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Female Hippocampus Vulnerability to Environmental Stress, a Precipitating Factor in Tau Aggregation Pathology

Ioannis Sotiropoulos[a,c,d,*], Joana Silva[c,d], Tetsuya Kimura[a,b], Ana Joao Rodrigues[c,d], Patricio Costa[c,d], Osborne F.X. Almeida[e], Nuno Sousa[c,d] and Akihiko Takashima[a,b]
[a]*RIKEN Brain Science Institute, Laboratory for Alzheimer's Disease, Wako-shi, Saitama, Japan*
[b]*Department of Aging Neurobiology, National Center for Geriatrics and Gerontology, Ohbu, Japan*
[c]*Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Campus Gualtar, Braga, Portugal*
[d]*ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal*
[e]*Max Planck Institute of Psychiatry, Munich, Germany*

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Abstract. Tau-mediated neurodegeneration is a central event in Alzheimer's disease (AD) and other tauopathies. Consistent with suggestions that lifetime stress may be a clinically-relevant precipitant of AD pathology, we previously showed that stress triggers Tau hyperphosphorylation and accumulation; however, little is known about the etiopathogenic interaction of chronic stress with other AD risk factors, such as sex and aging. This study focused on how these various factors converge on the cellular mechanisms underlying Tau aggregation in the hippocampus of chronically stressed male and female (middle-aged and old) mice expressing the most commonly found disease-associated *Tau* mutation in humans, *P301L-Tau*. We report that environmental stress triggers memory impairments in female, but not male, P301L-Tau transgenic mice. Furthermore, stress elevates levels of caspase-3-truncated Tau and insoluble Tau aggregates exclusively in the female hippocampus while it also alters the expression of the molecular chaperones Hsp90, Hsp70, and Hsp105, thus favoring accumulation of Tau aggregates. Our findings provide new insights into the molecular mechanisms through which clinically-relevant precipitating factors contribute to the pathophysiology of AD. Our data point to the exquisite sensitivity of the female hippocampus to stress-triggered Tau pathology.

Keywords: Chaperones, hippocampus, memory, mice, stress, tau aggregates

INTRODUCTION

Tau aggregation is a common feature in Alzheimer's disease (AD), frontotemporal dementia, and other tauopathies. The identification of *Tau* mutations has helped establish that Tau dysfunction is central to the neurodegenerative processes leading to dementia [1]. The most frequent human Tau mutation, *P301L* [2], results in the production of an aggregation-prone form of the protein. Expression of pathogenic *P301L-Tau* in mice results in the formation of Tau aggregates and neurofibrillary tangles, similar to those observed in AD brains; P301L-Tau was previously demonstrated to promote the assembly and accumulation of conformationally-abnormal and insoluble Tau that triggers neuronal degeneration and loss [3, 4].

Tauopathies are complex disorders with multiple precipitating factors. For example, age, sex, and stressful life events are known etiopathogenic factors in AD [5, 6]. While advanced age is the primary risk factor for developing AD, potential combinatorial effects are

*Correspondence to: Ioannis Sotiropoulos, Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Campus Gualtar, 4710 057 Braga, Portugal. Tel.: +351 253 604924; E-mail: ioannis@ecsaude.uminho.pt.

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likely, as illustrated by findings that: (i) the aged brain is more vulnerable to chronic stress [7], (ii) females are more vulnerable to stress-related disorders [8–10], and (iii) women are more prone to develop AD [11]. Notably, elevated levels of glucocorticoid secretion, such as those observed after stress, are associated with hippocampal degeneration and cognitive deficits in AD patients [12–14]; moreover, stress triggers AD-like pathology, including Tau hyperphosphorylation in rodents [15–17]. However, the interplay between these diverse risk factors in the establishment of Tau pathology is poorly understood.

In this study, middle-aged and old male and female transgenic P301L-Tau mice [3] were used to explore the mechanisms through which stress contributes to Tau aggregation. Analysis focused on the hippocampus, one of the first brain areas to display Tau pathology in the course of AD as well after exposure of experimental animals to stress [16, 17]. A key finding of this work is that chronic stress triggers a number of mechanisms, including caspase 3-driven truncation of au and abnormal Tau conformation that leads to Tau aggregation in female, but not male, P301L-Tau mice. Tau aggregation in female mice was accompanied by altered expression of molecular chaperones known to regulate the proper degradation and aggregation of Tau [18, 19], activation of apoptotic molecules, and impaired memory. Together, these results add new perspectives to our understanding of how stress and sex contribute to the precipitation of AD and other Tau-related pathologies.

MATERIALS AND METHODS

Animals and treatments

Middle-aged (12–14 months) and old (22–24 months) male and female P301L-Tau transgenic (Tg) mice and their wildtype littermates were used in this study. The expression of mutant human Tau is driven by CaMKII promoter avoiding any motor deficits [20, 21]. The previously-characterized transgenic line exhibits Tau aggregates in hippocampal neurons [3, 21, 22]. All animal experiments were performed according to Japanese Law, were approved by the Animal Care and Use Committee of RIKEN institute (Saitama, Japan), and conformed with US National Institutes of Health Guidelines on animal welfare and experimentation. Animals were subjected to prolonged stress over a period of 28 days [17]. Briefly, single stressors (overcrowding; restraint; placement on a rocking platform; i.p. injection of 0.9% saline 1 ml/100 g) were applied on a daily basis and in random order to prevent habituation. Efficacy of this protocol was verified by reduction of body weight ($p < 0.05$), increases in daytime serum corticosterone (CORT) levels ($p < 0.05$), and increases in anxiety levels measured by elevated plus maze test (reduction in entries and time in open arms, $p < 0.05$) (see Supplementary Table 1).

