Tau-mediated axonal degeneration is prevented by activation of the Wld^S pathway

[1]Katy Stubbs, [1]Megan Sealey, [1]Miguel Ramirez Moreno, [1]V Hugh Perry, [2*]Tracey A Newman and [1*]Amritpal Mudher

1. Biological Sciences, Faculty of Environment and Life Sciences, University of Southampton, Highfield Campus, Southampton, SO17 1BJ
2. Clinical and Experimental Science, Faculty of Medicine, University of Southampton, Highfield Campus, Southampton, SO17 1BJ

Joint corresponding authors: Dr Tracey Newman and Dr Amritpal Mudher.

Keywords: tauopathy, axonal degeneration, Wallerian degeneration, Wld^S

Abstract

Tauopathy is characterised by neuronal dysfunction and degeneration occurring as a result of changes to the microtubule associated protein tau. The neuronal changes evident in Tauopathy bear striking morphological resemblance to those reported in models of Wallerian degeneration. The mechanisms underpinning Wallerian degeneration are not fully understood although it can be delayed by the expression of the slow Wallerian degeneration (Wld^S) protein, which has also been demonstrated to delay axonal degeneration in some models of neurodegenerative disease. Given the morphological similarities between tauopathy and Wallerian degeneration, this study investigated whether tau-mediated phenotypes can be modulated by expression of Wld^S. In a *Drosophila* model of tauopathy in which expression of human Tau protein ($hTau^{0N3R}$) leads to progressive age-dependent phenotypes, activation of the pathway downstream of Wld^S completely suppressed tau-mediated degeneration. This protective effect was evident even if the pathway downstream of Wld^S was activated several weeks after hTau-mediated degeneration had become established. In contrast, Wld^S expression without activation of the downstream protective pathway did not rescue tau-mediated degeneration in adults or improve tau-mediated neuronal dysfunction including deficits in axonal transport, synaptic alterations and locomotor behaviour in $hTau^{0N3R}$ –expressing larvae. This collectively implies that the pathway mediating the protective effect of Wld^S intersects with the mechanism(s) of degeneration initiated by hTau and can effectively halt tau-mediated degeneration at both early and late stages. Understanding the mechanisms underpinning this protection could identify much-needed disease-modifying targets for tauopathies.

Introduction

Tau pathology is observed in numerous neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease (PD), motor neuron disease (MND) and a variety of other tauopathies such as fronto-temporal dementia, Pick's Disease, progressive supra-nuclear palsy and others. The axon is susceptible to tau pathology in these neurodegenerative diseases, with evidence of white matter changes indicative of axonal degeneration in tauopathies such as AD (1-3). Studies in animal models have demonstrated that axonal dysfunction in tauopathy is typified by disrupted axonal transport (4, 5) (6), due to tau hyperphosphorylation resulting in reduced cytoskeletal integrity (7). Axonal swellings and loss of white matter, hallmarks of axonal degeneration have been observed in P301L-tau mice, a model of familial fronto-temporal dementia (8) (9) (10).

Wallerian degeneration describes the sequential degeneration of axons following axonal injury which begins with breakdown of the cytoskeleton and ends with the fragmentation and loss of the separated distal axon (11). Wallerian degeneration and axonal degeneration in neurodegenerative disease share similarities including cytoskeletal breakdown (7) (12), disrupted axonal transport (13) (14), alterations to mitochondrial morphology (15) (16), and in the central nervous system (CNS), axonal swellings (12) (17). These similarities suggest that the mechanisms overlap and the term Wallerian-like may be used to describe degeneration that is not due to an acute injury.

The discovery of the slow Wallerian degeneration (Wld^S) protective mutation, which robustly delays Wallerian degeneration (18), identified a molecular pathway controlling axonal degeneration after injury [reviewed in (19) (20) (21) (22)]. Wld^S has been studied in experimental models to elucidate the molecular pathway and explore whether it underpins the

Wallerian-like degeneration observed in a variety of neurodegenerative conditions. This work has identified delayed degeneration in models of disease including: Multiple sclerosis (23), Parkinson's disease (24) (25), Charcot-Marie-Tooth disease type 1A (26) and 1B (27) and toxic neuropathy (28).

