Genetic reduction of tyramine β hydroxylase suppresses Tau toxicity in a Drosophila model of tauopathy

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A B S T R A C T

Tauopathies are a class of neurodegenerative diseases characterized by the abnormal phosphorylation and accumulation of the microtubule-associated protein, Tau. These diseases are associated with degeneration and dysfunction of the noradrenergic system, a critical regulator of memory, locomotion, and the fight or flight response. Though Tau pathology accumulates early in noradrenergic neurons, the relationship between noradrenaline signaling and tauopathy pathogenesis remains unclear. The fruit fly, Drosophila melanogaster, is a valuable model organism commonly used to investigate factors that promote Tau-mediated degeneration. Moreover, Drosophila contain the biogenic amine, octopamine, which is the functional homolog to noradrenaline. Using a Drosophila model of tauopathy, we conducted a candidate modifier screen targeting tyramine β hydroxylase (tyh), the enzyme that controls the production of octopamine in the fly, to determine if levels of this enzyme modulate Tau-induced degeneration in the fly eye. We found that genetic reduction of tyh suppresses Tau toxicity, independent of Tau phosphorylation. These findings show that reduction of tyh, a critical enzyme in the octopaminergic pathway, suppresses Tau pathogenicity and establishes an interaction that can be further utilized to determine the relationship between noradrenergic-like signaling and Tau toxicity in Drosophila.

1. Introduction

Tauopathies are a class of over 20 heterogeneous neurodegenerative diseases characterized by the abnormal phosphorylation and accumulation of the microtubule-associated protein, Tau [1]. These disorders, which include Alzheimer’s disease (AD), frontotemporal lobar degeneration (FTLD-Tau), and progressive supranuclear palsy (PSP), display region-specific Tau pathology that often follows a stereotyped progression of Tau deposition [2,3]. One region in particular, the locus coeruleus (LC), is known to display Tau pathology in the early stages of AD [3–7], PSP [8], and FTLD-Tau [9]. The LC is a brainstem nucleus that is the primary source of the biogenic amine, noradrenaline (norepinephrine), and contains noradrenergic neurons that innervate target regions widely throughout the brain, including the hippocampus and cortex. Activation of this noradrenergic system plays a critical role in movement, attention, and cognition [10], and dysfunction of the noradrenergic system is implicated in tauopathy pathogenesis [11].

In tauopathies, deposition of Tau pathology in the LC occurs early in the disease process [6,11,12], and LC degeneration correlates with disease progression [13,14]. Though dysfunction of the noradrenergic system correlates with behavioral impairments and cognitive decline in Tau-associated degenerative diseases [15], the causal links between altered noradrenergic signaling, Tau pathology, and tauopathy pathogenesis remains to be determined. Studies in cell culture and animal tauopathy models have implicated noradrenergic signaling in modulating Tau phosphorylation and toxicity using both pharmacological and genetic approaches [16–22]. Moreover, a recent examination of neurotransmitter processing in the mouse LC revealed that the noradrenergic metabolite, DOPEGAL, induces Tau-mediated degeneration [23], suggesting that increased noradrenergic activity, and subsequent neurotransmitter metabolism, promotes Tau pathology and subsequent neurodegeneration. Conversely, ablation of the LC, and resulting destruction of noradrenergic neuronal innervation, also exacerbates Tau pathology [24]. These seemingly conflicting reports underscore the
complex role of LC noradrenergic signaling in modulating Tau pathology and neurodegeneration. As a result, further experimentation into the relationship of noradrenergic signaling and Tau-mediated degeneration is needed.