Behavioral testing

Spatial reference memory was assessed in the Morris water maze at the end of the 28-day stress paradigm. As previously described [3], the maze consisted of a cylinder (1 m diameter) filled with water (24°C) made opaque with a white bio-safe dye. The cylinder contained a slightly submerged transparent escape platform (not visible because of the opaque water) and was placed in a room with landmark (reference) objects. Learning trials commenced by gently placing mice on the water surface close to the cylinder wall. Animals were tested over 9 consecutive days (3 trials/day; 60-s trial period). On the tenth day, the mouse had to search (60 s) for the escape platform that was absent during this "probe" test. Swim paths during these tests were monitored and recorded by a CCD camera, using Image J software (http://rsb.info.nih.gov/nih-image/). Data were subsequently analyzed using customized software based on Matlab (version 7.2, Mathworks Co Ltd, CA), with an image analysis tool box (Mathworks). Specifically, swim speed, distance from platform, and latency to reach the platform were computed. Learning was assessed by measuring the distance between the mouse and the platform at 0.5 s intervals until the mouse reached the platform or the session timed out. Next, we calculated the total distance traveled by the mouse by integrating the distance between the mouse and the platform, with the "integrated distance" value providing an "error score" as previously described [3, 21].

Western blot and immunohistological analysis

One day after the last behavioral test, half of the animals were killed by decapitation and their brains were rapidly excised; the hippocampi were dissected, snap-frozen and stored at –80°C until western blot analysis. The rest of the animals were deeply anesthetized with pentobarbital (50 mg/kg) and transcardially perfused with 10% PFA. Brains were postfixed for 16 h before embedding in paraffin, sectioning (4 μm) in the coronal plane. Western blot analysis was carried out on sarkosyl-insoluble and sarkosyl-soluble tissue

fractions, as previously described [3, 21]. Briefly, frozen hippocampi were homogenized in Tris-buffered saline (TBS; 10 mM Tris, 150 mM NaCl) including protease inhibitors (1 μg/ml antipine, 5 μg/ml pepstatin, 5 μg/ml leupeptin, 2 μg/ml aprotinin, and 0.5 μM 4-(2-aminoethyl)benzenesulfonylfluoride hydrochloride) and phosphataseinhibitors (1 mM NaF, 0.4 mM Na3VO4, and 0.5 mM okadaic acid). After centrifugation at 100,000 *g*, the supernatant (soluble fraction) was collected. Sarkosyl-insoluble, paired helical filament-enriched fractions were prepared from the TBS-insoluble pellets after re-homogenization in salt/sucrose buffer (0.8 M NaCl, 10 mM Tris/HCl, pH 7.4, 10% sucrose and protease and phosphatase inhibitors; see [5]), incubation with 10% sarkosyl (37°C, 1 h) and centrifugation (150,000 *g*). After SDS–PAGE electrophoresis, protein extracts were semi-dry transferred onto to nitrocellulose membranes which were subsequently incubated with the antisera listed in Supplementary Table 2. Signals were revealed by enhanced chemiluminescence (ECL, GE Healthcare) and evaluated using a LAS-3000 Bio-Imaging Analyzer System (Fujifilm). For immunohistochemistry, deparaffinized sections were exposed to antigen retrieval (citrate buffer) and 0.3% Triton-X before incubation with antiserum against MC1 (1 : 100) and appropriate secondary antibodies followed by DAB as previously described by our team [17]. Images were obtained on an Olympus confocal microscope. In addition, neuronal (CA3) densities were stereologically estimated by counting neurons in cresyl violet-stained serial coronal brain sections, using Neurolucida software (MBF Bioscience, Williston, VT) as previously described by us [23]. Furthermore, apoptotic cell death was monitored using "*in situ* cell death" TUNEL kit (Roche) on brain sections following manufacturer's instructions.

Statistical analysis

Numerical data are expressed as group means ± SEM. Descriptive statistics, mixed-design factorial and multifactorial analyses of variance (ANOVAs) were used for evaluation of main and/or interaction effects of the factors of interest. When significant interactions were detected, significance of simple effects was tested by pairwise comparisons of dependent and independent variables, using paired or unpaired *t*-tests, respectively. The nominal level of significance was set at $\alpha = 0.05$ and Bonferroni's procedure was applied in all posteriori tests to keep the type I error ≤ 0.05.