Considering that the axon is a site of tau-mediated dysfunction and degeneration, the aim of the present study was to investigate whether the axonal protection mediated by Wld^S was able to rescue tau-mediated axonal dysfunction and degeneration. *Drosophila melanogaster* has been used in the study of Wallerian degeneration and Wld^S (29) (30) (31) (32) (33) (34), and *Drosophila* models of tauopathy are similarly well-established (5) (35) (36) . Furthermore, several studies are beginning to implicate components of Wld^S (such as nicotinamide mononucleotide adenylyl transferase – NMNAT) in tau-mediated aggregation and degeneration in both rodent and *Drosophila* models (37) (38) (39). To investigate whether Wallerian-like degeneration in tauopathy is Wld^S-sensitive we studied the structural and functional effects of co-expression of human tau ($hTau^{0N3R}$) and Wld^S in *Drosophila*.

Our findings demonstrate that co-expression of Wld^S does not confer protection against hTau mediated dysfunction or degeneration. However, in stark contrast, activation of the pathway downstream of Wld^S results in profound protection, both preventing and arresting degeneration even in neurons already affected by tau-induced pathology.

Materials and methods

Fly stocks

Drosophila were raised and maintained on standard Bloomington media at 23°C with a 12/12 h light/dark cycle. UAS-$htau^{0N3R}$, elav-GAL4, D42-GAL4 and Oregon R flies were obtained from the Bloomington Drosophila Stock Centre (Indiana, IN, USA). The UAS-Wld^S and UAS-

mCD8::GFP, Or47b-GAL4/Cyo lines were obtained from Professor Liqun Luo (Stanford University, CA, USA (40)). The D42-GAL4, UAS-NPY::GFP line was generated previously (5), with UAS-NPY::GFP provided by Dr Ian Robinson (Plymouth University, UK). A homozygous htau^{0N3R};Wld^{S} line was generated for the current study by crossing UAS-htau^{0N3R} with UAS-Wld^{S} lines.

Axonal transport analysis

Transgenes were expressed using D42-GAL4, UAS-NPY::GFP, and third instar wandering larvae were selected for analysis. Larvae were anaesthetised using diethyl ether vapour (Thermo Fisher Scientific) and mounted in 1% agarose (Sigma-Aldrich) on glass slides, with the ventral surface facing the coverslip. Peripheral nerves were imaged using an Axioplan2 MOT upright fluorescence microscope (Zeiss) equipped with Micro Max CCD (Princeton Instruments) using MetaMorph acquisition software (Molecular Devices). Images were thresholded and the area covered by aggregates measured using Metamorph software.

Larval neuromuscular junction (NMJ) analysis

Transgenes were expressed using D42-GAL4, and third instar wandering larvae dissected, with internal organs removed and the skin pinned out and fixed in 4% formaldehyde (Sigma-Aldrich) for 90 mins at room temperature. Larval skins were then washed in 0.1% Triton X (Sigma-Aldrich) in phosphate-buffered saline (PBS-Tx; Thermo Fisher Scientific) prior to blocking in 5% goat serum, 3% horse serum and 2% bovine serum albumin (BSA; Sigma-Aldrich) in 0.1% PBS-Tx. Skins were incubated with goat anti-horseradish peroxidase (1:1000; ICN/Cappel), conjugated to fluorescein isothiocyanate. Skins were washed in 0.1% PBS-Tx and put through an ascending glycerol series (50, 70, 90 and 100%) before being mounted in Vectashield (Vector Laboratories) and imaged. NMJ's on muscle 4 from segments A3-5 were

imaged using a Leica SP2 scanning confocal microscope using the 488 argon laser. Maximum projections of Z stacks were generated for morphometric analysis; bouton size and interbouton axon width were measured using ImageJ with the assessor blinded to the sample number.

Larval locomotion

Larval behaviour was assessed as previously described (41). In brief, D42-GAL4 driven third instar wandering larvae were each placed in the centre of 0.3% Alsian Blue (Sigma-Aldrich), 1% agarose (Sigma-Aldrich) plates, and videos of larval behaviour recorded. Videos of larval locomotion were analysed using Ethovision 3.0 (Noldus) tracking software.