The fruit fly, Drosophila melanogaster is a useful model organism to study the molecular mechanisms of Tau-mediated degeneration [25–27]. Overexpression of human Tau in the Drosophila nervous system results in robust toxicity that lends itself well to forward and reverse genetic screens to identify pathways that promote Tau toxicity [25, 27–29]. Drosophila is also well-suited to investigate the relationship between noradrenergic signaling and Tau toxicity, as the fly contains octopamine, a functional and structural homolog to noradrenaline [30, 31]. Octopamine is generated from tyramine via the activation of the enzyme, tyramine β hydroxylase (τβh) in Drosophila. Octopamine is released widely throughout the brain where it activates G-protein coupled, noradrenaline-like receptors that mediate attention and learning, similar to the role of the noradrenaline system in vertebrates [31,32]. Given the similarities between Drosophila octopaminergic and vertebrate noradrenergic systems, and the importance of the noradrenergic system in tauopathy pathogenesis, we investigated the effects of modulating levels of τβh in a Drosophila model of tauopathy [27,33,34]. Considering that genetic reduction of the related noradrenergic synthesizing enzyme, dopamine β hydroxylase (dβh), in mouse tauopathy models ameliorated the toxic effects of Tau [23], we hypothesized that genetic reduction of the octopamine synthesizing enzyme, τβh, would suppress Tau toxicity in Drosophila.

2. Material and methods

2.1. Genetics & crosses

Flies were crossed and maintained at 25 °C, at 60 % relative humidity, on Nutri-Fly Bloomington formulation media (Genesee Scientific). The following stocks were obtained from the Bloomington Stock Center: w1118 (BL# 3605); GMR-GAL4 (BL# 9146); Crl1125 (BL# 60303); τβh1125 (BL# 76655). The following stocks were kindly provided: a double-balanced GMR-GAL4; UAS-MAPTΔN4RTau line (Dr. Mel Feany; heretofore referred to as GMR-GAL4; UAS-Tau); τβh2168, τβh866, and UAS-τβh (Dr. Maria Monastirioti). For each experiment, virgin female candidate modifiers were crossed with male GMR-GAL4 or GMR-GAL4; UAS-Tau flies. One-day-old adult flies from each cross were collected and processed for image acquisition or Western blotting (see below).

2.2. Fly eye image acquisition and analysis

One-day-old adult fly eyes were frozen overnight and affixed with the left eye facing upwards on a glass slide. Light microscopic images were acquired using SPOT software on a Nikon SMZ1500 stereo scope with 1x objective and 11.25 multiplier. Scanning electron microscopic images were acquired using a FEI Quanta 200 under low vacuum setting with an accelerating voltage of 15 kV.

The progeny from crosses between the candidate modifier lines and GMR-GAL4 and GMR-GAL4; UAS-Tau flies were used for analysis. At least 9 light microscopic images of fly eyes from the same genotype were used to quantify rough eye modification using the Flynotyper analysis plugin in ImageJ [41]. Briefly, the quick selection tool in Photoshop was used to isolate the fly eye, and this image was placed on a black background canvas. These eye images were analyzed with the Flynotyper ImageJ plugin using the light microscope setting to obtain a phenotypic score, where higher phenotypic scores represent more disordered, rougher eyes, while lower phenotypic scores represent more normal eyes [41].

2.3. Western blotting

Heads from frozen one-day-old flies were homogenized in 2X Laemmli buffer, boiled for 10 min, briefly centrifuged, and subjected to SDS-PAGE in 10 % separating gels. Proteins were transferred to a nitrocellulose membrane, blocked in 2 % milk in TBS containing 0.05 % Tween, and immunoblotted utilizing the following antibodies and dilutions: phosphorylation independent rabbit polyclonal anti-Tau C-terminal antibody (1:200,000, Dako), mouse anti-AT8 antibody (specific for phosphorylation at Ser 202/Thr 205) (1:1000, ThermoFisher), mouse anti-AT180 antibody (specific for phosphorylation at Thr231) (1:1000, ThermoFisher), mouse anti-AT270 antibody (specific for phosphorylation at Thr181) (1:1000, ThermoFisher), mouse anti-NRV antibody (1:1000, DSHB), and mouse anti-actin (1:5000, DSHB). Horseradish peroxidase-conjugated goat anti-rabbit and goat anti-mouse secondary antibodies (1:10,000, Jackson Immuno Research) were applied, and signal detected via chemiluminescence (BioRad). Membrane stripping was carried out by incubating membranes in stripping buffer (62.5 mM Tris–HCl pH 6.8, 2 % SDS, 1 % β-mercaptoethanol) at 50 °C for 30 min or Restore Western blot stripping buffer (ThermoFisher) at room temperature for 20 min.