RESULTS

Sex-specific susceptibility of P301L-Tau Tg mice to the memory-impairing effects of stress

Clinical evidence indicates that, besides aging, stress and sex influence the emergence of AD pathology, and that these potential risk factors may act in a combinatorial fashion [11, 13]. Our initial analysis of interactions between these factors involved examination of the learning curves in a hippocampus-dependent spatial learning-memory test (Fig. 1A–D). Mixed-designed factorial ANOVA revealed that all animals progressively learned the task over time (*Days*) [$F_{6.5,509.5} = 20.787$, $p \leq 0.001$]. Significant *Days* × *Sex* interactions were also found [$F_{6.5,509.5} = 2.334$, $p = 0.027$], with subsequent analysis showing that *P301L-Tau* females are significantly slower in acquisition of the task [$F_{1,78} = 8.65$, $p = 0.004$]. In addition, there were significant *Stress x Sex* interactions [$F_{1,78} = 11.03$, $p < 0.001$], attributable to the fact that only female mice with the P301L-Tau genotype were responsive to the deleterious effects of stress on this cognitive parameter. The factors *Age* and *Sex* also displayed a significant interaction [$F_{1,78} = 3.989$, $p = 0.049$] (cf. Fig. 1A, C). Furthermore, the probe test revealed a *Stress* × *Sex* interaction ($F_{1,78} = 4.372$, $p = 0.04$), and subsequent analyses clearly demonstrated that stress has a significant influence over the response of old female ($F_{1,44} = 7.08, p \leq 0.05$), but not in male ($F_{1,38} = 1.81$, $p = 1.87$), P301L-Tau animals (Fig. 1E, F). Stress did not exert any influence over the behavior of wild-type animals (data not shown). Overall, the above data demonstrate that stress induces cognitive deficits in female, but not male, P301L-Tau mice.

Stress aggravates the hippocampal burden of P301L-Tau in a sex-specific manner

Previous studies showed that stress induces Tau hyperphosphorylation and accumulation in neuronal cultures [24] and brain areas, such as the hippocampus, which are involved in the regulation of cognitive processes [16, 17]. Since formation and aggregation of insoluble Tau is the major pathological characteristic triggered by the P301L-Tau mutation [3, 22], it was of interest to examine whether stress alters Tau aggregation. To this end, we monitored the levels of sarkosyl-insoluble Tau aggregates in the hippocampi of P301L-Tau mice by western blot analysis since these aggregates are biochemically

