Mol Neurobiol (2011) 44:122–133
DOI 10.1007/s12035-011-8193-1

The Power and Richness of Modelling Tauopathies in *Drosophila*

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Received: 9 May 2011 / Accepted: 6 June 2011 / Published online: 17 June 2011

Abstract Tauopathies are a group of neurodegenerative disorders characterised by altered levels of phosphorylation or mutations in the neuronal microtubule protein Tau. The heterogeneous pathology of tauopathies suggests differential susceptibility of different neuronal types to wild-type and mutant Tau. The genetic power and facility of the *Drosophila* model has been instrumental in exploring the molecular aetiologies of tauopathies, identifying additional proteins likely contributing to neuronal dysfunction and toxicity and novel Tau phosphorylations mediating them. Importantly, recent results indicate tissue- and temporal-specific effects on dysfunction and toxicity coupled with differential effects of distinct Tau isoforms within them. Therefore, they reveal an unexpected richness of the *Drosophila* model that, coupled with its molecular genetic power, will likely play a significant role in our understanding of multiple tauopathies potentially leading to their differential treatment.

Keywords Tau · Alzheimer's · Frontotemporal dementia · Tauopathies · *Drosophila*

Drosophila as a Model for Neurodegenerative Diseases

Drosophila melanogaster has been the premier metazoan model organism for functional genomics because of the facility of its genetic manipulations and large-scale genetic screening, panoply of molecular genetic tools, well-annotated sequenced genome and its simple and inexpensive maintenance. *Drosophila* has been essential in addressing fundamental questions that led to our current understanding of biology including our own. Importantly for this review, the molecular and genetic facility of the system has led to its fruitful utilisation to probe the mechanisms governing neuronal function and dysfunction, including aspects characteristic of cognitive disorders, dementias, and neurodegenerative diseases [1, 2].

Different approaches have been employed to study neurodegeneration in *Drosophila*, such as forward genetic screens, inhibition of endogenous gene expression by RNAi or transposon-mediated mutagenesis methods. This led to the identification of genes that, when mutated, precipitate brain degeneration, such as *swisscheese* and *drop-dead* among others [3, 4]. Importantly, *Drosophila* models of a host of human neurodegenerative diseases have been established over the last 15 years. Generally, they entail expression of wild-type or disease-linked mutant human transgenes to explore the cellular and molecular mechanisms that underlie toxic gain-of-function [5–7] or loss-of-function phenotypes [8–11]. Finally, a pharmacological approach can also be used, where appropriate, to model neurodegenerative diseases and to test candidate therapeutics [12, 13]. Models of polyglutamine (polyQ) expansion diseases, such as Huntington's disease [6], Parkinson's disease [5] and ataxias such as SCA1 [14], have been established and used to gain significant insights into their pathobiology. Furthermore, accumulation of the Aβ40 and Aβ42 peptides or the microtubule binding protein Tau in the fly central nervous system (CNS) have been used to model aspects of the neurodegenerative dementia Alzheimer's disease (AD) [15, 16] and to search for

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pharmaceuticals that may ameliorate their toxicity and associated degeneration [17].

In models of neurodegenerative dementias, most approaches thus far have concentrated on recapitulation of toxicity and neuronal loss, aiming to gain insight into the cellular and molecular mechanisms of their pathogenesis. However, in contrast to vertebrate models, little attention has been paid to the effects of these conditions on motor dysfunction and behavioural and physiological neuroplasticity despite the rich repertoire of higher functions afforded by the fly.

The adult fly brain is thought to contain more than 300,000 neurons, which, like in mammals, are organised into structures broadly sub-serving functions such as vision, olfaction, locomotion and behavioural plasticities. In fact, *Drosophila* exhibit working memory, are capable of learning and remembering experiences gained in reward or punishment-mediated associative and non-associative conditioning paradigms [18, 19] and even remember to adjust their disposition towards other flies based on previous aggressive encounters [20]. Furthermore, *Drosophila* has been instrumental in the genetic and molecular dissection of learning and memory mechanisms with numerous mutants in these processes [21] and lately in functional imaging of these processes in the living animal [22].

This review will focus on the use of *Drosophila* to model tauopathies, a diverse class of neurodegenerative diseases that include dementias and motor dysfunctions [23–26]. We will emphasise the similarities and differences and advantages and disadvantages of using different tissues of the fly to model different aspects of these diseases, including neurotoxicity and neuronal dysfunction manifested as deficits in behavioural modules and plasticity.