2.4. Western blotting analysis

Densitometric analysis of bands was performed in ImageJ. Phosphorylated Tau levels were calculated as a ratio of the total Tau level in each corresponding lane. Total Tau levels were calculated as a ratio of each loading control, NRV or actin.

2.5. Statistical analysis

All statistical analyses were performed in GraphPad Prism 7.03 software, with error bars representing SEM. Statistical significance for Flynotyper eye images was assessed using one-way ANOVA with Dunnnett’s multiple comparison test, comparing the Tau alone condition to control and Tau + candidate modifiers. Statistical significance for densitometric analysis of Western blot images was assessed using unpaired t-tests comparing Tau alone to Tau + candidate modifier for each antibody.

3. Results

3.1. Genetic reduction of τβh suppresses Tau toxicity

The enzyme, τβh, generates octopamine from tyramine as part of the octopamine biosynthesis pathway in Drosophila (Fig. 1A). To determine whether reducing levels of τβh modifies Tau toxicity, in vivo, we utilized a well-established Drosophila tauopathy model that uses the eye-specific driver, GMR-GAL4, to drive expression of a human Tau responder (UAS-Tau) in the Drosophila eye [33,34,39]. This eye-specific Tau expression yields an abnormal eye, commonly referred to as a rough eye [33,34, 40]. Using these Tau transgenic flies, we asked whether a 50 % genetic reduction of τβh modulates the Tau rough eye. We first confirmed that progeny from the cross between GMR-GAL4 flies and the isogenic control, w1118, yielded a wild-type eye with normal ommatidial structure and arrangement (Fig. 1B), and that GMR-GAL4; UAS-Tau flies crossed to w1118 flies produced a moderate rough eye characterized by disorganized and fused ommatidia (Fig. 1C), consistent with previous findings [34,39]. This rough eye is an indicator of Tau-mediated degeneration, where the ommatidial disorganization can be suppressed or enhanced to identify genetic modifiers of Tau toxicity [33,34,40]. To determine if genetic reduction of τβh modulates the Tau rough eye, we
crossed Tau transgenic flies to $\beta_h^{\text{nM18}}$ mutant flies. This X-linked null allele was generated from imprecise p-element excision, and is the canonical $\beta_h$ allele used in the manipulation of the octopaminergic pathway [35-38]. We crossed male Tau transgenic flies to $\beta_h^{\text{nM18}}$ flies and found that $50\%$ genetic reduction of $\beta_h$ in female progeny ($\beta_h^{\text{nM18}}/\chi;\text{GMR-GAL4}/++;\text{UAS-Tau}/+$) restored ommatidial organization and suppressed the Tau rough eye phenotype (Fig. 1D). To confirm this suppression, and control for non-specific background genetic effects, we examined rough eye modification in the female progeny of Tau transgenic flies crossed to the revertant allele, $\beta_h^{\text{M6}}$. This $\beta_h^{\text{M6}}$ allele is the result of precise p-element excision, and was generated in the same mutagenesis screen that produced the $\beta_h^{\text{nM18}}$ allele [35]. Flies carrying the $\beta_h^{\text{M6}}$ revertant allele contain a functional copy of the $\beta_h$ gene and are fertile and produce normal levels of octopamine [35].

Crossing Tau transgenic flies to $\beta_h^{\text{M6}}$ flies produced female progeny ($\beta_h^{\text{M6}}/\chi;\text{GMR-GAL4}/++;\text{UAS-Tau}/+$) that exhibited a rough eye phenotype similar to the Tau rough eye of the GMR-GALA/++; UAS-Tau/+ flies (Fig. 1E), suggesting that the $\beta_h^{\text{M18}}$-mediated suppression is not due to the genetic background of the stock. Moreover, control flies (GMR-GALA) crossed with either $\beta_h^{\text{M18}}$ or $\beta_h^{\text{M6}}$ lines did not affect eye morphology (Fig. 1F, G) suggesting that reduction in $\beta_h$ levels on its own does not affect normal eye development. This Tau rough eye suppression in $\beta_h^{\text{M18}}$ heterozygous females was quantitatively confirmed by Flynotyper phenotypic analysis [41]. We found that genetic reduction of $\beta_h$, using the $\beta_h^{\text{M18}}$ allele, significantly reduced the rough eye phenotype compared to Tau alone controls, while the rough eye phenotype of flies containing the $\beta_h^{\text{M6}}$ allele was not statistically different than the Tau alone rough eye (Fig. 1H).