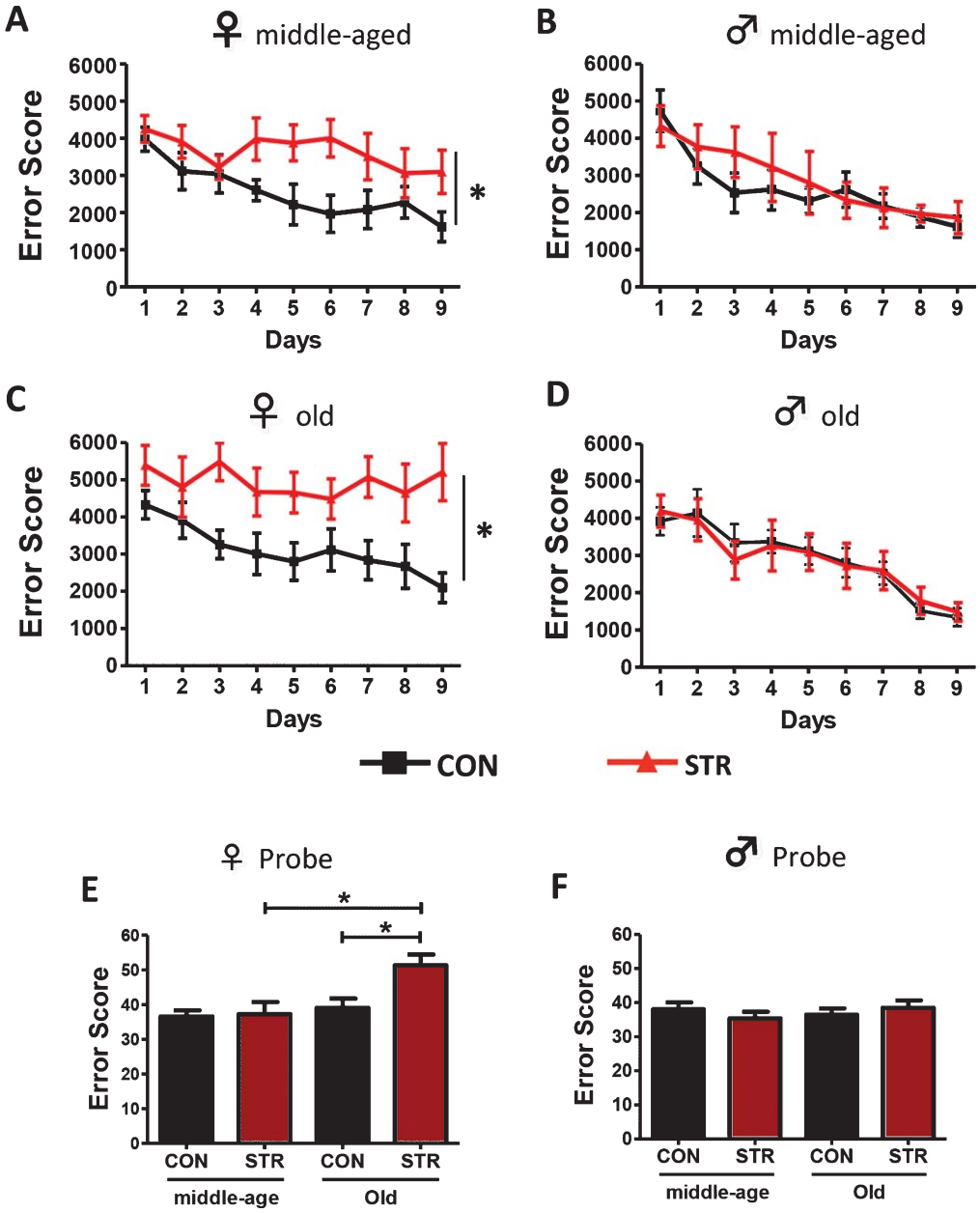

Fig. 1. Stress-induced cognitive impairment in female but not male P301L-Tau animals. A–D) Spatial reference memory was tested using the Morris water maze test for 9 consecutive days (3 trials per day) in middle-aged (A and B; 12–14 months old) and old (C and D; 22–24 months old) P301L-Tau Tg animals. Stress had a negative effect on the learning curve (represented by increased error score) of female (A, C) but not male (B, D) P301L-Tau mice. E, F) A probe test at the end of the acquisition period showed that the cognition-impairing effects of stress were more prominent in old versus middle-aged female mice; the error score in the probe test of stressed aged females was higher than the control (non-stressed) aged females, an effect not seen in middle-aged animals (E). All numerical data shown represent mean ± SEM ($n = 10$–12)*($p < 0.05$).

similar to those found in the neurofibrillary tangles that characterize tauopathies, including AD. We found that stress causes a significant increase in the amount of sarkosyl-insoluble Tau in the hippocampus of middle-aged and old female, but not male, P301L-Tau mice, with a significant *Stress x Sex* interaction [$F_{1,16(middle\text{-}aged)} = 6.314$, $p = 0.023$; $F_{1,16(Old)} = 5.635$, $p = 0.03$] (Fig. 2A). In addition, *post-hoc* testing after Bonferroni's correction showed that no differences were present between