Tau Biology

Tubulin-associated unit (Tau) is a major neuronal microtubule-associated protein, which was discovered, purified and characterised as a microtubule assembly promoting factor [27, 28]. By alternative splicing of the primary transcript, the single human *Tau* gene on chromosome 17 gives rise to six isoforms normally expressed in the adult CNS. The differences among them result from the presence of three (3R Tau) or four (4R Tau) imperfect microtubule-binding repeats in their carboxy-termini, as well as from the presence or absence of one or two inserts in the amino-terminal region [29–31]. Expression of these isoforms is developmentally regulated, and only the shortest isoform is present in foetal human brains. In normal adult human tissue, the ratio of 4R/3R Tau is ~1 [32, 33]. 4R Tau has higher affinity for microtubules than the 3R isoform, probably owing to the increased number of microtubule binding domains [34, 35].

Tau is distributed primarily in axons of the CNS [36, 37] and is essential for microtubule assembly, promoting and maintaining their stability. Microtubule stability is required for axonal transport, and Tau appears to interfere reversibly with the binding and regulation of motor proteins engaged in it [38–40]. Moreover, Tau has been shown to interact with actin, implicating it in the regulation of the microfilament cytoskeleton [37, 41]. In addition to its interactions with elements of the cytoskeleton, Tau has been reported to interact with the plasma membrane, an activity suggested to be important for neurite development [42, 43] and their outgrowth from the cell body [44, 45]. Interactions with the plasma membrane may also underlie the reported involvement of Tau in the migration of new neurons [46].

The diversity of Tau isoforms is further increased by various post-translational modifications, including phosphorylation, glycosylation, ubiquitinylation, nitration, deamidation, oxidation and glycation [23]. Moreover, the longest Tau isoform contains 80 potentially phosphorylatable serines and threonines, in addition to five tyrosine residues [47], and the overall phosphorylation status of the protein is controlled by the combined balanced activities of kinases and phosphatases [48–51]. Certain phosphorylation sites appear occupied both in normal and AD brains, while others appear predominantly in the disease state [47]. Phosphorylation at certain residues appears to precede and be required for occupation of additional sites [52, 53], underlying both the importance and dynamic nature of these modifications. It is thought that increased Tau phosphorylation reduces the amount of microtubule-bound protein and lowers its ability to promote microtubule assembly. Although a variety of kinases, including glycogen synthase-3 (GSK-3β), cyclin-dependent kinase 5 (cdk5), protein kinase A and microtubule-affinity regulating kinase (MARK) have been shown to regulate Tau phosphorylation in biochemical studies, the specific kinases that are responsible for its phosphorylation in the human CNS remain to be identified [54–57].

Tauopathies

Almost 20 years ago, Tau was demonstrated to be the major component of neurofibrillary tangles (NFTs), a neuropathological hallmark of AD [58, 59]. It has since become clear that Tau is integral to the pathogenesis of several dementing and motor dysfunction neurodegenerative diseases, grouped under the term tauopathies. Tauopathies and their characteristic pathologies have been reviewed extensively, and it is not our intention to recapitulate that here. Briefly, in addition to AD, tauopathies encompass disorders, including

Pick's disease (PD), progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD) among others. These tauopathies are characterised by altered 3R/4R ratios, while frontotemporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17) is the only tauopathy caused by mutations in the *Tau* gene [26, 47, 60–62]. Even though each tauopathy is characterised by particular pathological profiles and clinical features, they all have in common the presence of intraneuronal fibrillar inclusions of insoluble highly phosphorylated Tau isoforms [23, 62–64]. Since hyper-phosphorylated Tau exhibits much lower affinity for microtubules, the free protein is thought to aggregate into these filamentous structures in a series of steps of increasing complexity [64–66]. This seems to occur primarily in the somatodendritic compartment of neurons, while Tau depletion in the axons is thought to cause destabilisation of the microtubule network and disruption of axonal transport [39, 67–69]. Interestingly, although most FTDP-17-linked mutations do not affect phosphorylation sites, they nevertheless increase Tau phosphorylation and impair its ability to bind microtubules [68, 70], underscoring the toxicity of the hyper-phosphorylated protein. It is still unclear whether dysfunction and neuronal loss are consequences of excessive free hyper-phosphorylated Tau toxicity or result from depletion of the protein from the microtubules with catastrophic consequences on their integrity and function [62]. In addition, it is unclear whether all neuronal types are equally susceptible to Tau toxicity and degeneration, or they respond differentially in a manner reflected by the apparent localised effects of various tauopathies and the alterations in behaviour and pathology in patient brains.

Drosophila Tauopathy Models

In humans, accumulation of hyper-phosphorylated normal Tau [wild-type (WT)] isoforms characterises sporadic tauopathies like AD, Pick's disease, PSP and CBD among others. In most of these conditions, it is primarily the ratio of 4R to 3R isoforms that is altered, and it is currently unknown whether this change in the 4R or 3R isoform content is pathogenic. Therefore, it is unclear whether expression of one isoform would precipitate equivalent results with expression of the other in animal models of tauopathies. However, it seems reasonable to anticipate potential isoform-specific differences to emerge in transgenic systems where each of them is studied in isolation from the others. Moreover, because FTDP-17 is the only tauopathy characterised by *Tau* mutations [63], WT and mutant Tau isoforms should not be used interchangeably in tauopathy models, as they are associated with different diseases. Based on their clinically diverse symptomatology, WT and mutant Tau accumulation could have distinct consequences on dysfunction or degeneration in particular neuronal types.

