

# Caspase-3-Dependent Proteolytic Cleavage of Tau Causes Neurofibrillary Tangles and Results in Cognitive Impairment During Normal Aging

John C. Means<sup>1</sup> · Bryan C. Gerdes<sup>1</sup> · Simon Kaja<sup>1,2</sup> · Nathalie Sumien<sup>3</sup> · Andrew J. Payne<sup>1</sup> · Danny A. Stark<sup>1</sup> · Priscilla K. Borden<sup>1</sup> · Jeffrey L. Price<sup>4,5</sup> · Peter Koulen<sup>1,5,6</sup>

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**Abstract** Mouse models of neurodegenerative diseases such as Alzheimer's disease (AD) are important for understanding how pathological signaling cascades change neural circuitry and with time interrupt cognitive function. Here, we introduce a non-genetic preclinical model for aging and show that it exhibits cleaved tau protein, active caspases and neurofibrillary tangles, hallmarks of AD,

causing behavioral deficits measuring cognitive impairment. To our knowledge this is the first report of a non-transgenic, non-interventional mouse model displaying structural, functional and molecular aging deficits associated with AD and other tauopathies in humans with potentially high impact on both new basic research into pathogenic mechanisms and new translational research efforts. Tau aggregation is a hallmark of tauopathies, including AD. Recent studies have indicated that cleavage of tau plays an important role in both tau aggregation and disease. In this study we use wild type mice as a model for normal aging and resulting age-related cognitive impairment. We provide evidence that aged mice have increased levels of activated caspases, which significantly correlates with increased levels of truncated tau and formation of neurofibrillary tangles. In addition, cognitive decline was significantly correlated with increased levels of caspase activity and tau truncated by caspase-3. Experimentally induced inhibition of caspases prevented this proteolytic cleavage of tau and the associated formation of neurofibrillary tangles. Our study shows the strength of using a non-transgenic model to study structure, function and molecular mechanisms in aging and age related diseases of the brain.

John C. Means and Bryan C. Gerdes contributed equally to this work.

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✉ Peter Koulen  
koulenp@umkc.edu

- <sup>1</sup> Department of Ophthalmology, Vision Research Center, School of Medicine, University of Missouri—Kansas City, 2411 Holmes St., Kansas City, MO 64108, USA
- <sup>2</sup> Present Address: Department of Ophthalmology, Stritch School of Medicine, Loyola University Chicago, 2160 S First Ave., Maywood, IL 60153, USA
- <sup>3</sup> Center for Neuroscience Discovery, Institute for Healthy Aging, University of North Texas Health Science Center, 3500 Camp Bowie Boulevard, Fort Worth, TX 76107, USA
- <sup>4</sup> Department of Neurology and Cognitive Neuroscience, School of Medicine, University of Missouri—Kansas City, 2411 Holmes St., Kansas City, MO 64108, USA
- <sup>5</sup> School of Biological Sciences, University of Missouri—Kansas City, 5007 Rockhill Rd, Kansas City, MO 64110, USA
- <sup>6</sup> Department of Basic Medical Science, School of Medicine, University of Missouri—Kansas City, 2411 Holmes St., Kansas City, MO 64108, USA

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## Introduction

Tauopathies are a group of neurodegenerative disorders that are associated with the pathological aggregation of the tau protein in neurofibrillary tangles; one such disease is

Alzheimer's disease (AD). AD is a neurological disorder that leads to progressive cognitive decline. This includes memory loss, decline in cognitive control and a diminished capacity in visual–spatial skills [18]. A hallmark of AD is the accumulation of senile plaques and neurofibrillary tangles (NFTs), composed of abnormally processed tau [19].

Tau is a microtubule-associated protein that stabilizes the neuronal cytoskeleton and participates in vesicular transport [1]. Pathological alterations in tau occur in several neurodegenerative disorders, including AD and Parkinson's disease [24, 47, 48].

Several studies have demonstrated the activation of apoptotic machinery within the AD brain [4, 9]. Apoptosis is characterized by plasma membrane blebbing, chromosome condensation and DNA fragmentation and is initiated by caspases.

Caspases are a group of cysteine proteases that cleave after specific Asp residues and play a central role in the cell death pathway [45]. In addition to their established role in apoptosis there is evidence that caspases have non-apoptotic functions [25, 29]. Additionally, components of the neuronal cytoskeleton, including tau, are targeted by caspases.

There is evidence for increased caspase activation in AD brain [38]. Moreover, the presence of caspase-cleaved tau has also been detected in AD brain. The caspase cleavage of tau plays an important role in the oligomerization and formation of a pathological tau species in AD. This cleavage generates a highly fibrillogenic tau species, which aggregates more freely and to a greater degree than the full length tau and also assists in aggregate formation of the full length tau [13]. Antibodies recognizing truncated tau at Asp421 showed that cleaved tau (Asp421), active caspase-3 and fibrillary tau colocalize in AD brains [36]. In addition, a mouse model of tauopathies showed that the majority of cells with active caspases also had NFTs [42]. Moreover, experiments in tissue culture provide evidence that the Asp421 cleaved tau is toxic to neurons [5, 28, 35].

Mouse models of age related cognitive decline such as seen in AD and other tauopathies are important for understanding how pathological cascades change neural circuitry and in time interrupt cognitive function [41]. In this study we used an established non-genetic preclinical model for aging [21, 22] and showed that in cognitively impaired mice, cleaved tau, active caspases and NFTs were present and this was significantly correlated with behavioral deficits. To our knowledge this is the first report of a non-transgenic, non-interventional mouse model displaying structural, functional and molecular aging deficits associated with AD and other tauopathies in humans.

