. *Acta Neuropathol* 2014; **127**: 109–135.
7 Czeh B, Simon M, Schmelting B, Hiemke C, Fuchs E. Astroglial plasticity in the hippocampus is affected by chronic psychosocial stress and concomitant fluoxetine treatment. *Neuropsychopharmacology* 2006; **31**: 1616–1626.
8 Bessa JM, Ferreira D, Melo I, Marques F, Cerqueira JJ, Palha JA *et al*. The mood-improving actions of antidepressants do not depend on neurogenesis but are associated with neuronal remodeling. *Mol Psychiatry* 2009; **14**: 764–773, 739.
9 Mateus-Pinheiro A, Pinto L, Bessa JM, Morais M, Alves ND, Monteiro S *et al*. Sustained remission from depressive-like behavior depends on hippocampal neurogenesis. *Transl Psychiatry* 2013; **3**: e210.
10 O'Leary OF, Cryan JF. A ventral view on antidepressant action: roles for adult hippocampal neurogenesis along the dorsoventral axis. *Trends Pharmacol Sci* 2014; **35**: 675–687.
11 Fuster-Matanzo A, Llorens-Martin M, Jurado-Arjona J, Avila J, Hernandez F. Tau protein and adult hippocampal neurogenesis. *Front Neurosci* 2012; **6**: 104.
12 Morris M, Maeda S, Vossel K, Mucke L. The many faces of tau. *Neuron* 2011; **70**: 410–426.

© 2017 Macmillan Publishers Limited, part of Springer Nature.