Therefore, we will organise the text below based on the transgenic Tau isoform utilised and the neuronal type where it is expressed to avoid generalisations across WT and mutant isoforms and fly neuronal types expressing them. In our view, this increases the utility of *Drosophila* in the study of tauopathies and other neurodegenerative dementias, as the advanced tools for spatiotemporal control of transgene expression permit relatively expedient analyses in diverse cell types under different conditions and levels of transgene expression.

WT Isoform Toxicity in the CNS

With the aim of generating a tauopathy model amenable to genetic analysis, Feany and co-workers utilised the binary UAS/GAL4 system for tissue-specific transgene expression [71, 72] of the WT human Tau 0N4R isoform throughout the *Drosophila* CNS [73]. The model does not fully emulate sporadic tauopathies because the *Tau* transgene was expressed in the CNS since its formation in the embryo [74]. Nevertheless, these flies displayed tauopathy-like phenotypes in that they died a lot earlier than controls, apparently due to late onset progressive vacuolisation and Tau accumulation in their brains [73]. Moreover, in accord with AD patients, the vacuolisation in the brains of flies expressing the 0N4R pan-neuronally was age dependent, targeted preferentially cholinergic neurons and was associated with increased immunostaining for a series of NFT-specific epitopes [73]. The vacuolisation probably reflects *bona fide* degeneration, as it seems to affect pre-existing neurons since few if any vacuoles were observed in young flies. Moreover, a number of TUNEL-positive neurons were detected in these brains, at least in part associated with activation of the JNK signalling pathway [75]. However, insoluble fibrils could not be recovered from the brains of these flies, indicating that the Tau-dependent neurotoxicity in this model is due to alterations that occur before the formation of large aggregates. It is possible then that *Drosophila* models of tauopathies might be particularly amenable to analysis of early, pre-tangle events of Tau-associated neurodegeneration (i.e. hyper-phosphorylation).

As for human tauopathies, in addition to the preferential degeneration of cholinergic neurons [73], another neuronal type was severely affected by pan-neuronal expression of human WT Tau. The mushroom bodies (MB) are bilaterally symmetrical assemblies of over 2,000 neurons per hemisphere essential for learning and memory in insects and thought functionally analogous to the vertebrate hippocampus [18, 76]. Pan-neuronal expression of 2N4R or 0N4R Tau throughout development, reduced to near complete loss the number of MB neurons in adult brains (Fig. 1a),