## Methods

### Animals

The animal experiments performed were approved by the local Institutional Animal Care and Use Committee. Young (6 months; analogous to an approximately 34 years old human) and old (24 months; analogous to an approximately 70 years old human) male C57BL/6 J mice were obtained from the National Institute on Aging. Individual animals were maintained in the institutional vivarium at ambient temperature ( $23 \pm 1$  °C), under a 12-h light/dark cycle starting at 0600 h. Mice had ad libitum access to food and water, except during testing.

### Behavioral Assays

#### *Spatial Learning and Memory*

The behavioral outcome measures were described by us previously [21]. Spatial learning and memory were determined using a swim maze test as described by us previously [43]. Briefly, performance in the swim maze test was assessed using a learning index. The learning index was defined as the relative improvement in performance normalized to the average performance of the young mice using path length. The pretraining phase involved the mouse learning the motor components of swimming and climbing onto the platform, without learning the location of the platform in the tank. Afterwards, the mice were tested for their ability to learn the location of the platform. This involved four acquisition sessions and for each session there were five trials during which the mouse had to swim to the platform from a different starting point in the tank.

#### *Bridge Walking*

In short, the latency to fall was determined for each mouse after being placed on one of four bridges (1 cm square, 1 cm round, 2 cm square, 2 cm round) mounted 45 cm above a padded surface. The maximum latency to fall was set at 60 s. Mice were placed on each bridge three times and the mean latency to fall was used as a measure of performance for each bridge.

### Caspase Activity Assay

Caspase activity was measured using the caspase-3 substrate Ac-DEVD-AFC (Santa Cruz Biotech, sc-311274A) as the caspase substrate. To measure caspase activity in SH-SY5Y, cells were harvested 30 min after UV-irradiation and spun down at  $2000\times g$ . The cell pellets were

resuspended in 100  $\mu$ L Caspase Buffer (20 mM HEPES, pH 7.5, 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 1 mM EDTA, 1 mM EGTA, 1 mM DTT supplemented with protease inhibitor cocktail) and lysed by 3 cycles of freeze thaw. To detect caspase activity in mouse forebrain and cerebellum, 1  $\mu$ g of lysate was brought up to a volume of 100  $\mu$ L with caspase buffer. The lysates were then incubated for 1 h at 37 °C. 0.2  $\mu$ M Ac-DEVD-afc (in DMSO) was added and the reaction mixed. The reactions were then analyzed fluorometrically (excitation 405 nm, emission 535 nm) and activity was expressed in relative arbitrary fluorescence units.

### Antibodies

To examine Tau cleavage, mouse anti-Tau, caspase cleaved (truncated at Asp421) antibody (Millipore, MAB5430) (1:1000) was used. Previous work indicated that caspase-3 targets Tau at Asp421 [20]. For a loading control mouse anti-Actin (Millipore, MAB1501R) (1:1000) was used.

### Immunoblot Analysis

Mice were euthanized using carbon dioxide asphyxiation and brains were then dissected into forebrain, olfactory bulb, and cerebellum and snap frozen using liquid nitrogen. Total protein concentration was determined using the method of Lowry. Twenty-five micrograms of total protein in SDS loading buffer were separated electrophoretically and transferred to nitrocellulose membrane as described previously [21]. Protein level quantification was performed by densitometry using ImageJ software (NIH). Density values were corrected for background and TAU expression normalized for endogenous actin expression.

### Thioflavin S Stain

To examine NFT formation in SH-SY5Y cells (ATCC, Manassas, VA, USA), cells were stained with Thioflavin S (Sigma, T1892). Thioflavin S was used at a concentration of 0.05 % diluted in distilled water. Cells were rinsed in distilled water and incubated in 0.05 % Thioflavin S for 5 min. After 5 min, the cells were washed in 70 % Ethanol for 5 min and rinsed with distilled water overnight. To examine brain sections for Thioflavin S-positive cells, the same procedure was used except Thioflavin S was used at a concentration of 0.01 %.

### Brain Immunostaining

For tissue processing and fixation, brains were dissected and separated in two halves by cutting along the midline section

with a disposable razorblade. The left hemisphere was used for preparation of tissue lysates, essentially as described previously [21, 22]. In brief, total protein was extracted from frozen samples using CytoBuster tissue lysis reagent (EMD Millipore, Bellerica, MA) in the presence of protease inhibitors (Roche Applied Science, Indianapolis, IN). The right hemisphere was immersion fixed in freshly prepared 4 % PFA in PBS pH7.4 overnight, and cryoprotected by sequential immersion in 10, 20 and 30 % sucrose in PBS pH 7.4 supplemented with 0.01 % sodium azide. Tissue was embedded in Tissue-Tek<sup>®</sup> cryoembedding compound (Takura Finetek USA, Torrance, CA) and 12  $\mu$ m sections were cut on a Vibratome Ultra Pro 5500 cryostat (North Central Instruments, Minneapolis, MN).

Mouse forebrain tissue sections were blocked at room temperature for an hour, followed by incubation with anti-cleaved TAU for 3 days at 4 °C. After primary incubation tissue sections were washed with Phosphate buffer and incubated with goat anti-mouse Alexa Fluor<sup>®</sup> 488 (1:2000 dilution; Life Technologies, Carlsbad, CA) secondary antibody for 1 day at 4 °C. Tissue was co-labeled with 4',6-diamidino-2-phenylindole (DAPI; 1:50000; Thermo Fisher Scientific Inc., Waltham, MA) to identify cell nuclei. After secondary incubation tissue sections were washed and mounted using AquaPolymount (Polysciences Inc., Warrington, PA). Images were acquired using a Leica SP5X WLL confocal microscope (Leica Microsystems Inc., Buffalo Grove, IL).