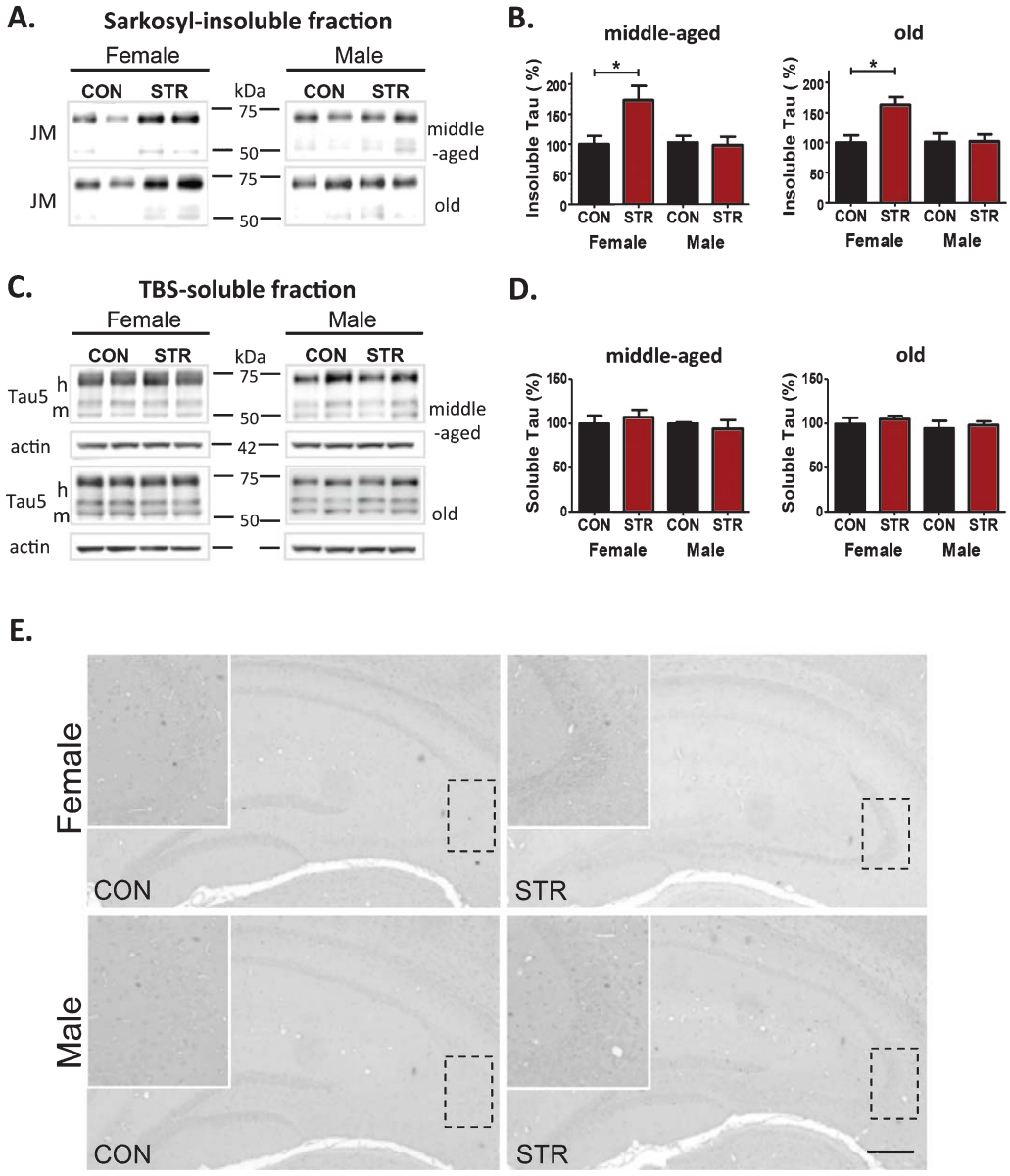

Fig. 2. Stress increases levels of sarkosyl-insoluble Tau in the female, but not male, hippocampus. Representative immunoblots and quantification of both sarkosyl-insoluble (A, B) and TBS-soluble (C, D) Tau levels in the hippocampus of male and female P301L-Tau Tg mice. Stress specifically increased the levels of insoluble Tau (64 kDa Tau, detected by JM antibody) in the female hippocampus without exerting an effect on the male hippocampus (A, B). In contrast, stress did not influence the levels of soluble Tau, detected by Tau5 antibody (C, D; h and m refer to human and mouse Tau, respectively). All numerical data shown represent mean ± SEM values ($n = 7$–8), depicted with respect to data obtained in control tissues (CON), set at 100% *($p < 0.05$). E) Increased MC1 staining in stressed female hippocampus when compared to control females; this stress effect was not obvious in males. Insets are high-power magnifications (20×) of the rectangular areas marked in the respective low-magnification micrographs (4×).; bar 200 μm.

stressed and non-stressed males whereas stressed and non-stressed females were significantly different ($p_{\text{middle-aged}} = 0.008$; $p_{\text{old}} = 0.007$). The greater abundance of insoluble Tau in the hippocampi of stressed female P301L-Tau mice is in line with the greater cognitive impairments found in these animals (cf. Fig. 1A–D). Interestingly, hippocampal levels of total soluble Tau were not influenced by stress in any of the experimental groups (Fig. 2C, D). In addition, stress also did not alter the phosphorylation pattern of soluble Tau, as assessed by a panel of different phospho-dependent antibodies (Supplementary

Fig. 1). Together, our molecular analysis indicates that stress specifically exerts its effect on insoluble Tau species, without affecting soluble forms, suggesting a detrimental effect of stress on Tau aggregation.

In a next step, we monitored levels of abnormally-conformed Tau since abnormal Tau conformation is an important step in Tau aggregation pathology [25]. Using the MC1 antibody which specifically stains abnormally-folded Tau, an early pathogenic conformation of Tau [26], we observed a greater number of MC1-immunoreactive neurons in the hippocampal CA3 subfield of female P301L-Tau mice that had been exposed to the chronic stress protocol (Fig. 2C). Furthermore, as P301L-Tau and its aggregates have been shown to have neurotoxic properties [26] and AD pathology in humans is characterized by reduction in neuronal number, we estimated hippocampal cell number using stereological tools. We found that stress resulted in a significant reduction of CA3 cell density in old P301L-Tau females (CON 7.539 ± 0.15 and STR 6.796 ± 0.13; $p < 0.05$) without affecting neuronal numbers in male P301L-Tau mice (CON 7.562 ± 0.16 and STR 7.711 ± 0.27). The greater severity of cognitive deficits observed in old stressed P301L-Tau females (compared to middle-agedP301L-Tau females) (Fig. 1A) suggests that mechanisms other than cell loss alone contribute to stress-triggered impairments of memory during the early stages of Tau aggregation.

Stress-triggered degenerative mechanisms in the hippocampus of female P301L-Tau transgenic mice

We previously observed that exposure of neural cells to glucocorticoids, the main stress hormones, decreases Tau turnover resulting in its cytoplasmic accumulation [24]. Interestingly, glucocorticoid receptors partner with various molecular chaperones and co-chaperones [27], also implicated in the clearance of misfolded proteins, including Tau [18, 19]. Thus, it was of interest to monitor stress-induced changes in the levels of some molecular chaperones that are critically involved in protein clearance and/or aggregation. Using a panel of antibodies against relevant molecular chaperones, we here observed that stress in female P301L-Tau mice leads to significant decreases in hippocampal levels of Hsp70, and its co-chaperone Hsp105 ($p < 0.05$; Fig. 3A, B), both of which are suggested to play an essential role in the degradation of misfolded Tau [18]. In contrast, stress resulted in increased levels of Hsp90 (Fig. 3A, B); this chaperone was previously shown to increase the stability of P301L-Tau and its aggregates [28, 29]; levels of other chaperones examined (Hsp60, Hsp27, and 40) were not altered by stress (Fig. 3A, B). These changes were not observed in the hippocampus of stressed males (Fig. 3E, F).

While many studies suggest activation of the apoptotic machinery in AD brains, it is presently debated whether the finding of apoptosis-related markers in the postmortem brains of AD patients [30] reflects neuronal loss through apoptotic mechanisms ([31, 32]. We found that exposure to stress evoked a significant increase in the protein levels of the pro-apoptotic molecule Bax with small decreases in Bcl-xL and Bcl-2 levels in the female hippocampus (Fig. 3A, C), the net result of which was an increase in the pro-apoptotic: anti-apoptotic protein ratio ($p \leq 0.05$; Fig. 3D); note that this effect of stress was not found in the male P301L-Tau hippocampus (Fig. 3E, G, H) Consistent with the findings of Spines-Jones et al. [31] and de Calignon et al. [32], we failed to observe marked histochemical signs of apoptosis in the hippocampi of stressed P301L mice. However, stress led to a significant increase in the levels of C-terminal caspase 3-cleaved Tau (Tau truncated at D421, also referred Tau-C3) when P301L female mice were exposed to stress (Fig. 3C); again, stress did not increase truncated Tau levels in the male P301L-Tau hippocampus (Fig. 3G). Since Tau-C3 is more prone to fibrillization and aggregation [25, 32], it is highly plausible that stress-driven increases in Tau-C3 also significantly contribute to the increases of Tau aggregates in the hippocampi of stressed female P301L-Tau mice.