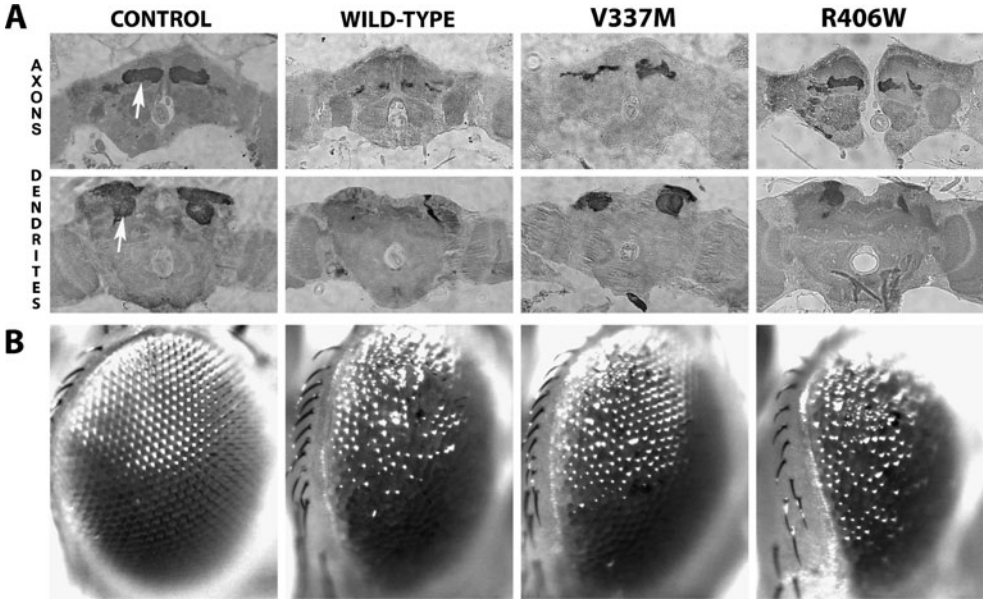

Fig. 1 **a** Carnoy's-fixed paraffin-embedded frontal sections stained with anti-Leonardo from animals accumulating the indicated WT and mutant Tau isoforms under the control of the pan-neuronal driver Elav-GAL4. *Top* Sections at the level of the mushroom body axons of the γ-subtype-lobe in the anterior of the head. *Bottom* Sections at the level of the mushroom body dendrites (calyces) in the posterior of the head. The FTDP-17-linked mutants yield milder deficits than WT Tau. *WT* represents the 0N4R isoform and control animals are heterozygotes for the drivers in the absence of transgenes. The *arrows* indicate the normal size and structure of the axons and dendrites in the control animals for comparison with the Tau-expressing ones. **b** Retinal toxicity induced by expression of *Tau* transgenes in the eye under the control of the GMR-GAL4 driver. Flies were raised at 25°C, and all *crosses* were performed in parallel. Accumulation of the R406W FTDP-17 mutant appears to be more damaging than either WT or V337M

whereas 0N3R did not [52], suggesting isoform-specific effects. Toxicity appeared confined to the MBs, as neighbouring (i.e. protocerebral bridge) or distant (i.e. ellipsoid body, optic lobes) neuropils remained unaffected. This was a consequence of specific toxicity of hyper-phosphorylated Tau on the neuroblasts that give rise to MB neurons (MBNB) and required phosphorylation at least on Ser^{238}, Thr^{245}, Ser^{262} and Ser^{356} [52, 77]. These results are in agreement with the heterogeneous clinical and cognitive profiles of human tauopathies, which likely reflect differential vulnerability of particular neuronal populations to the overall levels of specific Tau isoforms and their phosphorylation status therein [63]. However, Tau-dependent ablation of the MBs does not reflect a degenerative process because toxicity of the hyper-phosphorylated protein ostensibly blocked the MBNB to MB neuron transition in embryos, rather than induce loss of already extant ones. This type of toxicity may be akin to Tau-dependent loss of mammalian adult neural stem cells, a process that has been proposed to contribute to neuronal loss in tauopathies (reviewed in [78]).

Another CNS cell type affected in certain tauopathies is the glia [79, 80]. *Drosophila* glia perform similar functions with their vertebrate counterparts and are critical for maintaining neuronal viability [81]. Expression of the 0N4R isoform in adult glia decreased longevity and resulted in accumulation of hyper-phosphorylated Tau with significant contribution to the latter by the JAK/STAT signalling pathway [82]. Importantly, and in contrast with its accumulation in neurons, hyper-phosphorylated Tau in glia formed fibrillar aggregates readily, which are highly reminiscent of NFTs and similar to the glial tangles observed in tauopathies, such as CBD and PSP [83]. Because fibrillar Tau and NFTs have not been described in *Drosophila* CNS neurons thus far, this result suggests that in agreement with observations from vertebrate nervous systems, the degree of Tau aggregate formation and their characteristics vary depending on the particular cell type targeted in *Drosophila* (CNS versus glia). This in turn supports the notion that perhaps the proteome of particular cell types or cells under specific conditions (i.e. cellular stress) may be conducive to NFT formation. The phenotypic consequences of Tau expression in glia and neurons were similar and additive, supporting the notion that distinct molecular pathways are likely employed to yield phenotypically similar pathologies in different cells of the fly CNS.

WT Tau-Mediated Dysfunction in the CNS

A prominent clinical feature of many tauopathies is cognitive impairment, including deficits in learning and memory. The cognitive deficits are particularly interesting,

as they may occur before any evidence of neuronal degeneration or aggregate formation [84]. Supporting this idea learning deficits become ameliorated despite NFT presence in a mouse tauopathy model [85]. Because the MBs are essential for learning and memory in *Drosophila*, the effects of human Tau in these neurons were investigated independently of the toxicity phenotypes described above. The negatively reinforced associative conditioning paradigm coupling electric foot-shocks to aversive odours [21, 86] was used to assess learning and memory in flies expressing Tau in their MBs.

To bypass the preferential Tau toxicity on MB development [52], 0N4R human Tau, the 2N4R bovine Tau and the *Drosophila* endogenous 0N5R-like protein were expressed specifically in the MBs from late pupal stages onto adulthood. Enrichment of the MBs with any of these Tau proteins precipitated associative learning and memory deficits [87]. These deficits reflect Tau-dependent dysfunction and were not a consequence of degenerating MBs because the protein was not expressed during embryogenesis when the MBNBs are differentially sensitive and deficient learning characterised young flies. MB-confined neurodegeneration was detectable only in few animals over 45 days old [87], whereas aggregates were not detectable neither immunohistochemically nor biochemically [87, 88]. Therefore, it appears that the behavioural deficits are a consequence of toxic gain-of-function-like effects brought about by Tau overabundance within MB neurons. However, the 0N3R protein had little effect on associative learning, suggesting specificity of Tau isoform on CNS dysfunction potentially mediated by the increased microtubule binding capacity of the 4R isoforms.