13 Brandt R. The tau proteins in neuronal growth and development. *Front Biosci* 1996; **1**: d118–d130.
14 Shahani N, Brandt R. Functions and malfunctions of the tau proteins. *Cell Mol Life Sci* 2002; **59**: 1668–1680.
15 Kalil K, Dent EW. Branch management: mechanisms of axon branching in the developing vertebrate CNS. *Nat Rev Neurosci* 2014; **15**: 7–18.
16 Kimura T, Whitcomb DJ, Jo J, Regan P, Piers T, Heo S *et al.* Microtubule-associated protein tau is essential for long-term depression in the hippocampus. *Philos Trans R Soc Lond Ser B* 2013; **369**: 20130144.
17 Ittner LM, Ke YD, Delerue F, Bi M, Gladbach A, van Eersel J *et al.* Dendritic function of tau mediates amyloid-beta toxicity in Alzheimer's disease mouse models. *Cell* 2010; **142**: 387–397.
18 Mondragon-Rodriguez S, Trillaud-Doppia E, Dudilot A, Bourgeois C, Lauzon M, Leclerc N *et al.* Interaction of endogenous tau protein with synaptic proteins is regulated by *N*-methyl-D-aspartate receptor-dependent tau phosphorylation. *J Biol Chem* 2012; **287**: 32040–32053.
19 Kimura T, Yamashita S, Fukuda T, Park JM, Murayama M, Mizoroki T *et al.* Hyperphosphorylated tau in parahippocampal cortex impairs place learning in aged mice expressing wild-type human tau. *EMBO J* 2007; **26**: 5143–5152.
20 Mu Y, Gage FH. Adult hippocampal neurogenesis and its role in Alzheimer's disease. *Mol Neurodegener* 2011; **6**: 85.
21 Demars M, Hu YS, Gadadhar A, Lazarov O. Impaired neurogenesis is an early event in the etiology of familial Alzheimer's disease in transgenic mice. *J Neurosci Res* 2010; **88**: 2103–2117.
22 Crews L, Rockenstein E, Masliah E. APP transgenic modeling of Alzheimer's disease: mechanisms of neurodegeneration and aberrant neurogenesis. *Brain Struct Funct* 2010; **214**: 111–126.
23 Sotiropoulos I, Catania C, Riedemann T, Fry JP, Breen KC, Michaelidis TM *et al.* Glucocorticoids trigger Alzheimer disease-like pathobiochemistry in rat neuronal cells expressing human tau. *J Neurochem* 2008; **107**: 385–397.
24 Sotiropoulos I, Catania C, Pinto LG, Silva R, Pollerberg GE, Takashima A *et al.* Stress acts cumulatively to precipitate Alzheimer's disease-like tau pathology and cognitive deficits. *J Neurosci* 2011; **31**: 7840–7847.
25 Pinheiro S, Silva J, Mota C, Vaz-Silva J, Veloso A, Pinto V *et al.* Tau mislocation in glucocorticoid-triggered hippocampal pathology. *Mol Neurobiol* 2015; **53**: 4745–4753.
26 Dawson HN, Ferreira A, Eyster MV, Ghoshal N, Binder LI, Vitek MP. Inhibition of neuronal maturation in primary hippocampal neurons from tau deficient mice. *J Cell Sci* 2001; **114**(Part 6): 1179–1187.
27 Cerqueira JJ, Mailliet F, Almeida OF, Jay TM, Sousa N. The prefrontal cortex as a key target of the maladaptive response to stress. *J Neurosci* 2007; **27**: 2781–2787.
28 Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T *et al.* Fiji: an open-source platform for biological-image analysis. *Nat Methods* 2012; **9**: 676–682.
29 McHugh TJ, Jones MW, Quinn JJ, Balthasar N, Coppari R, Elmquist JK *et al.* Dentate gyrus NMDA receptors mediate rapid pattern separation in the hippocampal network. *Science* 2007; **317**: 94–99.
30 Sahay A, Scobie KN, Hill AS, O'Carroll CM, Kheirbek MA, Burghardt NS *et al.* Increasing adult hippocampal neurogenesis is sufficient to improve pattern separation. *Nature* 2011; **472**: 466–470.
31 Kim WY, Wang X, Wu Y, Doble BW, Patel S, Woodgett JR *et al.* GSK-3 is a master regulator of neural progenitor homeostasis. *Nat Neurosci* 2009; **12**: 1390–1397.
32 Romine J, Gao X, Xu XM, So KF, Chen J. The proliferation of amplifying neural progenitor cells is impaired in the aging brain and restored by the mTOR pathway activation. *Neurobiol Aging* 2015; **36**: 1716–1726.
33 Vossel KA, Xu JC, Fomenko V, Miyamoto T, Suberbielle E, Knox JA *et al.* Tau reduction prevents Abeta-induced axonal transport deficits by blocking activation of GSK3beta. *J Cell Biol* 2015; **209**: 419–433.
34 Llorens-Martin M, Jurado J, Hernandez F, Avila J. GSK-3beta a pivotal kinase in Alzheimer disease. *Front Mol Neurosci* 2014; **7**: 46.
35 Jessberger S, Clark RE, Broadbent NJ, Clemenson GD Jr., Consiglio A, Lie DC *et al.* Dentate gyrus-specific knockdown of adult neurogenesis impairs spatial and object recognition memory in adult rats. *Learn Mem* 2009; **16**: 147–154.
36 Gould E, McEwen BS, Tanapat P, Galea LA, Fuchs E. Neurogenesis in the dentate gyrus of the adult tree shrew is regulated by psychosocial stress and NMDA receptor activation. *J Neurosci* 1997; **17**: 2492–2498.
37 Lucassen PJ, Meerlo P, Naylor AS, van Dam AM, Dayer AG, Fuchs E *et al.* Regulation of adult neurogenesis by stress, sleep disruption, exercise and inflammation: Implications for depression and antidepressant action. *Eur Neuropsychopharmacol* 2010; **20**: 1–17.
38 Lopes S, Teplytska L, Vaz-Silva J, Dioli C, Trindade R, Morais M *et al.* Tau deletion prevents stress-induced dendritic atrophy in prefrontal cortex: role of synaptic mitochondria. *Cerebr Cortex* 2016.