### UV Irradiation of Cells

SH-SY5Y cells (ATCC<sup>®</sup> number: CRL-2266) were plated in a 6-well plate (35 mm dish) at  $1 \times 10^6$  cells per well and allowed to adhere overnight. The next day cells were treated with the pan-caspase inhibitor zVAD-fmk at 100 $\mu$ M concentration. One hour after treatment cells were UV irradiated (UV-C, 254 nm) for 5 min using a 3UV transilluminator (BioChemi System, UVP, Upland, CA, USA). One hour after UV treatment, cells were harvested for immunoblot analysis, caspase activity assays or stained for NFT formation using Thioflavin S.

### Data Analysis and Statistics

Protein expression levels normalized to mouse ACTB were quantified by densitometry using ImageJ software (NIH). Prism5 software (GraphPad Inc., La Jolla, CA) was used for statistical analysis. Student's t test was used to compare data and the correlation analyses between protein level and behavioral measurements were performed by calculating a Pearson product-moment correlation coefficient ( $r$ ) to evaluate the strength of the association [21].

## Results

### Cleaved Tau is Present in the Forebrain of Aged Mice

A growing body of evidence highlights the importance of tau truncation in initiation and potentiation of tau aggregation [8, 10, 11, 13, 33, 34, 37, 49]. To determine if truncated tau was present we performed quantitative immunoblotting to measure the amount of cleaved tau in the forebrain. We detected significantly greater levels of truncated tau in the aged forebrains compared to the young (Fig. 1a, b) ( $p = 0.0362$ ).

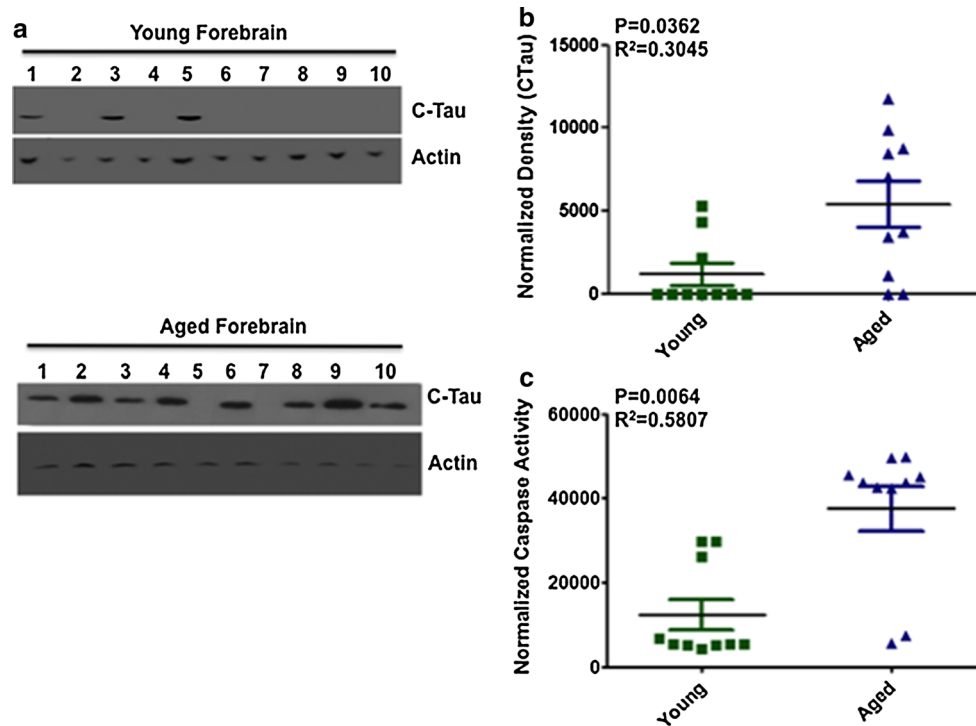
Tau truncated at amino acid D421 has been detected in AD and other tauopathies [2, 16, 37]. Truncation of tau introduces a conformational change that contributes to aggregation. Caspases are cysteine aspartic proteases typically considered to be activated during apoptosis, but can also be activated without cell death [30]. In this regard, while caspase activation precedes and promotes tangle formation, tangle-bearing neurons can survive for extended

periods [7, 32]. Cleavage of tau at D421 is mediated by caspase-3 [13, 37]. To determine if there was increased caspase activity we used the caspase-3 substrate DEVD-afc to measure the caspase activity in the forebrain of aged and young mice. Caspase activity was significantly higher in the forebrain of aged mice compared to young (Fig. 1c) ( $p = 0.0064$ ).

### Cognitive Deficits Correlate with Increased Levels of Truncated Tau and Active Caspases in the Forebrain of Aged Mice

Since the forebrain is the region of the CNS that contributes to the generation of spatial learning and memory and motor function [21], we wanted to examine the correlation between truncated tau and active caspases with behavior in the forebrain.

We identified a statistically significant association between truncated tau expression and behavioral performance in both the bridge walking (assessed as the latency of fall, LTF) and swim maze test (assessed by the learning



**Fig. 1** Increased caspase activity and cleaved tau expression in the forebrain of aged mice. **a** Representative immunoblots from 10 animals for each group (young and aged mice forebrain) showing cleaved tau (CTau) and actin (as a loading control) expression in the forebrain. Each well number represents an individual animal. **b** Quantitative densitometry indicated that cleaved tau expression is significantly higher ( $p = 0.0362$ ,  $R^2 = 0.3045$ ) in the forebrain of aged mice compared to young. Tau density was normalized to actin. Normalized density is given along the ordinate and the two groups

(young vs. aged) are given along the abscissa. **c** DEVD-afc, a substrate used to measure caspase-3 activity indicated that caspase activity is significantly higher ( $p = 0.0064$ ,  $R^2 = 0.5807$ ) in the forebrain of aged mice compared to young. Caspase activity was normalized (ordinate) to buffer alone. Data are shown as mean  $\pm$  SEM. *Green squares* represent young individuals, *blue triangles* represent aged.  $n = 10$  mice per group. Blots were confirmed in duplicate (Color figure online)

index, LI). Truncated tau expression in the forebrain showed a significant, strong negative association with the learning index (Fig. 2a). In addition, there was a significant negative association with truncated tau expression and the latency to fall (Fig. 2b).