Collectively, the results suggest that elevation of 4R Tau in the MBs precipitates temporally separable toxicity and dysfunction. In the MB neuroblasts, Tau elevation either blocks their proliferation or kills them. However, in terminally differentiated neurons as in adult MBs, Tau elevation results in dysfunction but does not appear to cause their degeneration. In agreement with this notion, strong conditional expression in the adult CNS of the highly toxic to MBNBs 2N4R or the slightly less toxic 0N4R isoform [52], using the TARGET system [89, 90], impaired associative learning but did not affect MB morphology [77]. Significantly, two novel putative phosphorylation sites on WT Tau, Ser^{238} and Thr^{245} seem essential for its toxic effects on MBNBs. Blocking their putative phosphorylation by replacing these residues with non-phosphorylatable alanines yielded animals with structurally normal MBs, even though 2N4R-S238A/T245A was expressed pan-neuronally throughout development. However, adult MBs are unable to support associative learning in the presence of 2N4R-S238A/T245A [52]. Ser^{262} and Ser^{356}, which are targeted by the kinase Par-1, are also important for MB function. Par-1 encodes the fly ortholog of mammalian MARK, previously shown to phosphorylate Tau and regulate microtubule dynamics [91]. Ser^{262} and Ser^{356} are hyper-phosphorylated in 2N4R-S238A/T245A, whereas Tau variants with both sites blocked by alanine substitutions do not mediate structural or functional MB deficits [52, 77].

Collectively, the evidence from the CNS is highly supportive of the hypothesis that Tau-mediated neuronal toxicity is dissociable from dysfunction [92]. Whether glial expression of WT Tau precipitates behavioural phenotypes is currently unknown, but in *Drosophila*, these cells appear to play a role in processes, leading to long-term memory [93].

WT Tau-Mediated Toxicity in the PNS

Drosophila offers a number of tools to assess the toxicity of Tau *per se* or proteins and signalling pathways engaged in the process as mentioned above, but the fly retina is by far the most commonly utilised tissue for this type of analyses. The adult fly retina is comprised of approximately 800 identical units, called ommatidia, arranged in a hexagonal array. Unlike vertebrates, cells that comprise the adult *Drosophila* retina are derived from the larval eye-antennal imaginal disk and not from the neuroblasts that give rise to the CNS [94, 95]. Hence, we will consider the photoreceptors and other cell types that comprise the retina as part of the PNS. Toxicity is manifested in the retina as a "rough" eye phenotype, characterised by reduced external eye size, loss of the regular ommatidial organisation and often neuropil degeneration in the medulla. Accumulation of 2N4R and 0N4R Tau in this tissue under a GAL4, a driver expressed in all retinal cell types precipitated eye malformation to varying degrees [88, 96] (Fig. 1b). Similar, albeit milder phenotype is revealed when the photoreceptor neurons are targeted specifically with the pan-neuronal driver [88]. Notably, cell fate in the retina and formation of ommatidia occur during a relatively brief period in the larva [95] and further elaborated during the pupal stage [94]. Therefore, emergence of adult animals with rough eyes likely represents Tau-dependent toxicity potentially akin to that described for MBNBs above, rather than degeneration *per se*. In contrast, ommatidia of normal appearance upon adult emergence, which die or become dysmorphic in an age-dependent manner, would be consistent with a degenerative phenotype.

The power of fly models lies in the ability to perform large-scale forward genetic screens to dissect the cellular signalling pathways involved in the disease process. One great advantage of the retina model is the ease of visualising components that promote Tau toxicity (enhancers of the rough eye phenotype) or suppress it (suppressors of the

rough eye phenotype) in such genetic screens. This is facilitated by the ease of detecting changes in ommatidial morphology, often by simple visual inspection. Utilisation of the retinal system resulted in expedient identification of the importance of GSK-3β-mediated phosphorylation for Tau toxicity and differentiated the action of the kinase on Tau with canonical wingless pathway signaling. In fact, when GSK-3β was co-expressed with WT Tau, large NFT-like aggregates were observed in photoreceptor neurons and enhanced toxicity [96]. This is the only case of NFT-like aggregate formation other than in glia as described above. In a similar independent study, over-expression of *Drosophila* Chk2 kinase enhanced the toxicity of WT Tau, but NFTs or other type of aggregates were not reported in this case [97].