Given that a $50\%$ reduction in $\beta_h$ suppressed the Tau rough eye, we explored whether flies null for $\beta_h$ would show similar Tau rough eye suppression. To test this, we assessed eye morphology in male progeny of Tau transgenic flies crossed to $\beta_h^{\text{nM18}}$ mutant alleles. As the $\beta_h$ gene is on the X chromosome, male flies harboring the mutant $\beta_h^{\text{nM18}}$ allele on their one X chromosome are hemizygous null for $\beta_h$. We again confirmed that GMR-GALA and GMR-GAL4; UAS-Tau flies crossed to $\beta_h^{\text{nM18}}$ flies yielded a wild-type (Fig. 2A) and moderate Tau rough eye (Fig. 2B), respectively. Similar to heterozygous females, we found that hemizygous male progeny of $\beta_h^{\text{nM18}}$ flies crossed to Tau transgenic flies ($\beta_h^{\text{nM18}}/\chi;\text{GMR-GAL4}/++;\text{UAS-Tau}/+$) suppressed the Tau rough eye (Fig. 2C), while male progeny of $\beta_h^{\text{M6}}$ revertant flies crossed to Tau transgenic flies ($\beta_h^{\text{M6}}/\chi;\text{GMR-GAL4}/++;\text{UAS-Tau}/+$) did not modify the Tau rough eye (Fig. 2D). The lack of suppression in $\beta_h^{\text{M6}}$ male mutant flies further indicates that the $\beta_h^{\text{M18}}$-mediated rough eye suppression is not due to genetic background of the fly line. Additionally, male progeny from control flies (GMR-GALA) crossed with either $\beta_h^{\text{M18}}$ or $\beta_h^{\text{M6}}$ lines did not affect eye morphology in the absence of Tau expression (Fig. 2E, F). The Tau rough eye phenotype and subsequent suppression of Tau toxicity in hemizygous $\beta_h^{\text{M18}}$ male progeny was quantitatively confirmed by Flynotyper phenotypic analysis (Fig. 2G) [41], and mirrors the suppression observed in female heterozygous flies.

P-element mutagenesis experiments are known to generate second-site mutations, independent of the proposed mutation [42]. To
confirm that the $\tau_{\beta h}^{18}$-mediated Tau rough eye suppression was specific to a mutation in the $\tau_{\beta h}$ gene, and not a result of a second-site mutation, we examined Tau rough eye modification in a mutant line from the Trojan-GAL4 ($T2A$-GAL4) collection. These fly strains carry a cassette that contains a stop signal that disrupts expression of the gene into which the cassette is inserted, and can function as a null allele \cite{43,44}. We crossed the line predicted to disrupt $\tau_{\beta h}$ ($\tau_{\beta h}^{T2A}$) to Tau transgenic and control flies, and compared the progeny from these crosses to progeny from crosses to a $T2A$-GAL4 control line ($Ctrl^{T2A}$), which contains the cassette in a non-gene disrupting site on the X chromosome.

Flynotyper analysis of progeny from these crosses showed that the $\tau_{\beta h}^{T2A}$ line suppressed the Tau rough eye in both heterozygous females and hemizygous males, compared to Tau transgenic flies crossed to the $Ctrl^{T2A}$ line (Supplemental Fig. 1), confirming that our observations using the $\tau_{\beta h}^{18}$ allele are not a result of a second-site mutation.

### 3.2. Genetic reduction of $\tau_{\beta h}$ does not alter Tau phosphorylation

Given that reductions in $\tau_{\beta h}$ attenuated the Tau-induced rough eye, we assessed if the $\tau_{\beta h}^{18}$-mediated suppression of Tau toxicity is associated with an alteration in Tau phosphorylation levels. Densitometric analysis of western blots of $\tau_{\beta h}^{18}$/x heterozygous female and $\tau_{\beta h}^{18}$/y hemizygous male Tau transgenic flies displayed similar levels of phosphorylated Tau at AT8, AT180, and AT270 phospho-epitopes, and total Tau levels were similar when quantified using either NRV or actin as loading controls (Fig. 3). These data suggest that the $\tau_{\beta h}^{18}$-mediated suppression of Tau toxicity occurs independent of Tau phosphorylation.