Immunohistochemical analysis of axonal degeneration

Adults were collected 0-2 days after eclosion from UAS-mCD8::GFP, Or47b-GAL4 driven crosses and aged to the relevant time point. Flies were anaesthetised with CO_2, heads were ligated and the brains dissected and placed in 4% formaldehyde and fixed at room temperature for 45 minutes. Following fixation, brains were washed in 0.1% PBS-Tx before either mounting in Vectashield or proceeding for staining. Those to be stained were blocked in 5% goat serum, 3% horse serum, 2% BSA in 0.1% PBS-Tx and stained with rabbit anti-human tau antibodies (1:1000; Dako) or mouse anti-phospho tau PHF-1 (1:1000 – thermofisher), washed and incubated in goat anti-rabbit or anti-mouse Alexa Fluor 563 (1:1000; Invitrogen; Thermo Fisher Scientific). Brains were washed and mounted in Vectashield prior to imaging on an Axioplan2 MOT upright Epifluorescence microscope (Zeiss) equipped with a QImaging Retiga 3000 CCD Camera (Photometrics) and images were acquired using Metamorph software (Molecular Devices). Images were quantified in ImageJ with the assessor blinded to genotype

and timepoint. For axonal swellings, images were thresholded and the coverage of swellings measured.

Axon injury to activate the pathway downstream of Wld^S (referred to as Wld^S pathway-activation)

The third antennal segment was removed from flies, 1 or 3 weeks after eclosion from Or47b-GAL4 driven crosses, under CO_2 anaesthesia using Dumont #5 forceps. This induced an axonal injury in olfactory receptor neurons (ORNs), whose cell bodies are located in the third antennal segment. At the relevant time points, brains were dissected as described above. Degeneration was quantified by previously described methods (42). Briefly, with the assessor blind to genotype and time point, the presence of the commissural axons was recorded (Y/N) and the percentage of brains of each genotype at each time point with intact axons was calculated. The intensity of GFP signal within glomeruli was measured using ImageJ and the background intensity was subtracted.

Statistics

Statistical analysis was conducted using GraphPad Prism, version 6.0 (GraphPad Software, Inc.), using analysis of variance and the Bonferroni correction for the comparison of groups. The Mantel-Cox test was used for survival analysis, with the Bonferroni correction used for the comparison of multiple groups. Values are presented as the mean ± standard error. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Activation of the pathway downstream of Wld^S protects against $hTau^{0N3R}$-induced degeneration

Though previous studies of injury models indicate that the presence of Wld^S within the axon is crucial for its protection (33-35), the findings from chronic models of disease do not show any

consistent or significant Wld^S-mediated protection despite clear evidence of Wallerian-like degeneration in these models (31) (32) (34). One explanation for this lack of rescue could be that the pathway that Wld^S is acting in is not "activated" in these models of chronic degeneration, raising the possibility that the protein may require some form of injury to unmask its protective effect. Indeed, in all cases where Wld^S has been reported to rescue axonal degeneration, the neurons are injured by default as part of the experimental paradigm (22) (26) (35) (36).

To explore whether injury-induced "activation" was required for the Wld^S pathway to protect against tau-mediated degeneration, $hTau^{0N3R}$;Wld^S axons of the olfactory receptor neurons (ORNs) expressing membrane bound GFP were injured (axotomised) by removal of the third antennal segments as previously described (42). Adult brains were analysed after ecclosion at hourly (h) or weekly (w) time points post-axotomy induced Wld^S pathway activation (referred to as "pa" from here on). This revealed that control and tau expressing axons had degenerated at 2w after eclosion/1wpa. In contrast $hTau^{0N3R}$;Wld^S expressing axons were intact at this time point (data not shown) confirming that the axotomy paradigm "activated" the pathway downstream of Wld^S. To ascertain the extent to which activation of the Wld^S pathway protected against tau-mediated degeneration, the prominent degenerative features of tau-expressing axons were quantified. Axonal swellings, which are characteristic of tau-mediated axonal degeneration, were present in naïve $hTau^{0N3R}$;Wld^S axons (arrowheads Fig 1ai). In contrast these were not found in hTau-animals of the same genotype after activation of the Wld^S pathway (Fig. 1aii). The progressive accumulation of axonal swellings is evident in hTau expressing animals within 2 weeks after eclosion and trebles by week 5. In contrast swellings were not seen at any time point in $hTau^{0N3R}$;Wld^S expressing animals where the activation of the Wld^S pathway was elicited through axotomy (Fig. 1b). This illustrates that once activated,