DISCUSSION

Despite considerable progress in the understanding of the pathophysiology and neurobiology of neurodegenerative disorders, AD remains a complex, multifactorial disorder with many risk factors that include age, sex, and stressful life events [5, 13, 14], whose effects occur through largely undefined mechanisms. While both AD and the endocrine and behavioral response to stress display clear sex-specific profiles (females appear more vulnerable) [6, 8, 10], there is a paucity of research aimed to explain these differences (National Institute of Mental Health 2011). The present study considered clinically relevant risk factors of AD pathology, namely aging, sex, and stress on the progression of Tau pathology.

The present results demonstrate that exposure to chronic stress aggravates Tau pathology by increasing

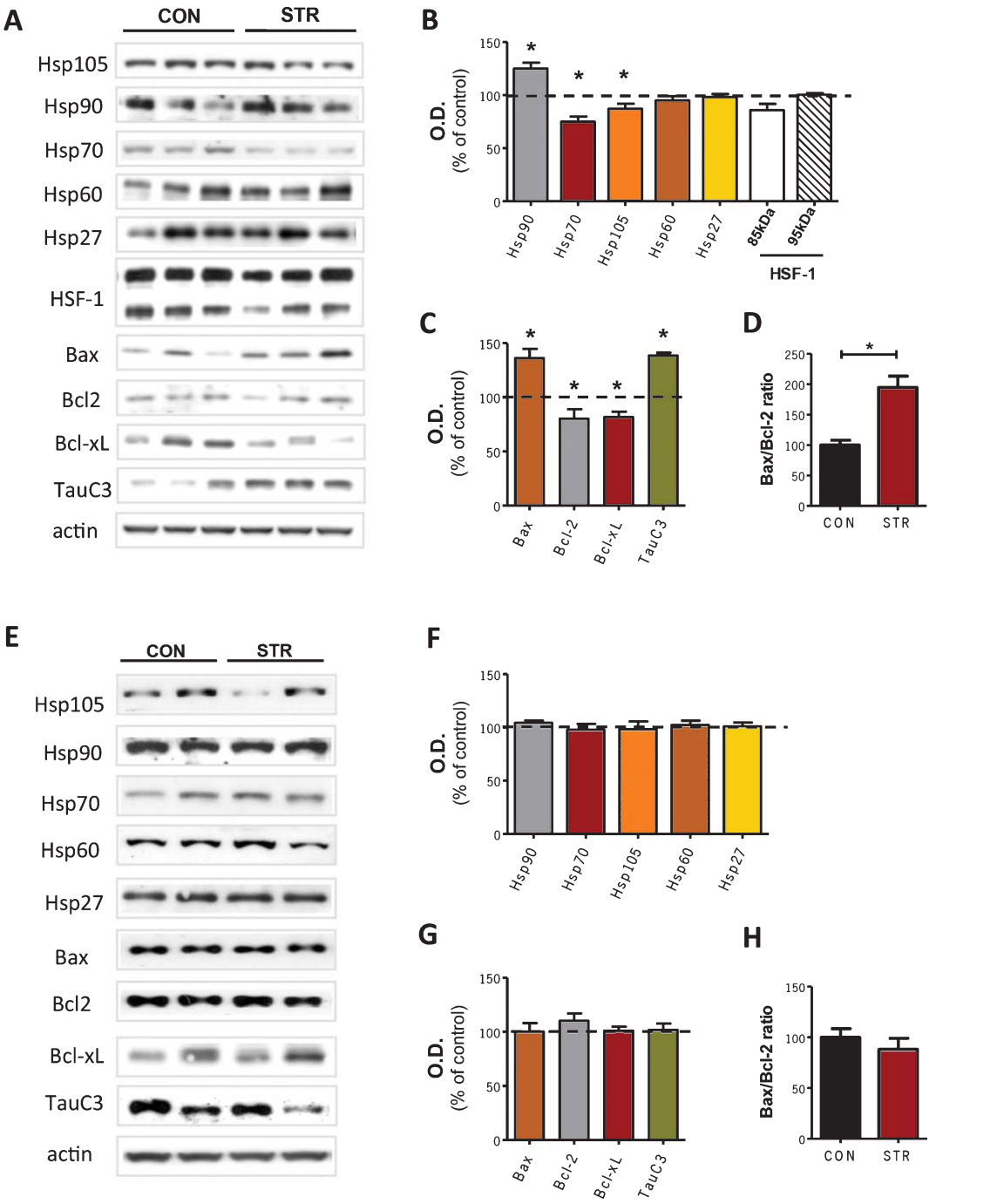

Fig. 3. Stress-induced changes in molecular chaperones and apoptotic markers in the hippocampus of female P301L-Tau mice. Representative blots and quantitative analysis showing the effects of stress on the hippocampus of female (A–D) and male (E–H) P301L-Tau mice. Stress reduced the protein levels of the molecular chaperones Hsp70 and Hsp105 and (B) increased those of Hsp90 in female P301L-Tau animals; stress did not exert any effect on the expression of these proteins in P301L-Tau males (F). In addition, stress activated the biochemical machinery associated with apoptosis in female hippocampus (C), significantly increasing Bax and reducing Bcl-2 and Bcl-xL protein levels, thus favoring an increase in the Bax/Bcl-2 ratio (D); similar changes were not observed in the male hippocampus (G, H). Truncated Tau levels (detected by TauC3 antibody) were increased by stress in the hippocampus of female (C), but not male (G), P301L-Tau Tg animals. All numerical data shown represent mean ± SEM values ($n = 6$–7), depicted with respect to data obtained in control tissues (CON), set at 100% ($^*p < 0.05$).