### 3.3. Overexpression of $\tau_{\beta h}$ does not affect Tau toxicity or phosphorylation

Since the genetic reduction of $\tau_{\beta h}$ suppressed the Tau rough eye, we investigated the effect of conversely overexpressing the $\tau_{\beta h}$ enzyme using a $UAS$-$\tau_{\beta h}$ transgene \cite{45}. As before, control progeny yielded a normal eye (Fig. 4A) and Tau transgenic flies yielded a moderate rough eye (Fig. 4B). However, overexpression of $\tau_{\beta h}$ did not affect the Tau rough eye (Fig. 4C), nor did it alter the morphology when crossed with control flies (Fig. 4D). The absence of Tau rough eye modification was
quantified with Flynotyper phenotypic analysis and confirmed to be no different from Tau alone controls (Fig. 4 E). These findings show that overexpression of the \( t\beta h \) enzyme in the fly eye does not influence Tau toxicity and is not toxic on its own. Moreover, western blot analysis of \( t\beta h \)-overexpressing Tau transgenic flies (\( x/x; \text{GMR-GAL4/+; UAS-Tau/UAS-t}\beta h) \), compared to Tau transgenic flies alone (\( x/x; \text{GMR-GAL4/+; UAS-Tau/UAS-t}\beta h \)), did not reveal a difference in phosphorylated Tau or total Tau levels (Fig. 4 F, G) demonstrating that overexpression of \( t\beta h \) in the eye does not influence Tau phosphorylation.

4. Discussion

Tauopathies are characterized by early Tau pathology in the noradrenergic nucleus, LC [6,11,12], and LC degeneration and subsequent loss of noradrenergic innervation correlates with disease progression [13,14]. Conversely, pharmacological manipulation of downstream receptors suggest that increased noradrenergic signaling may also exacerbate Tau pathology [23]. These studies raise questions about whether the noradrenergic system suppresses or promotes tauopathy pathogenesis. Using the noradrenaline homolog in Drosophila, octopamine, this study aimed to assess whether genetic reduction of \( t\beta h \), the enzyme that controls the catalysis of octopamine, modulates Tau toxicity in a Drosophila tauopathy model. We find that genetic reduction of \( t\beta h \) suppresses Tau toxicity independent of Tau phosphorylation, while increasing \( t\beta h \) expression in the eye does not impact toxicity. These findings identify a novel interaction between octopamine synthesis and Tau toxicity in modulating the Tau rough eye phenotype.

In Drosophila, the octopaminergic system shares structural and functional similarities to the vertebrate noradrenergic system, where it plays a critical role in regulating the flight or fight response, learning, and memory [46]. Octopamine production is controlled by \( t\beta h \), and \( t\beta h \) mutants are devoid of octopamine [31,35]. We identified a functional role of \( t\beta h \) in modifying Tau toxicity by observing a suppression of Tau toxicity in \( t\beta h^{M18} \) flies. Specifically, complete ablation of \( t\beta h \) in

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A \quad \text{Tau} \quad \text{Tau + } t\beta h^{M18} \quad \text{Ctrl}
\]

\[
p\text{Tau (AT8)} \quad \text{p\text{Tau (AT180)}} \quad \text{p\text{Tau (AT270)}} \quad \text{Total Tau} \quad \text{NRV} \quad \text{Actin}
\]

\[
B \quad \text{Female}
\]

\[
\text{Relative protein levels (Normalized to Tau flies)}
\]

\[
\begin{array}{cccc}
\text{ATB} & \text{AT180} & \text{AT270} & \text{Total Tau (NRV)} & \text{Total Tau (actin)}
\end{array}
\]

\[
\text{Ctrl} \quad \text{Tau} \quad \text{Tau + } t\beta h^{M18}
\]