Q3
39 Lopes SVJ, Pinto V, Dalla C, Kokras N, Bedenk B, Mack N *et al.* Tau protein is essential for stres-induced brain pathology. *Proc Natl Acad Sci USA* 2016; **113**: E3755–E3763.
40 Gu Y, Oyama F, Ihara Y. Tau is widely expressed in rat tissues. *J Neurochem* 1996; **67**: 1235–1244.
41 Yuan A, Kumar A, Peterhoff C, Duff K, Nixon RA. Axonal transport rates *in vivo* are unaffected by tau deletion or overexpression in mice. *J Neurosci* 2008; **28**: 1682–1687.
42 Pallas-Bazarra N, Jurado-Arjona J, Navarrete M, Esteban JA, Hernandez F, Avila J *et al.* Novel function of Tau in regulating the effects of external stimuli on adult hippocampal neurogenesis. *EMBO J* 2016; **35**: 1417–1436.
43 Harada A, Oguchi K, Okabe S, Kuno J, Terada S, Ohshima T *et al.* Altered microtubule organization in small-calibre axons of mice lacking tau protein. *Nature* 1994; **369**: 488–491.
44 Roberson ED, Scearce-Levie K, Palop JJ, Yan F, Cheng IH, Wu T *et al.* Reducing endogenous tau ameliorates amyloid beta-induced deficits in an Alzheimer's disease mouse model. *Science* 2007; **316**: 750–754.
45 Arendt T, Stieler J, Strijkstra AM, Hut RA, Rudiger J, Van der Zee EA *et al.* Reversible paired helical filament-like phosphorylation of tau is an adaptive process associated with neuronal plasticity in hibernating animals. *J Neurosci* 2003; **23**: 6972–6981.
46 Leon-Espinosa G, Garcia E, Gomez-Pinedo U, Hernandez F, DeFelipe J, Avila J. Decreased adult neurogenesis in hibernating Syrian hamster. *Neuroscience* 2016; **333**: 181–192.
47 Bullmann T, de Silva R, Holzer M, Mori H, Arendt T. Expression of embryonic tau protein isoforms persist during adult neurogenesis in the hippocampus. *Hippocampus* 2007; **17**: 98–102.
48 Bullmann T, Holzer M, Mori H, Arendt T. Pattern of tau isoforms expression during development *in vivo*. *Int J Dev Neurosci* 2009; **27**: 591–597.
49 Schoch KM, DeVos SL, Miller RL, Chun SJ, Norrbom M, Wozniak DF *et al.* Increased 4R-Tau induces pathological changes in a Human-Tau Mouse Model. *Neuron* 2016; **90**: 941–947.
50 Iovino M, Agathou S, Gonzalez-Rueda A, Del Castillo Velasco-Herrera M, Borroni B, Alberici A *et al.* Early maturation and distinct tau pathology in induced pluripotent stem cell-derived neurons from patients with MAPT mutations. *Brain* 2015; **138** (Part 11): 3345–3359.
51 Hamilton LK, Aumont A, Julien C, Vadnais A, Calon F, Fernandes KJ. Widespread deficits in adult neurogenesis precede plaque and tangle formation in the 3xTg mouse model of Alzheimer's disease. *Eur J Neurosci* 2010; **32**: 905–920.
52 Boekhoorn K, Joels M, Lucassen PJ. Increased proliferation reflects glial and vascular-associated changes, but not neurogenesis in the presenile Alzheimer hippocampus. *Neurobiol Dis* 2006; **24**: 1–14.
53 Chen S, Townsend K, Goldberg TE, Davies P, Conejero-Goldberg C. MAPT isoforms: differential transcriptional profiles related to 3R and 4R splice variants. *J Alzheimer's Dis* 2010; **22**: 1313–1329.
54 Sennvik K, Boekhoorn K, Lasrado R, Terwel D, Verhaeghe S, Korr H *et al.* Tau-4R suppresses proliferation and promotes neuronal differentiation in the hippocampus of tau knockin/knockout mice. *FASEB J* 2007; **21**: 2149–2161.
55 Komuro Y, Xu G, Bhaskar K, Lamb BT. Human tau expression reduces adult neurogenesis in a mouse model of tauopathy. *Neurobiol Aging* 2015; **36**: 2034–2042.
56 Wagner P, Wang B, Clark E, Lee H, Rouzier R, Pusztai L. Microtubule associated protein (MAP)-Tau: a novel mediator of paclitaxel sensitivity *in vitro* and *in vivo*. *Cell Cycle* 2005; **4**: 1149–1152.
57 Cho JH, Johnson GV. Primed phosphorylation of tau at Thr231 by glycogen synthase kinase 3beta (GSK3beta) plays a critical role in regulating tau's ability to bind and stabilize microtubules. *J Neurochem* 2004; **88**: 349–358.
58 Hernandez F, Lucas JJ, Cuadros R, Avila J. GSK-3 dependent phosphoepitopes recognized by PHF-1 and AT-8 antibodies are present in different tau isoforms. *Neurobiol Aging* 2003; **24**: 1087–1094.
59 Inestrosa NC, Arenas E. Emerging roles of Wnts in the adult nervous system. *Nat Rev Neurosci* 2010; **11**: 77–86.
60 Dwyer JM, Duman RS. Activation of mammalian target of rapamycin and synaptogenesis: role in the actions of rapid-acting antidepressants. *Biol Psychiatry* 2013; **73**: 1189–1198.
61 Polman JA, Hunter RG, Speksnijder N, van den Oever JM, Korobko OB, McEwen BS *et al.* Glucocorticoids modulate the mTOR pathway in the hippocampus: differential effects depending on stress history. *Endocrinology* 2012; **153**: 4317–4327.
62 Howell KR, Kutiyanawalla A, Pillai A. Long-term continuous corticosterone treatment decreases VEGF receptor-2 expression in frontal cortex. *PLoS ONE* 2011; **6**: e20198.
63 Zhong P, Wang W, Pan B, Liu X, Zhang Z, Long JZ *et al.* Monoacylglycerol lipase inhibition blocks chronic stress-induced depressive-like behaviors via activation of mTOR signaling. *Neuropsychopharmacology* 2014; **39**: 1763–1776.