the aggregation of sarkosyl-insoluble Tau, C-terminal truncation of Tau by caspase-3 and, abnormal conformation of Tau in the hippocampus of female P301L-Tau mice. Indeed, both truncation and abnormal conformation of Tau precede its aggregation and formation of neurofibrillary tangles [25, 26, 32], thus serving as early markers of disease. It is suggested that the Tau-C3 species contributes to misfolding of Tau into a conformation that can nucleate and recruit other Tau molecules into aggregates [25, 32, 33]. Consistent with a previous suggestion that Tau aggregation does not depend on Tau phosphorylation [33], we observed that stress-triggered Tau aggregation is not accompanied by increased Tau hyperphosphorylation. We also found that stress impairs cognition in the absence of neurofibrillary tangles, a finding in line with the observation of initial deposition of mutant P301L-Tau in pre-tangle aggregates of human subjects expressing the P301L-Tau mutation [34].

Disturbances in the degradation of misfolded Tau protein are suggested to underlie the aggregation of Tau [18]. Tau degradation critically depends on the binding of Tau to molecular chaperones which target the protein for degradation [18, 35, 36]. The present study showed that stress downregulates hippocampal levels of Hsp70 and its co-chaperone, Hsp105. This finding is consistent with the reported inverse correlation between increased insoluble Tau and decreased Hsp70 protein in the postmortem brains from AD patients [37] as well as *in vitro* observations that Hsp70 promotes the degradation of insoluble, but not soluble, Tau [18]. Further, we found that stress leads to an increase in hippocampal levels of Hsp90. This chaperone is known to stabilize both mutant P301L-Tau protein [29] and its aggregates [28]. Pharmacological inhibition of Hsp90 was previously, shown to upregulate Hsp70 levels [35] and to reduce the levels of insoluble Tau [22]; thus, the dynamic cycling of the two chaperones with opposing actions may play an important role in the clearance of misfolded proteins; whereas Hsp70 promotes substrate ubiquitination, Hsp90 inhibits ubiquitination [38]. Interestingly, Hsp90 and Hsp70 serve to maintain the glucocorticoid receptor (GR) in a high affinity state; upon binding of ligand (glucocorticoid) to the GR, these heat shock proteins dissociate from the Hsp-GR complex, facilitating nuclear import of liganded GR where it initiates transcription; Hsp90 has also been implicated in GR recycling [39, 40]. The stress-induced increases in Hsp90 observed here indicate that GR are constantly available for binding ligand, thus adding impetus to the vicious cycle initiated by stress.

We previously showed that glucocorticoids stimulate apoptosis in the hippocampus. Here, we report that hippocampi of stressed P301L-Tau mice displayed increases in the ratio of pro-apoptotic (Bax) versus anti-apoptotic (Bcl-2 and BCl_{XL}) protein expression with concomitant increases in caspase-dependent truncated Tau (Tau-C3), but without any overt signs of ongoing apoptosis. While many studies support the involvement of Tau aggregates in neuronal injury and degeneration [3, 4] and AD brains exhibit markers of caspase activation and apoptosis (e.g., active caspase-3 and Bax), the role of apoptosis in AD remains controversial [32] as apoptosis does not seem to be a major contributor to neuronal death in human tauopathies [41] and Tg mouse models [42], although it should also be noted that apoptotic cells are rapidly cleared, making their detection in chronic stress difficult. Recent evidence suggests other functions (e.g., regulation of synaptic function and plasticity) for proteins, otherwise, best known for their role in apoptosis [43, 44]; further, caspase-cleaved Tau is suggested to contribute to synaptic deficits [44, 45], compromised mitochondrial function [46], and cellular demise [25]. Given that Tau-accumulating neurons survive for many years [47], it has been suggested that active-caspase-3 and other apoptosis-related enzymes may contribute to disease progression without necessarily triggering acute neuronal death [31, 32, 46]. Indeed, Rohn et al. [48] demonstrated that expression of the anti-apoptotic Bcl-2 protein in 3xTg-AD mice reduced caspase activation, Tau truncation, and tangle formation, providing an additional causal role for the above molecules in the pathological mechanisms involved in Tau aggregation.

The Tau pathology observed in the hippocampus of female P301L-Tau mice that had been exposed to stress translated into deficits in spatial reference memory, a hippocampal dependent task. This effect was accentuated by age; detailed statistical analyses on the present data point to complex interactions between genotype, stress, and sex, all of which have been previously identified as individual factors contributing to AD [7, 11, 12]. Notably, the lack of Tau aggregation-associated cognitive impairment in non-Tg animals highlights these interactions and their convergence to amplify the phenotype of P301L-Tau mice. In addition, future studies are needed to clarify the interaction between stress and glutamate release on the development and spread of Tau pathology since i) glutamate-dependent extracellular Tau seems to be involved in disease propagation [49] and, ii) glutamate plays an important role in mediating the cellular and electrophysiological actions

of glucocorticoids [50, 51] that are released during stress.