The retina system has been extensively used to probe the role of Tau phosphorylation on particular sites on its toxicity. The steady-state pattern of Tau phosphorylation in the adult retina differs somewhat from that in the CNS [98]. However, work in the retina has provided insights into the mechanisms of Tau toxicity in the brain as well. WT Tau toxicity in the retina appears to be mediated by the orchestrated phosphorylation of several serines and threonines, rather than a single specific site. Expressing transgenes carrying single or double blocking mutations in 15 known phosphorylation sites linked with Tau pathologies did not visibly suppress the rough eye phenotype as long as the remaining 14 could be occupied [99]. In contrast, a construct that was pseudophosphorylated at these sites caused more severe toxicity than the WT protein [100]. This argues against a dominant role of particular sites and suggests cooperativity among at least these sites in mediating toxicity, dysfunction and degeneration. Intriguingly, further analysis on the role of various Tau-targeting kinases in the retina revealed that as in the CNS, blocking phosphorylation by Par-1 decreased Tau toxicity independently of subsequent phosphorylations by GSK-3β or Cdk5. However, blocking GSK-3β phosphorylation appeared to enhance Tau toxicity as well as its binding to microtubules [101]. This suggests that binding to microtubules may contribute to toxicity, independent of phosphorylation on major sites already putatively linked with pathology. Significantly, this is potentially applicable to the effects of Tau on neuronal dysfunction in the CNS as detailed above.

Finally, Tau-induced toxicity has also been modelled in the *Drosophila* notum [102]. Expression of WT Tau and various mutants in the notum resulted in a bristle loss phenotype of varying degrees. This system, which is also amenable to easily monitored toxicity enhancement and suppression, was also used for drug screening. Hyperphosphorylation seems to be important for Tau toxicity in this system as well, since co-expression of a dominant negative allele of GSK-3β or of the Bβ regulatory subunit of PP2A, but not the B′ regulatory subunit (Wdb) with WT Tau ameliorated the bristle-loss phenotype. In the retina, however, PP2A appears to require the B′ regulatory subunit as over-expression of a dominant negative Wdb construct resulted in enhanced toxicity of WT Tau in photoreceptor neurons [103]. This further supports the notion of a cell-type-specific regulation of Tau and its toxicity.

WT Tau-Mediated Dysfunction in the PNS

It has been proposed that hyper-phosphorylated Tau cannot bind microtubules, and this is thought to precipitate the disruption of microtubule-based functions, including axonal transport and synaptic transmission [62]. Interestingly, these defects may become apparent prior to any evidence of filament formation or neuronal death. The *Drosophila* larval motor neuron and neuromuscular junction (NMJ) system represent a well-characterised, attractive system to explore potential effects of Tau on these processes. Over-expression of 0N3R WT Tau within larval motor neurons disrupted axonal transport causing transport vesicles to aggregate and resulted in decline of larval locomotor function [104]. Consistent with these findings pan-neuronal expression of 0N4R Tau resulted in age-dependent deficits in adult locomotor function (Skoulakis, unpublished data) assessed as the ability of the flies to climb on the side of the vial that houses them as described by Hirth [105]. Interestingly, 0N3R becomes hyper-phosphorylated [104], but the phosphorylation pattern in the motor neurons differs somewhat from that in other tissues [98]. Intriguingly, phosphorylation by GSK-3β is important for Tau-dependent axonal transport defects as it is for retinal toxicity, suggesting at least some pathogenic mechanisms in common. Expression of 0N4R-WT Tau also induces changes in morphology of the NMJs and a dramatic defect in synaptic transmission [106]. It would be interesting to assess the effects of different 4R Tau isoforms in this system to investigate potential WT isoform specific effects in various neuronal populations.

The findings presented above are also consistent with earlier reports of axonal degeneration in sensory neurons [107] upon expression of a bovine WT Tau isoform introduced as an axonal marker in *Drosophila* [108]. Moreover, Williams et al. [107] demonstrated axonal degeneration upon expression of the 0N3R WT human Tau.

Mutant Tau Isoform Toxicity in the CNS

In addition to WT Tau, transgenes carrying FTDP-17-linked mutations have also been generated and used in most of the experiments described above. Because of the differences in the human symptoms, pathology and molecular aetiology of FDTP-17 and the other tauopathies, we consider the two sets of results separately.

Pan-neuronal expression of the common FTDP-17 associated R406W mutant 0N4R Tau resulted in significantly more reduced longevity than that observed upon expression of WT Tau, enhanced age-dependent vacuolisation and cholinergic neuron loss in the brain [73]. It appears that the R406W mutant protein renders CNS neurons particularly susceptible to oxidative stress [75]. This constitutes a potential explanation of the enhanced toxicity of this mutant protein and a difference with the WT isoform. Furthermore, oxidative stress did not appear to act on Tau toxicity by changing the phosphorylation status of R406W, at least in commonly surveyed sites, but rather to promote cell cycle activation in the CNS neurons expressing this protein [75]. This suggested yet another Tau-dependent toxicity mechanism as terminally differentiated neurons attempting entry in the cell cycle become apoptotic [109]. However, additional common FTDP-17-linked mutations, such as V377M or P301L, were not tested in these experiments, so it is currently unknown whether these observations are specific to the R406W mutation or characterise all such Tau mutants.