There was also a significant association between active caspases and behavioral performance in both the bridge walking and swim maze tests. Active caspases in the forebrain showed a strong negative association with the learning index and the latency to fall (Fig. 2c, d).

### Correlation Between Cognitive Deficits and Increased Caspase Activity and Truncated Tau in the Cerebellum

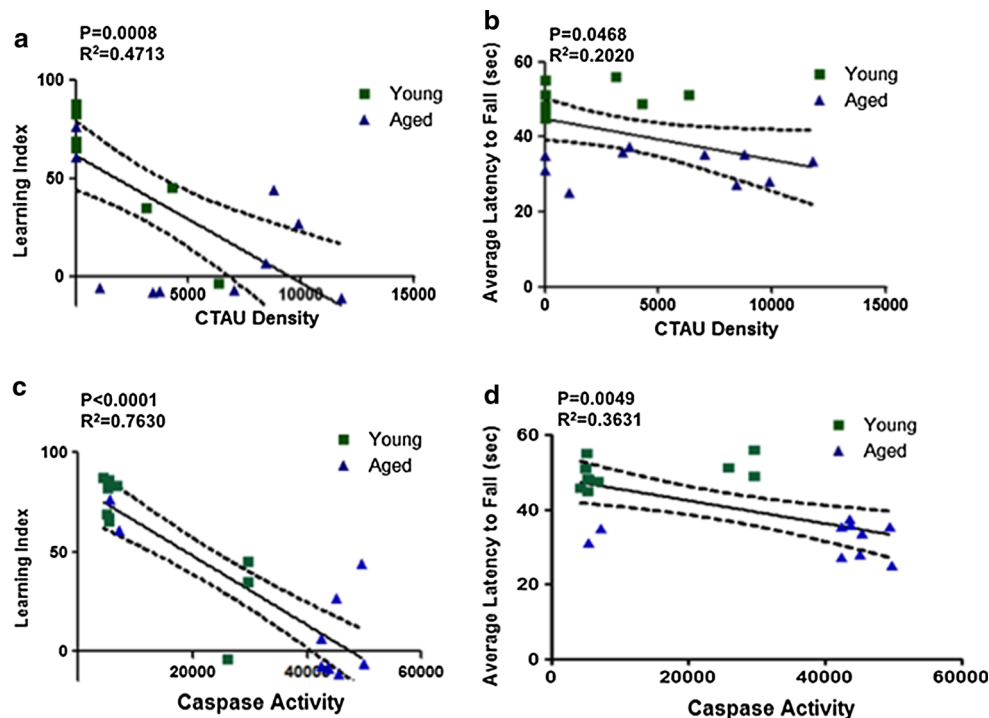
The main role of the cerebellum is to regulate motor function. We quantified truncated tau expression in the cerebellum of aged and young mice by quantitative immunoblotting. In the cerebellum of young mice, truncated tau expression was not detectable (Fig. 3a, b), while truncated tau expression was measured in 30 % of aged cerebella (Fig. 3a, b). In addition, the cerebellum of aged mice had detectable caspase activity (Fig. 3c). The caspase

activity was not significantly different from aged to young mice. However, the aged mice that had increased caspase activity also had truncated tau expression.

Consequently, we examined the association between behavioral performance and truncated tau expression and caspase activity in the cerebellum. There was a significant negative association between caspase activity and the latency to fall (Fig. 4d). In addition, although not statistically significant, truncated tau expression did show a weak negative association with the latency to fall (Fig. 4b). In contrast, there were no significant correlations of either caspase activity or truncated tau expression with the learning index (Fig. 4a, c). This is expected since the main role of the cerebellum is to regulate motor function.

### A Weak Learning Index Correlates with Increased Caspase Activity and Tau Cleavage: Scalability of Molecular Determinants in the Model of Cognitive Impairment During Normal Aging

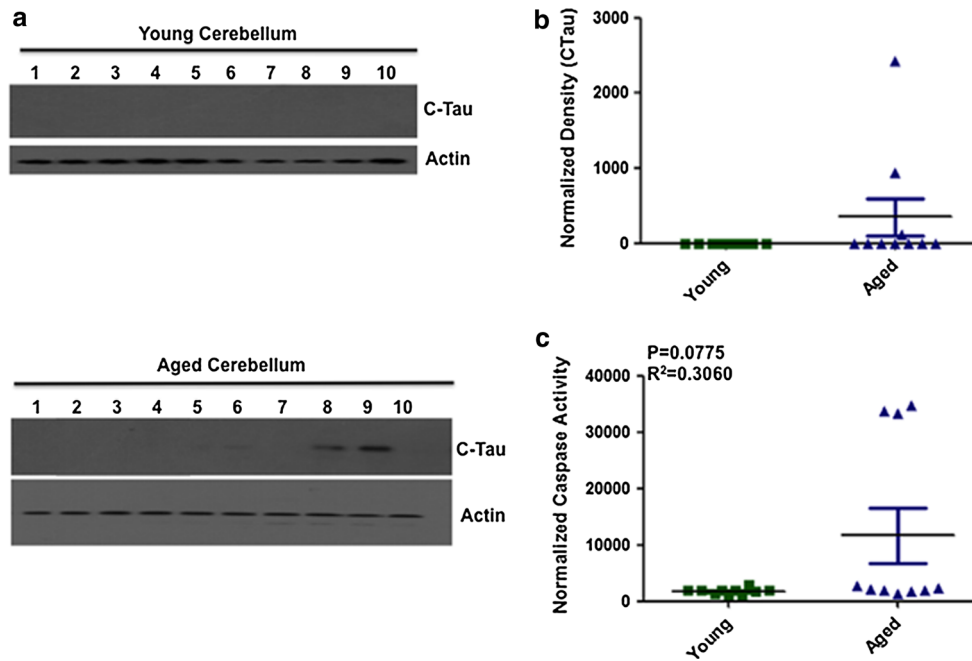
In examining the forebrain of young mice we observed three outliers in which truncated tau and increased caspase activity were observed. Upon further examination, we