194 the Wld^S pathway protects against the initiation and development of tau-mediated axonal
195 degeneration.
196
197 To study a more disease-relevant situation we investigated whether activation of the Wld^S
198 pathway protected against already established tau-mediated axonal degeneration. The Wld^S
199 pathway was activated at 3w after eclosion, a time at which axonal swellings are already
200 established in $hTau^{0N3R}$-expressing axons. In $hTau^{0N3R}$;Wld^S axons where Wld^S pathway was
201 not activated, a progressive increase in axonal swellings is evident with time, such that axonal
202 swellings at 4w after eclosion are 4-fold greater than those seen at 3w after eclosion, with this
203 increasing further by 6w after eclosion ($P<0.001$; Fig 2a). At these later time points the
204 swellings in the naïve $hTau^{0N3R}$;Wld^S axons are significantly greater than those seen in the
205 naïve Wld^S axons which serve as the controls. In contrast activation of the Wld^S pathway halts
206 the development of axonal swelling. Olfactory receptor neurons in flies expressing hTau;Wld^S
207 showed the anticipated accumulation of axonal swellings at 3w after eclosion, prior to Wld^S
208 pathway activation, but any further accumulation was halted once the Wld^S pathway was
209 activated (Fig. 2b) with no progression in pathology seen after this time. Once the pathway
210 downstream of Wld^S was activated, the axonal swellings in $hTau^{0N3R}$;Wld^S animals were not
211 significantly different to those seen in controls at any time point. This indicates that in addition
212 to preventing the emergence of tau-mediated axonal degeneration (as shown in Fig 1),
213 activation of the Wld^S pathway can also halt the progression of tau-mediated axonal
214 degeneration once it has begun.
215

Expression of Wld^S without "activation" of the downstream pathway is insufficient to protect against $hTau^{0N3R}$-induced phenotypes

The results indicate that activation of the pathway downstream of Wld^S potently suppresses hTau-mediated degeneration. This supports our hypothesis that the lack of protection through co-expression of Wld^S in chronic models of degeneration (31) (32) (33) (34) is because the pathway that Wld^S acts in is not normally "activated" in otherwise naïve axons. Acute injury to an axon "activates" it unmasking its protective effect. However, as the previous studies were conducted in rodents, we sought to ascertain whether the "protection requires pathway activation" phenomenon that we described in Figs 1 and 2 holds true in our invertebrate model as well.

To prove that expression of Wld^S is insufficient for protection against $hTau^{0N3R}$-mediated degeneration and that "activation" of the pathway downstream is required, degeneration in naïve $hTau^{0N3R}$;Wld^S flies was studied in the absence of "activation". ORNs expressing membrane-bound GFP underwent progressive age-related axonal degeneration in all $hTau^{0N3R}$ expressing flies. This was characterised by the appearance of axonal swellings at 2-3 weeks after eclosion, which increased in number and size as the flies aged (Fig. 3a/b $hTau^{0N3R}$ column). Axonal swellings were also evident in controls and Wld^S flies, but only at older, 5-7 week time points (Fig. 3a control and Wld^s columns). Noticeably, these swellings were also apparent in $hTau^{0N3R}$;Wld^S flies (where the Wld^s pathway had not been activated - Fig 3a $hTau^{0N3R}$;Wld^s column). Quantification confirmed that there was no significant difference in onset, extent or progression of axonal swellings in the $hTau^{0N3R}$;Wld^S flies when compared with $hTau^{0N3R}$ alone (Fig. 3a/b).

These results suggest that simply co-expressing Wld^S does not protect against tau-mediated degeneration. We next sought to investigate whether this is also the case in larvae, where tau-

mediated neuronal dysfunction manifests in profound Wallerian-like axonal phenotypes incuding disrupted axonal transport and destabilisation of the cytoskeleton (5) (7). Using a *Drosophila* line expressing GFP-tagged neuropeptide Y in motor neurons, axonal transport was visualised using microscopy in live intact third instar larvae. As reported previously, numerous large vesicular aggregates were found in tau-expressing larvae indicative of axonal transport disruption (Fig. 4a). However, these aggregates were also observed in $hTau^{0N3R}$;Wld^{S} larvae. Quantification of the coverage areas of the aggregates indicated that the aggregates were not significantly reduced in $hTau^{0N3R}$;Wld^{S} larvae compared with $hTau^{0N3R}$ larvae (Fig. 4b).