In contrast to these results, the 0N4R-R406W and V377M mutations precipitated a milder effect than that of WT isoforms on the MBNBs [52]. Resultant adult MBs were not as severely perturbed as upon WT Tau expression, and in the case of V377M, the structural changes were minimal (Fig. 1a). Therefore, the effects of WT and FTDP-17-linked mutant Tau appear isoform and cell-type-specific in broad agreement with clinical data [26]. The functional effects of 0N4R, R406W and V377M mutations on associative learning and memory have not yet been studied in isolation from the structural defects they cause on the MBs, albeit minimal. However, even with the associated structural defects, the effects of these mutations on associative learning were less severe than those of the WT protein [88], further underscoring the functional differences between the WT and mutated proteins.

Mutant Tau Isoform Toxicity in the PNS

Expression of FTDP-17-linked mutations yielded significant toxicity in the retina, which is generally more severe and with somewhat different morphology than that obtained with WT isoforms (Fig. 1b) [53, 88, 110]. A forward genetic screen with the 0N4R-V337M mutant isoform identified 24 modifiers of its toxicity, seven of which were kinases and phosphatases including Par-1 [110]. Surprisingly, Par-1 over-expression suppressed V377M toxicity, while in an independent study, it was shown to enhance R406W-mediated retinal toxicity as expected [53]. Moreover, blocking Par-1 phosphorylation sites by mutating Ser^{262} and Ser^{356} to Ala completely abolished R406W toxicity [53]. This discrepancy likely reflects differences even among FDTP-17-linked mutations possibly in the temporal order of phosphorylations or of mutation-specific subtle biochemical or structural consequences. In agreement with this notion, while targeting Ser^{262} and Ser^{356} by Par-1 is essential for subsequent phosphorylations by GSK-3β and Cdk-5 in the context of the R406W mutation [53], prior occupation of these sites is not a prerequisite for the phosphorylation by GSK-3β of the WT isoform as discussed above [101]. Because phosphorylations seem to act cooperatively for the WT isoforms [99], such differences may be inconsequential in the context of disease manifestation.

Two forward genetic screens using the retina as read-out have identified components of the actin cytoskeletal network as enhancers of V337M neurotoxicity [110, 111]. Accordingly, genetic reduction of actin levels suppressed V377M-induced toxicity, while increasing actin levels enhanced the phenotype [112]. The same authors showed accumulation of filamentous actin and the formation of actin-rich rods, analogous to Hirano bodies, in the *Drosophila* brain and retina upon over-expression of the R406W-mutant Tau [112]. Interestingly, however, detectable changes in actin were not observed in flies expressing WT Tau, potentially another manifestation of differences in the consequences of the mutant and WT protein accumulation.

Genetic interaction studies have also linked Tau toxicity to both cell-cycle regulation and oxidative stress. Khurana et al. demonstrated that abnormal expression of cell-cycle markers is recapitulated in the brain of flies that express pan-neuronally WT and R406W mutant Tau [100]. Cell-cycle activators enhanced, and cell-cycle inhibitors suppressed, the WT as well as the V337M-induced toxicity in the retina. Furthermore, the authors showed that cell-cycle activation appears to follow Tau phosphorylation and that activation of the TOR pathway by Tau resulted in toxicity closely emulating neurodegeneration in a cell-cycle-dependent manner. Collectively, these studies strongly suggest a role for Tau-dependent ectopic activation of the cell cycle and consequent apoptotic death in the retina and the CNS. This may underlie, at least in part, the described toxicity and brain vacuolisation upon Tau accumulation.

Fly Tau

The single *Drosophila Tau* gene is expressed both in the developing and adult nervous systems, where it is most prominent in photoreceptors [113]. Possibly because of redundancy with other microtubule-associated proteins, dTau loss is not fully lethal [114]. Interestingly, the degenerative phenotype of hypomorphic alleles of the MAP1B ortholog Futsch was suppressed by dTau over-expression in the nervous system [115]. The endogenous

dTau is expressed at low levels within the MBs [113]; however, its over-expression specifically in these neurons induced a decrease in olfactory learning as described in a previous session [87]. Elevation of dTau in the retina resulted in toxicity resembling the phenotype caused by expressing WT human Tau, suggesting that the toxic properties of the fly and human protein are conserved [116]. In the same study, comparison of larval proteomes and genetic screening revealed that approximately half of the interactions of the human and dTau proteins with other molecules in the fly eye were identical. Likewise, expression of dTau or human Tau in larval motor neurons disrupted axonal transport and neuromuscular junction morphology, giving rise to significant impairments in locomotor function [117]. In addition, both transgenic constructs caused significant synaptic bouton reduction at the larval NMJ, while the human WT protein was also shown to reduce the number of mitochondria within the remaining boutons and perturb synaptic transmission [118]. Finally, in the context of AD, dTau appears to be an important downstream mediator of Aβ42 toxicity, since its loss partially reduces Aβ42 pathology in *Drosophila* [119]. Similarly, bristle loss in the fly notum induced by human WT Tau expression was less pronounced when expression of endogenous dTau was reduced [102]. However, complete removal of dTau did not modify the toxicity associated with expression of human Tau proteins in the eye [120].