\[
\text{(B) Densitometric analysis of immunoblots quantifying phosphorylated Tau levels as a ratio of total Tau, and total Tau levels as a ratio of NRV or actin. (n = 6-16, unpaired t-tests, all pairwise comparisons between Tau and Tau + } t\beta h^{M18} \text{ are not significant). (C) Representative western blot images of phosphorylated Tau (ATB, AT180, and AT270), total Tau, NRV, and actin levels from brain lysates of female Tau transgenic flies alone (Tau: } x/x; \text{GMR-GAL4/+; UAS-Tau/+}) \text{ or heterozygous for } t\beta h \text{ (Tau + } t\beta h^{M18}; \text{UAS-Tau/+). Control flies (Ctrl) lack the UAS-Tau transgene ( } x/x; \text{GMR-GAL4/+). (D) Densitometric analysis of immunoblots quantifying phosphorylated Tau levels as a ratio of total Tau, and total Tau levels as a ratio of NRV and actin. (n = 7-15, unpaired t-tests, all pairwise comparisons between Tau and Tau + } t\beta h^{M18} \text{ are not significant). Error bars represent } \pm \text{ SEM. All flies 1-day-old.}
\]

\[
C \quad \text{Tau} \quad \text{Tau + } t\beta h^{M18} \quad \text{Ctrl}
\]

\[
p\text{Tau (AT8)} \quad \text{p\text{Tau (AT180)}} \quad \text{p\text{Tau (AT270)}} \quad \text{Total Tau} \quad \text{NRV} \quad \text{Actin}
\]

\[
D \quad \text{Male}
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\[
\text{Relative protein levels (Normalized to Tau flies)}
\]

\[
\begin{array}{cccc}
\text{ATB} & \text{AT180} & \text{AT270} & \text{Total Tau (NRV)} & \text{Total Tau (actin)}
\end{array}
\]

\[
\text{Ctrl} \quad \text{Tau} \quad \text{Tau + } t\beta h^{M18}
\]

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\text{(D) Densitometric analysis of immunoblots quantifying phosphorylated Tau levels as a ratio of total Tau, and total Tau levels as a ratio of NRV and actin. (n = 7-15, unpaired t-tests, all pairwise comparisons between Tau and Tau + } t\beta h^{M18} \text{ are not significant).}
\]
of octopamine reduction in heterozygous females (confirmed in males to undetectable levels [35, 47], but the precise level of octopamine is still functionally active [35], similar suppression was observed indicating that a complete loss of octopamine is not necessary to suppress the Tau rough eye phenotype. Previous studies using the UAS-MTH null mutants have confirmed its efficacy in reducing octopamine concentrations in males to undetectable levels [35, 47], but the precise level of octopamine reduction in heterozygous females (UAS-MTH/x) has not been established. Given that we did not directly confirm the extent to which specific concentrations of octopamine contribute to the suppression of Tau toxicity. This limitation notwithstanding, our UAS-MTH-mediated suppression, coupled with the absence of effect in flies carrying the UAS-MTH revertant allele, provide a control to demonstrate the specificity of our octopamine pathway manipulation and further supports an interaction between the octopaminergic pathway and Tau toxicity. Pharmacological experiments aimed at determining the degree to which levels of octopamine, and its precursor tyramine, modulate Tau toxicity are therefore warranted.

Relatedly, overexpression of τh did not affect Tau toxicity. This suggests that increasing τh protein levels in the eye is not sufficient to enhance Tau toxicity. One explanation for this may be that the functional role of τh in promoting Tau toxicity is already at maximum efficacy with further increases in enzyme availability unable to enhance the Tau rough eye. However, it should be noted that we did not directly evaluate τh levels in these experiments, limiting our ability to directly assess a dose-response effect. Tau-mediated cell death is typically apoptotic [33, 48], and given that our studies do not identify a functional mechanism linking τh to Tau toxicity, it remains unknown whether the rough-eye suppression effects of τh reduction are due to direct enzyme interaction with Tau or a result of reduced octopamine synthesis and subsequent Tau pathogenicity. Moreover, τh is predominantly expressed in octopaminergic neurons localized to the subesophageal ganglion and thoraco-abdominal ganglia [31], therefore overexpression of τh in the eye may not translate to increased octopamine production in our Tau transgenic flies. Though neurons in the eye may not produce octopamine, these cells have been shown to contain the full complement of octopaminergic receptors [49], and are innervated by octopaminergic neurons [31, 50]. This suggests that the τh-mediated suppression of Tau toxicity may be due to reduced octopamine secretion from octopaminergic neurons, outside of the eye. Future experiments utilizing dual binary expression systems, where eye-specific Tau expression is coupled with activation or inactivation of octopaminergic neurons would assist in better defining the relationship between τh activity, octopamine
levels, and Tau toxicity.