$HTau^{0N3R}$ expression is associated with altered synaptic morphology, characterised by thinning of the inter-bouton axons and the appearance of minisatellite boutons (43). These features were observed in the current study (Fig. 4c) and this phenotype was not improved by co-expression of Wld^{S}. No significant difference in the thickness of the inter-bouton axon (Fig. 4d) or the proportions of bouton of each size (Fig. 4e) were seen in the neuromuscular junctions of either $hTau^{0N3R}$;Wld^{S} or $hTau^{0N3R}$ expressing animals .

Disruption of axonal transport and altered synaptic morphology are associated with alterations in locomotor behaviour. Using an open field behavioural assay, the crawling behaviour of third instar larvae was investigated. When placed in the centre of an arena, control larvae quickly move towards the edge of the arena, following a straight path. In contrast, tau expressing larvae move more slowly and take a confused and twisting path, demonstrated by an increase in meander (turning per distance moved; Fig. 4f) and in angular velocity (turning per time elapsed; Fig. 4g) and the reduction in overall velocity (Fig. 4h). However, the co-expression of Wld^{S} with $hTau^{0N3R}$ did not improve locomotor behaviour, with no significant difference between $hTau^{0N3R}$;Wld^{S} and $hTau^{0N3R}$ expressing larvae (Fig. 4f-h).

The adult and larval data collectively shows that without "activation" of the pathway downstream of Wld^S, the protective effect of Wld^S on $hTau^{0N3R}$-mediated dysfunction (in larvae) or degeneration (in adult flies) is not uncovered.

Activation of the Wld^S-pathway protects against the effects of $hTau^{0N3R}$ without influencing total or phosphorylated tau levels

The most parsimonious explanation for this curious phenomenon of injured-activated protection against $hTau^{0N3R}$ pathology, may simply be that hTau is lost from injured $hTau^{0N3R}$;Wld^S axons and therefore cannot exert it's detrimental effects to cause axonal degeneration. To investigate this, hTau immunoreactivity was assessed in hTau-expressing animals with and without Wld^S-pathway activation and both the amount of hTau and its cellular localisation was examined. No significant differences were found in hTau distribution or total hTau expression between these two groups; hTau staining persisted in injured $hTau^{0N3R}$;Wld^S axons even 5 weeks after Wld^S activation (Fig. 5a) and there was no difference in total Tau levels (Fig 5b). This is remarkable because it implies that despite expression within the axon for 6 weeks, hTau has not caused degeneration in the $hTau^{0N3R}$;Wld^S axons once the Wld^S pathway is activated. This begs the question as to how activation, of the Wld^S-pathway protects against the human tau induced degeneration across this length of time.

Since hyper-phosphorylation has been shown to mediate tau toxicity in many *Drosophila* models (5) (7) (44), it is conceivable that the activated Wld^S-pathway is altering the degenerative changes by reducing the levels of phosphorylated hTau. To investigate this, hTau phosphorylated at the PHF-1 site was quantified in $hTau^{0N3R}$;Wld^S axons with and without Wld^S-pathway activation. There was a trend for a reduction in the PHF-1 signal in the $hTau^{0N3R}$;Wld^S flies where Wld^S was activated but this was not significant (Fig 5c).

Discussion

The axonal compartment of neurons is susceptible to tau-mediated dysfunction and degeneration making it a potential therapeutic target in the treatment of neurodegenerative disease. This study demonstrates that when the pathway downstream of Wld^S is "activated" in $hTau^{0N3R}$;Wld^S axons, tau-mediated axonal swellings *were prevented from forming.* Significantly, in animals allowed to develop axonal swellings due to $hTau^{0N3R}$ expression any further progression of pathology was halted after the Wld^S-pathway was activated. This protective effect was seen without alterations in total tau and importantly was dependent on the activation of pathways downstream of Wld^S. Understanding the mechanisms by which activation of the Wld^S-protective pathway negates tau-mediated axonal degeneration could yield important insight into how axons degenerate in tauopathy and other similar chronic degenerative conditions, and provide novel disease-modifying targets that emulate this protective effect.

Variable impact of Wld^S overexpression in previous models of neurodegeneration: can this be explained by the need to "activate" pathway downstream of Wld^S to uncover neuroprotection?