Uncorrected proof

© 2017 Macmillan Publishers Limited, part of Springer Nature.

64 Jernigan CS, Goswami DB, Austin MC, Iyo AH, Chandran A, Stockmeier CA *et al*. The mTOR signaling pathway in the prefrontal cortex is compromised in major depressive disorder. *Progr Neuro-Psychopharmacol Biol Psychiatry* 2011; **35**: 1774–1779.
65 Morita T, Sobue K. Specification of neuronal polarity regulated by local translation of CRMP2 and Tau via the mTOR-p70S6K pathway. *J Biol Chem* 2009; **284**: 27734–27745.
66 Egeland M, Zunszain PA, Pariante CM. Molecular mechanisms in the regulation of adult neurogenesis during stress. *Nat Rev Neurosci* 2015; **16**: 189–200.
67 Sousa N. The dynamics of the stress neuromatrix. *Mol Psychiatry* 2016; **21**: 302–312.
68 Mayer JL, Klumpers L, Maslam S, de Kloet ER, Joels M, Lucassen PJ. Brief treatment with the glucocorticoid receptor antagonist mifepristone normalises the corticosterone-induced reduction of adult hippocampal neurogenesis. *J Neuroendocrinol* 2006; **18**: 629–631.
69 Cameron HA, Gould E. Adult neurogenesis is regulated by adrenal steroids in the dentate gyrus. *Neuroscience* 1994; **61**: 203–209.

Supplementary Information accompanies the paper on the Molecular Psychiatry website (http://www.nature.com/mp)

Uncorrected Proof

© 2017 Macmillan Publishers Limited, part of Springer Nature.