**Fig. 2** Increased levels of truncated tau expression and caspase activity in the forebrains of aged mice correlates with age-related loss of memory and motor function. **a–b** Correlation analysis revealed that cleaved tau (CTau) expression in the forebrain was negatively associated with bridge walking performance ((A) Learning Index) ( $p = 0.0008$ ,  $R^2 = 0.4713$ ) and swim maze performance ((B) Average Latency to Fall) ( $p = 0.468$ ,  $R^2 = 0.2020$ ). **c** Similarly, increased

caspase activity also negatively associated with bridge walking (Learning Index) ( $p < 0.0001$ ,  $R^2 = 0.7630$ ) and **d** swim maze performance (Average Latency to Fall) ( $p = 0.0049$ ,  $R^2 = 0.3631$ ). *Green squares* represent young mice, *blue triangles* represent aged mice. Linear regressions are shown as *solid lines*; *dotted lines* represent 95 % confidence intervals (Color figure online)

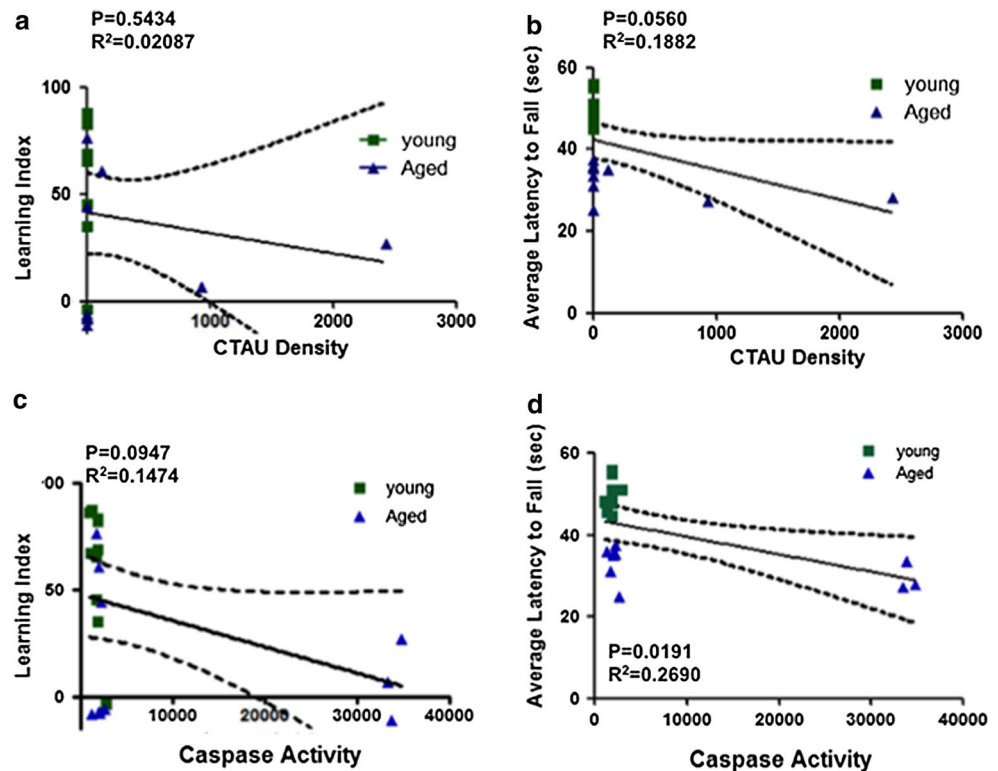




**Fig. 3** Cleaved tau immunoreactivity and caspase activity in the cerebellum showed no difference between young and aged mice. **a** Representative immunoblots from 10 animals for each group showing immunoreactivity for cleaved tau (CTau) and actin (loading control) in the cerebellum of young and aged mice. Each well number represents an individual animal. **b** Quantitative densitometry revealed no significant difference in cleaved tau levels in the cerebellum of young and aged mice. Tau density was normalized to actin.

**c** Similarly, caspase activity, measured using the substrate DEVD-afc showed no significant difference ( $p = 0.0775$ ,  $R^2 = 0.3060$ ) in the cerebellum of aged mice compared to young. Caspase activity was normalized to buffer alone. Data are shown as mean  $\pm$  SEM. *Green squares* represent young individuals, *blue triangles* represent aged.  $n = 10$  mice per group. Blots were confirmed in duplicate (Color figure online)

**Fig. 4** Correlation analysis between cleaved tau expression and caspase activity in the cerebellum with behavior. **a** Correlation analysis revealed that truncated tau (CTau) levels showed no significant association with the learning index ( $p = 0.5434$ ,  $R^2 = 0.02087$ ) or **b** the latency to fall ( $p = 0.0560$ ,  $R^2 = 0.1882$ ). **c** Similarly, increased caspase activity showed no significant association with the learning index ( $p = 0.0947$ ,  $R^2 = 0.01474$ ). **d** In contrast increased caspase activity showed a significant negative association with the latency to fall ( $p = 0.0191$ ,  $R^2 = 0.2690$ ). *Square symbols* represent young, *triangles* represent aged.  $n = 10$  mice per group



found a correlation with the learning index. In all three instances the young mice that had a low learning index indicative of cognitive impairment also had increased caspase activity and truncated tau (Fig. S1). Similarly, in aged mice that had no detectable truncated tau or caspase activity there was a higher learning index indicative of normal cognitive performance (Fig. S1).