Previous studies of Wld^S in models of chronic neurodegeneration have indicated that expression of Wld^S has variable effects on disease phenotypes. This was also evident in our study where no rescue of $hTau^{0N3R}$-mediated neuronal dysfunction or degeneration was seen in either larvae or adults stages following mere co-expression of Wld^S. Similar results were reported in the *SOD1-G93A* model of MND (45). Like this, there is a large body of conflicting evidence of Wld^S sensitivity in chronic neurodegenerative diseases displaying Wallerian-like degeneration. Wld^S did not alter axonal degeneration in models of prion disease (46), MND (45) (47) and hereditary spastic paraplegia (48). In contrast, in models that investigate

315 degeneration with an acute onset, such as toxic neuropathy (28), ischaemic injury (49) and
316 MPTP induced-Parkinsonism (24) (50) a protective effects of Wld^S is reported.
317
318 One interpretation of the dissimilar effect of Wld^S in models of acute and chronic
319 neurodegeneration and their variable sensitivity to Wld^S could be a different mechanism of
320 axonal degeneration occurring in acute compared with chronic neurodegenerative conditions.
321 Another explanation for the variable sensitivity to Wld^S in models of chronic
322 neurodegeneration could simply be that its protective effect is a general delaying of
323 degeneration which is not always apparent in the time period assayed in the chronic models in
324 question. Our data imply that this is unlikely to be the case since no protective effect emerged
325 at even very late time points when Wld^S was simply expressed with $hTau^{0N3R}$ (Fig 1). Instead,
326 we propose and that another explanation may be provided by a key difference between the
327 experimental paradigms employed to study acute degeneration, which is missing in the models
328 of chronic neurodegeneration. This is that in all acute models, injury has to be simulated to
329 create the acute condition and this may set in motion a series of events that "activate" the Wld^S
330 protective pathway. This is never done in models of chronic neurodegeneration so it is
331 conceivable that in those models the protective effect of Wld^S is not induced due to inadequate
332 "activation" of the pathway that Wld^S acts upon. This would limit the impact of Wld^S on the
333 ensuing neurodegeneration. Where there is partial rescue of phenotype in chronic models, the
334 Wld^S pathway may start to become "activated" as the degeneration sets in. "Activation" of the
335 Wld^S pathway by simulating injury or established neurodegeneration is a novel concept. There
336 is no precedence for this idea from studies published to date because no one has reported
337 overlaying an acute injury in a chronic model. Our data indicates that Wld^S behaves differently
338 in uninjured axons compared to injured ones – the mechanisms responsible for this need to be
339 elucidated.

Activation of the Wld^{S}-protective pathway prevents as well as halts progression of tau-mediated degeneration

The axonal degeneration observed in hTau^{0N3R} transgenic flies was characterised by axonal swellings, which are indicative of the early stages of axonal degeneration caused by human tau. Axonal swellings are not a feature of Wallerian degeneration in the peripheral nervous system, however they have been described following injury in the CNS (13) (51). Axonal swellings have also been observed in models of neurodegeneration, including AD (51) (52) (53) and tauopathy (54) (9), leading to suggestions that Wallerian-like degeneration is occurring in neurodegenerative diseases.

Upon activation of the Wld^{S} pathway, we did not observe axonal swellings and there was a lack of any other feature of tau-mediated degeneration in hTau^{0N3R};Wld^{S} axons. These tau-expressing axons looked as normal as the Wld^{S} expressing controls, which previous studies have shown to be both morphologically normal, as well as physiologically functional (30). This protective effect was evident even when the Wld^{S} pathway was activated in the hTau^{0N3R};Wld^{S} axons at a time point after tau-mediated degeneration was established. This demonstrates that once activated, the Wld^{S} protective pathway prevents emergence of tau-mediated degeneration as well as halting progression of already established degeneration.

What is the mechanism by which activated Wld^{S} pathway protects against hTau?