It appears then that transgenic expression of human Tau proteins in the *Drosophila* CNS and PNS and the resultant toxicity and dysfunction phenotypes occur largely as a consequence of excess WT or mutant Tau in addition to the resident dTau in tissues mentioned above. This may be reflected in the sensitivity and often unique results obtained in the retina where dTau is particularly abundant in the photoreceptors. As such, systematic study of the interaction of the human and *Drosophila* Tau proteins and its effects on the structure and function of the cytoskeleton are likely to be particularly informative as already suggested by a recent study [121, 122].

Conclusions

The results from the *Drosophila* tauopathy models described above demonstrate that WT and mutant Tau proteins are processed differentially and often have distinct consequences even within the same cell types (Table 1). Furthermore, these results provide particularly salient examples of the differences that must occur among these neurodegenerative disorders even if the underlying pathogenic mechanisms are broadly similar. It appears, therefore, that the isoform and mutant status of Tau are likely critical in determining the clinical and pathological differences of the various tauopathies in humans as well. Therefore, WT and mutant Tau proteins cannot be used interchangeably as models of a single disorder.

Moreover, the neuronal type where WT and mutant Tau are expressed is important as revealed by tissue-specific differences in phosphorylation patterns [98] or reports that modifiers of toxicity in the *Drosophila* retina did not mediate changes in CNS pathology [123]. Such cell-specific effects on Tau isoforms could provide an explanation for the differential sensitivity of various neuronal populations in tauopathies.

Fly and mouse models of tauopathies could be utilised in a complementary manner to validate conclusions or to identify and verify modifiers of Tau toxicity. For example, in a mouse model of FTDP-17, Karsten and colleagues

Table 1 Differential effects of WT and mutant Tau after selective expression in CNS and PNS neurons

Isoform	Retina (rough eye)	MB structure	MB function (learning)	NMJ (axonal transport)	Notum (bristle loss)	Glia	References
dTau	Moderate	Normal	Impaired	Disrupted	nd	nd	[84, 111, 112]
2N4R	Strong	Ablated	Impaired	nd	nd	nd	[49, 75, 97]
0N4R	Strong	Ablated	Impaired	Disruptred	Moderate	Fibrillar inclusions	[49, 80, 85, 98]
0N3R	Mild	Normal	Normal	Disruptred	nd	nd	[75, 85, 100]
0N4R-R406W	Very strong	Mild defects	Mild impairment	Disruptred	Strong	nd	[49, 85, 98, 120]
0N4R-V337M	Strong	Mild defects	Mild impairment	Normal	Moderate	nd	[49, 85, 98, 106]
0N4R-R406W S2A	Normal	Normal	Normal	nd	nd	nd	[49, 50, 75]
2N4R-S2A	Normal	Normal	Normal	nd	nd	nd	[75, 97]
2N4R-STA	Moderate	Normal	Impaired	nd	nd	nd	[49, 75]

The first column refers to the transgenic proteins over-expressed in the neuronal subtypes listed in the following columns. The "References" column lists the main references for each transgene and its effects

nd non-defined

found that puromicine sensitive aminopeptidase (PSA) was upregulated in the cerebellum of mice expressing the FTDP-17-linked P301L mutant Tau transgene compared to non-transgenic controls [124]. They hypothesised that since its expression was changed in a region relatively resistant to neurodegeneration, PSA could have a protective role against Tau P301L toxicity. For expedient validation of this hypothesis, they utilised a *Drosophila* tauopathy model and showed that over-expression of PSA suppressed, whereas PSA loss-of-function exacerbated the WT-Tau-induced toxicity in the retina. Interestingly, whereas mild suppression of the WT-Tau phenotype was observed with co-expression of PSA, the phenotype of P301L was suppressed more dramatically.

Finally, the powerful genetics of the fly have and will continue to provide a wealth of suppressors and enhancers of the tauopathy modelled providing significant insights into disease susceptibility genes in humans and ultimately offer new therapeutic targets and platforms for rapid drug screening as summarised in a recent review [17]. The short generation time of *Drosophila* coupled with the development of automated or semi-automated behavioural assays will probably yield high-through-put screening for pharmaceuticals aimed at ameliorating the effects of WT or mutant Tau excess. Since flies like patients are freely moving behaving animals with a blood/brain barrier that compounds have to cross, it seems reasonable to suggest that such a system will be much more powerful than similar screening systems utilising cultured cells.

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