### Higher Caspase Activity and Cleaved Tau in the Forebrain of Aged Mice

In examining the caspase activity and truncated tau expression in both the forebrain and cerebellum we observed that the forebrain had a significantly higher amount of both active caspases and truncated tau compared to the cerebellum (Fig. S2A–B). In addition, since we detected both increased caspase activity and truncated tau in the forebrain of aged mice we wanted to determine if there was a correlation between the two. In the forebrain of aged mice, there was a significantly strong positive association between increased caspase activity and cleaved Tau expression (Fig S2C).

### Presence of Cleaved Tau and Thioflavin S-Positive NFTs is Present in Cortical Layers of Aged Mice

To visually observe cleaved tau and NFT formation we performed immunohistochemistry on the forebrains of aged and young mice. We stained forebrains using the caspase cleaved tau antibody and observed cleaved tau staining in aged mice, but not young (Fig. 5a). Interestingly, in young mice that demonstrated behavioral impairment we also observed cleaved tau staining in the forebrain.

One pathological hallmark of tauopathies is aggregation of the microtubule-associated protein tau. To examine this, we stained aged and young forebrains with the histological dye, Thioflavin S. Thioflavin S stains tau-based neurofibrillary tangles/paired helical filaments [26, 39, 44] as well as amyloid  $\beta$  plaques [3, 27, 46]. In aged, behaviorally impaired mice we detected Thioflavin S-positive forebrains, while in young behaviorally-nonimpaired mice there was no detectable Thioflavin S staining (Fig. 5b). In addition, young behaviorally impaired mice also stained Thioflavin S-positive. The same was observed in the cerebellum (Fig. 5c, d).

### Tau Cleavage is Caspase-3-Dependent and Leads to the Formation of Neurofibrillary Tangles

To investigate the upstream and downstream mechanisms of tau cleavage, we tested whether caspases can cleave tau and whether truncated tau can form NFTs in a cellular

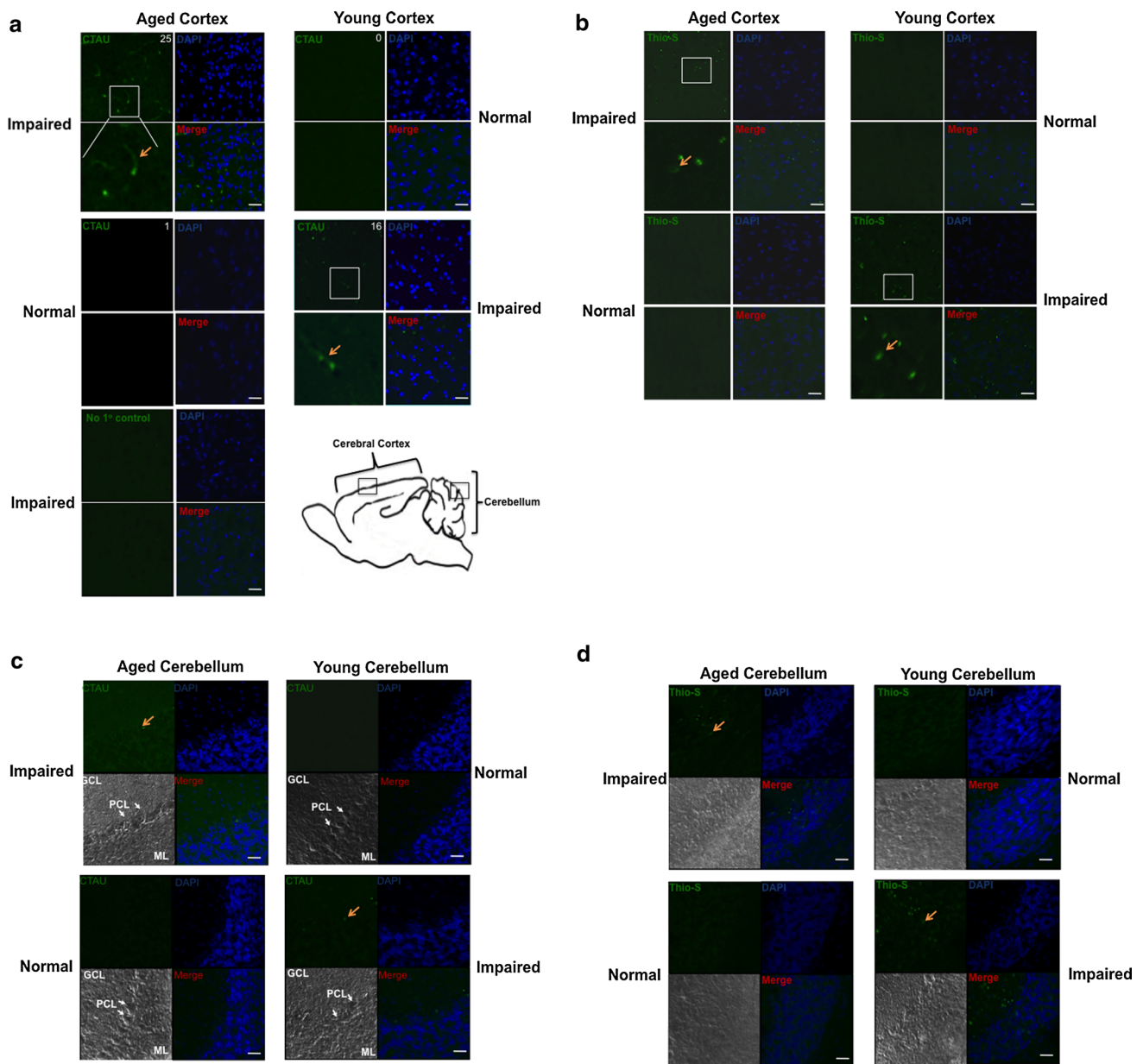
in vitro model, the human neuroblastoma cell line, SH-SY5Y. Caspase activity was induced by UV irradiation. One hour after UV treatment, cells were harvested for immunoblot analysis. SH-SY5Y cells that were UV irradiated showed cleaved Tau present along with activated caspases (Fig. 6a, c). However, when cells were treated with the broad caspase inhibitor zVAD-fmk prior to UV treatment, caspase activation along with cleaved Tau was not detected. To determine if neurofibrillary tangles were being formed, cells were stained with Thioflavin S and imaged. Cells that were UV irradiated showed Thioflavin S-positive staining and this was inhibited by pretreatment with zVAD-fmk (Fig. 6d).