Insight into the neurobiological basis of sex differences in susceptibility to develop AD in humans is difficult to obtain. However, a recent report that showed that stress induces the overproduction of amyloid-β, another key player in AD pathology, in the hippocampus of female (but not of male) 5xTg mice [52] supports our findings with respect to the increased vulnerability of the female hippocampus to stress-triggered Tau pathology. Age-related decrease in neuroprotective estrogen and progesterone (and its derivatives) is a plausible explanation for the propensity of post-menopausal women to develop AD [11, 53, 54]. Comparisons between the human female and mouse reproductive cycles are not justified for many reasons (see [55]). This study did not specifically investigate the role of reproductive aging in stress-triggered pathology. Nevertheless, it should be noted that sex steroids are notoriously difficult to measure using standard methods in female rodents [56], although there are reports that aged female rodents may secrete low levels of estrogen even after the loss of regular ovarian cyclicity after about 1 year of age ([57, 58] and reviewed in [55]). Our results show that, contrary to males, both middle-age (12 months old), and old (22 month old) stressed females displayed similar behavioral and biochemical patterns, suggesting that age-related reproductive senescence is unlikely to be the main underlying factor for the herein-observed sex-related differences in the response of P301L-Tau mice to stress; on the other hand, even slight age-related reductions in sex hormone levels could potentially exacerbate the effects of stress in the old versus middle-aged female animals (see Fig. 1). Nevertheless, it would be important to know whether sex *per se* determines a given pathological trajectory; for example, future studies could examine whether the herein observed sex differences arise from the organizing actions of sex steroids during early development and/or are dependent on the activating actions of sex steroids for their manifestation over the reproductive life cycle [8, 10, 59]. Interestingly, a recent study showed that de-masculinization of neonatal male 3xTg mice narrows the gender gap in terms of amyloid-β pathology [60].

In summary, the current study provides new information on the cellular mechanisms through which chronic stress may precipitate Tau aggregation pathology and cognitive impairment (see Fig. 4). In light of current AD clinical trials aiming to block Tau aggregation, this study adds to our knowledge of disease

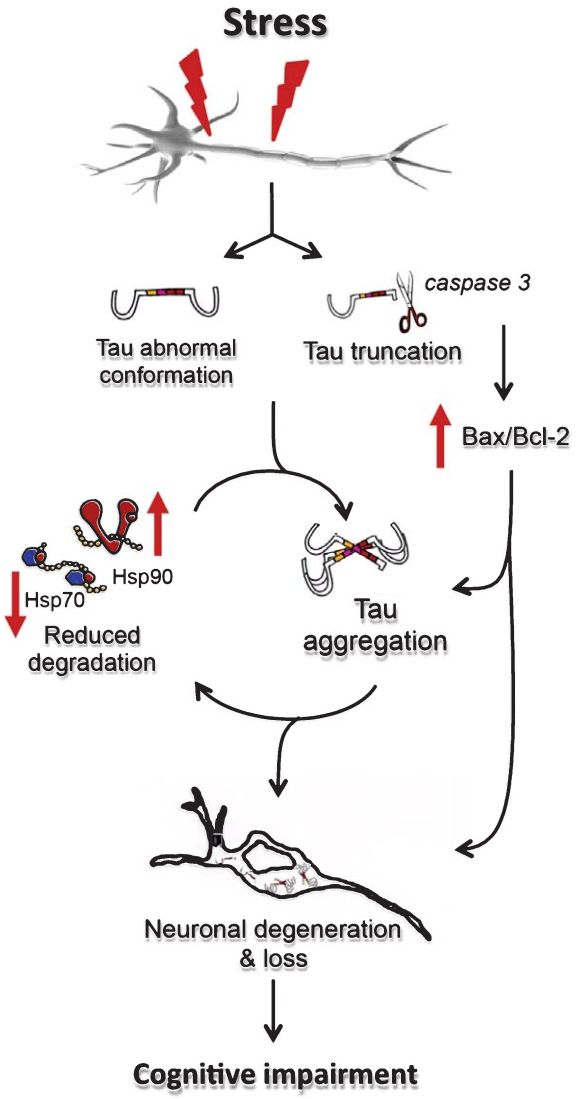

Fig. 4. The role of chronic stress on cellular pathways that result in Tau aggregation pathology. This working model summarizes how stress might trigger different parts of the Tau aggregation machinery. Exposure to chronic stress triggers caspase 3-mediated Tau truncation and abnormal conformation; two cellular phenomena suggested to be involved in Tau oligomerization and ultimately, aggregation, that eventually result in neuronal loss and cognitive impairments [3, 20, 25, 31, 32]. In parallel, stress dysregulates the molecular chaperone machinery by increasing Hsp90 and decreasing Hsp70 levels, thus reducing Tau degradation. Note that Hsp90 is a suggested therapeutic target as its inhibition upregulates Hsp70 and reduces insoluble Tau [22, 33]. In addition, stress activates the apoptotic machinery which may contribute to Tau truncation and aggregation [46], as well as synaptic deficits [38, 43], compromised mitochondrial function and cell death [25, 44].

etiopathogenesis and highlights the important interplay between stress and other clinically-relevant precipitating factors for Tau pathology development supporting the emerging idea that the female hippocampus is

inherently more vulnerable to stress-related disorders of the brain.

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Authors' disclosures available online (http://www.j-alz.com/disclosures/view.php?id=2416).

SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: http://dx.doi.org/10.3233/JAD-140693.

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