Our results show that Tau toxicity can be suppressed independent of alterations to Tau phosphorylation at three proline-directed phosphorylation sites (AT8, AT180, and AT270). These-phospho-epitopes correlate with tauopathy disease [51], and while increased Tau phosphorylation is typically thought to promote toxicity, specific phosphorylation sites have been shown to be protective [52,53]. Importantly, there are upwards of 85 potential phosphorylation sites on the human Tau protein [51], and while we chose to examine three key proline-directed sites implicated in pathology, there is still the possibility that reductions in \( \tau / h \) alters phosphorylation at any of the other dozens of phosphorylation sites on Tau. Given that \( \tau / h \) reduction is able to suppress Tau toxicity without altering its phosphorylation status at a subset of disease-associated epitopes. Whether or not reduction of \( \tau / h \) could regulate Tau phosphorylation in other brain regions remains to be determined.

Notably, octopaminergic signaling is mediated by G-protein coupled receptors that can modulate cAMP and/or calcium levels [31,54,65]. Both cAMP and calcium levels can influence cell viability [56–58], and our data suggest that manipulation of these pathways, independent of their ability to modulate tau phosphorylation, may be sufficient to overcome the pathological effect of Tau expression. Additional experiments aimed at understanding how reduction of \( \tau / h \) levels promotes cell viability in the context of phosphorylated Tau levels may offer therapeutic value.

Despite the differences in biogenic amine signaling between humans and the \textit{Drosophila} fly model, there are some overlapping and evolutionarily conserved mechanisms that are relevant to our findings. For one, octopamine is a trace amine found at low levels in human brains, and octopamine is similarly generated from tyramine in both humans and \textit{Drosophila}. However, humans utilize the enzyme, \textit{dih}, to perform this reaction, while \textit{Drosophila} utilize \textit{tih}. Overall, \textit{dih} exhibits 39 % identity with \textit{Drosophila} \textit{tih}, with certain regions showing up to 68 % identity [35]. The major role for \textit{dih} is to convert dopamine into noradrenaline, while in human octopamine production plays a comparatively minor role. This raises the question as to whether the \textit{tih}-mediated effects observed in our human Tau-expressing fly model translates to the actions of \textit{dih} in the mammalian brain. Recently, genetic reduction of \textit{dih} was shown to reduce Tau cleavage, aggregation, and toxicity in a murine tauopathy model [23]. In this study, the effect of \textit{dih} reduction on Tau was identified to be due to the reduction of noradrenaline and its metabolite, DOPEGAL, highlighting an interaction between noradrenergic signaling and Tau toxicity [23]. While the role of octopamine was not examined in this study, octopamine and other trace amines have been implicated in neurodegenerative disease [59,60], underscoring the need for further exploration into the interaction between octopamine metabolism and tauopathy-related pathophysiology.

5. Conclusions

Our data demonstrate that genetic reduction of \( \tau / h \) suppresses Tau toxicity in \textit{Drosophila}, independent of Tau phosphorylation. These findings identify a novel interaction that may offer therapeutic value in the context of tauopathy pathogenesis.

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CRediT authorship contribution statement

Varuna Nangia: Conceptualization, Data curation, Formal analysis, Methodology, Writing - original draft. Julia O’Connell: Data curation, Formal analysis. Kusha Chopra: Data curation, Methodology, Formal analysis. Yaling Qing: Methodology, Formal analysis. Camille Reppert: Data curation. Cynthia M. Chai: Conceptualization, Data curation. Kesshni Bhasin: Methodology, Formal analysis. Kenneth J. Colodner: Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Writing - review & editing.

Declaration of Competing Interest

The authors have no competing interests to declare.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.neulet.2021.135937.

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