### Discussion

The present study provides the first evidence for a non-transgenic mammalian model of tauopathies during normal aging. Normally aged mice that are not genetically modified and have not been subjected to neuronal damage show behavioral cognitive impairment and a high level of active caspase compared to young animals of the same strain. In addition, we detected truncated tau in aged mice, which was most prominent in the forebrain. These increased levels of caspase activity and cleaved tau correlated with cognitive deficits associated with aging. This was similar to what was reported in a fly model of AD. In that model, aged wildtype flies showed increased caspase activity, tau truncation and behavioral impairment [31]. One of the hallmark characteristics of tauopathies is NFT formation. Thioflavin S is used to stain tau-containing neurofibrillary tangles/paired helical filaments [26, 39, 44] as well as amyloid  $\beta$  plaques [3, 27, 46]. We detected NFTs using Thioflavin S in all cognitively-impaired mice.

Activated caspases cleave tau, the main component of NFTs. When cleaved in vitro by caspases, tau “seeds” filamentous aggregates [6]. In addition, tau adopts the MC1 conformation when cleaved, one of the earliest pathologic events in tangle formation [6]. Truncated tau occurs early in the development of tangles within AD brains and in a transgenic mouse model of AD. This truncated form of tau is therefore likely to initiate or accelerate NFT development.

Tau aggregation is a hallmark of several neurodegenerative diseases, including AD [40]. The mechanism underlying tau aggregation is still unclear. Recent reports have shown that tau cleavage plays an important role in tau aggregation and neurodegeneration [17]. Truncation of tau may generate tau fragments that initiate tau aggregation, which can lead to toxicity or result in tau fragments, which induce neurodegeneration through mechanisms not yet known, independent of aggregation.



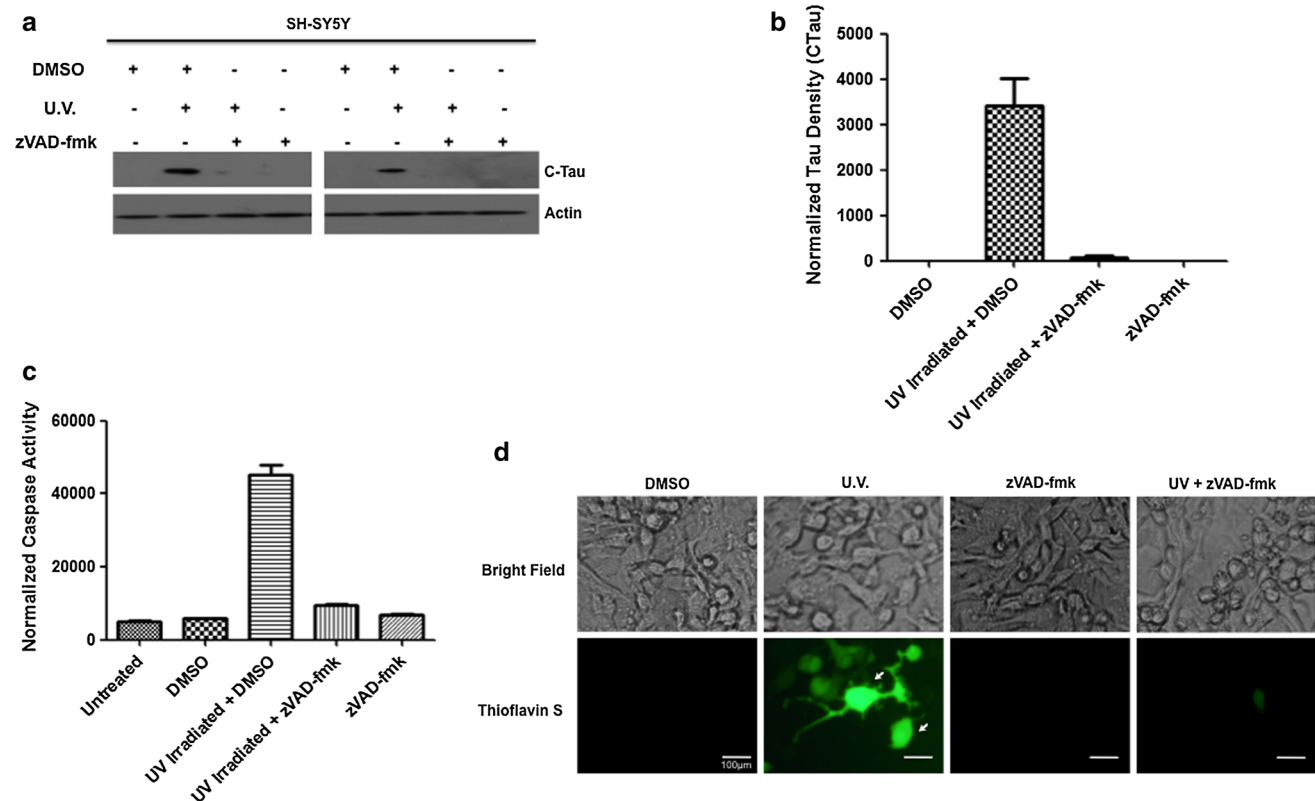
**Fig. 5** The aged cortex of cognitively impaired mice is positive for cleaved tau and NFTs. **a** Aged and young forebrains were immunostained for cleaved tau (CTAU) (green). Aged mice that showed behavioral impairment also were positive for cleaved tau. Average number of cleaved TAU/area is indicated at top right of CTAU panel. **b** Similarly, the aged behaviorally impaired mice were also positive for NFTs based on Thioflavin S staining (Thio-S) (green). DAPI was used to stain nuclei (blue). Images are representative fields of the outer cortical layers of the forebrain (the region where images were taken is identified by a box in panel A). The CTAU or Thio-S images

were overlaid with the DAPI stain and shown in the merge panels, respectively. **c** Aged and young cerebellum were immunostained for cleaved tau (CTAU) (green). Aged mice showing behavioral impairment were positive for cleaved tau. **d** Similarly, aged behaviorally impaired mice were Thioflavin S positive (Thio-S) (green). Cerebellum sections were counterstained with DAPI (blue) as a nuclear marker. Red arrows indicate CTAU or Thio-S positive cells. The region where images were taken is identified by a box in panel A. ML Molecular layer, PCL Purkinje cell layer, GCL Granule cell layer. Scale bars represent 25  $\mu$ M (Color figure online)

In addition, SH-SY5Y cells transfected with a tau mutant are vulnerable to apoptosis [12]. It is possible that the C-terminal truncation of tau eliminates an as yet unidentified motif that functions to modulate cell death.

It is interesting to note that the inclusions that form in cells containing cleaved tau stain for Thioflavin S. This indicates that the aggregates have some ordered structure, which based on previous reports is likely filamentous.





**Fig. 6** Active caspases cleave tau in the human neuroblastoma SH-SY5Y cell line. **a** SH-SY5Y cells were UV irradiated in the presence of the general caspase inhibitor zVAD-fmk (100  $\mu$ M) and immunoblotted for cleaved tau (CTau). Cells that were UV irradiated had detectable cleaved tau but this was blocked by the caspase inhibitor zVAD-fmk. Actin served as a loading control. **b** Quantitative densitometry indicates SH-SY5Y cells show a higher amount of cleaved tau (normalized to actin) compared to control and zVAD-fmk

treated cells. **c** SH-SY5Y cells that have been UV irradiated show increased levels of caspase activity (normalized to buffer), measured using the caspase substrate DEVD-afc and this is inhibited by pretreatment with zVAD-fmk. **d** SH-SY5Y cells that have been UV irradiated stain Thioflavin S-positive (green) and this is inhibited by treatment with zVAD-fmk. Arrows indicate Thioflavin S-positive cells (green). Scale bars represent 100  $\mu$ M (Color figure online)

We propose a model on the role of caspases in the cleavage of tau in tangle development. Following the activation of caspases by a stimulus, tau is cleaved predominantly at Asp421 [13]. The truncated tau undergoes a conformational change, leading to increased filament formation and tau aggregation on the microtubule. To compensate for this, tau is phosphorylated, which leads to tau being removed from the microtubules. The hyperphosphorylated tau then leads to paired helical filament (PHF) formation and microtubule destabilization [14, 15]. Although microtubule destabilization may be acting as a neuroprotective mechanism or is an attempt of the neuron to initiate neuroprotective signaling, the disruption of intracellular trafficking leads to apoptosis. Based on the role of caspase mediated cleavage of tau in promoting NFT formation in AD, blocking the truncation of tau could provide a promising therapeutic approach for the treatment of AD or other tauopathies.

In the mice we examined we identified outliers from both the aged and young groups. The aged outliers showed

no significant caspase activity or tau cleavage. Interestingly, these outliers had fewer cognitive deficits based on the learning index. Conversely, the young outliers that showed significant caspase activity and tau cleavage also had impaired cognitive function suggesting that the same mechanism is at play regardless of age. These outliers are expected in a system that has not been genetically or chemically modified. As with many neurodegenerative disorders such as AD, the older population is more prone to disease onset, as age represents the primary predisposing condition. However, the younger population can also be susceptible, although at a lower rate and to a smaller extent. We consider this finding as highly relevant as it not only contributes to a statistically highly significant finding, but also models the human clinical situation of AD very accurately.

Transgenic mouse models of AD have allowed researchers to examine potential mechanisms involved in the development of disease based on human mutations in the genes for presenilins (PSEN1 and PSEN2) and amyloid

beta precursor protein (APP). Some of these models also use mutations in MAPT, the gene for the cytoskeletal protein tau linked to tauopathies such as frontotemporal dementia [23]. However, because of the large discrepancy in the behavioral findings observed across the AD mouse models, a question that arises is whether we are really any closer today to determining what these mechanisms are. In addition, another argument that complicates the use of animal models based solely on APP and/or tau mutations is that other mechanisms may be at play.

In using a non-genetic aging model to examine AD-like pathologies we remove the variability that is associated with transgenic animals. For instance, transgene integration is apparently random. Also, experiments reveal that the genetic surrounding of the inserted transgenic construct is modulating the expression pattern of the transgene itself both quantitatively and qualitatively. In our system we allow for normal aging and disease onset to occur instead of forcing the system to a disease state.

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