Tau-mediated degeneration is dependent upon factors including total tau levels (55), phosphorylation at pathological sites (56) (35) and tau aggregation (57). The presence of human tau within hTau^{0N3R};Wld^{S} axons, even weeks after activation of the Wld^{S} pathway, indicates that the protection seen was not due to a reduction in total tau level as a result of loss of human tau from the axon. Nor is it likely to be due to any significant reduction in its phosphorylation status at the one pathological site, PHF1 (ser/thr 396 and 404) that we

examined. This surprising observation implies that upon activation, the Wld^S-pathway acts to negate the degenerative effects of tau, despite the persistence of pathologically phosphorylated human tau within the axon. Nonetheless it is possible that this protective effect was conferred by reduced misfolding or phosphorylation at other pathological sites that have previously been implicated in tau-mediated degeneration in other *Drosophila* models of tauopathy, such as MC1 (57), AT100 (Thr212/Ser214) (58) or 12E8 (ser262/356) (35) or reduced aggregation of human tau remains to be determined by future studies. It is conceivable that Wld^S pathway activation may influence tau phosphorylation at other sites as well as modulate tau aggregation, as several isoforms of NMNAT, one of which is a component of Wld^S fusion protein, have been shown to act as potent chaperones of phosphorylated tau, preventing its aggregation *in vitro* (37). This report, and others like it which implicate NMNAT in modulation of tau-mediated toxicity in other experimental models (38) (39), suggest that activation of Wld^S pathway does not act downstream of tau, and instead there are some key points of intersection.

A better understanding of the mechanisms that underpin the Wld^S protective pathway may highlight commonalities with hTau mediated degeneration and thus identify potential points of intersection at which protection is conferred. A key component of the Wld^S pathway is the NAD^+ salvage pathway, which has recently also been linked with AD (59). Wld^S contains nicotinamide mononucleotide adenylyl transferase 1 (NMNAT1) (60) (61), which is the final enzyme in the NAD^+ salvage pathway in mammals, and the biosynthetic activity of NMNAT1 is required for the full Wld^S protective phenotype (62) (63). One isoform in mammals (NMNAT2) and the sole *Drosophila* homolog dNMNAT are rapidly lost upon injury and this is associated with degeneration (64). Wld^S is believed to compensate for the loss of NMNAT in injured Wld^S expressing axons, thereby preventing loss of NAD^+ and activation of the downstream pro-degenerative pathway. dSarm/Sarm1 (32) and Highwire/PHR1 (34) are

endogenous mediators of axon degeneration that affect levels of NMNAT, with Axundead (30) and Pebbled (65) identified downstream of the loss of NMNAT. Knockout of these endogenous mediators results in axon survival after injury. We postulate that the Wld^S protective pathway would not be activated in uninjured hTau;Wld^S expressing axons, and so its protective effects would not be evident. Upon activation, the Wld^S pathway may confer protection against tau-mediated degeneration because its downstream components are switched on and can interact directly with pathological tau. Supporting this, it has been shown that NMNAT prevents tau-mediated aggregation *in vitro*, acts as chaperone for proteosis of tau in rodent models and promotes clearance of tau oligomers leading to suppression of tau-induced degeneration in *Drosophila* models of tauopathy (38) (37, 39) (39). Alternatively, protection may be conferred indirectly due to downstream neuroprotective effects of Wld^S pathway activation, such as enhanced mitochondrial calcium buffering (66), or reduced oxidative stress (67) negating tau-mediated dysregulation in intracellular calcium and/or tau-mediated mitochondrial dysfunction (68) (59) or oxidative stress (43). Future studies will be required to identify the exact point at which the activated Wld^S pathway intersects with and therefore protects against tau-mediated degeneration. In particular it will be vital to explore whether expression of downstream meditors of the Wld^S pathway that potentially block injury-induced axon degeneration (such as NMNAT, dSarm, axed, highwire, or even NAD^+) also modulate tau-mediated degeneration to emulate Wld^S pathway activation.

Conclusion

We show that activation of the Wld^S-pathway reliably protects against tau-mediated axonal degeneration, almost abolishing it. It is vital to understand how engagement of this pathway and its downstream mediators interact with tau, whether directly or indirectly, to halt tau-mediated axonal degeneration. This will yield important clues about the mechanisms underpinning tau-mediated axonal degeneration as well as enable identification of novel drug

targets that can emulate the complete protection we report here, to truly halt tau-mediated degeneration in all tauopathies.

Acknowledgements

This study was funded by The Gerald Kerkut Charitable Trust.

Conflicts of Interest

The authors declare no conflicts of interest.

References

1. McAleese KE, Walker L, Graham S, Moya ELJ, Johnson M, Erskine D, et al. Parietal white matter lesions in Alzheimer's disease are associated with cortical neurodegenerative pathology, but not with small vessel disease. Acta Neuropathol. 2017;134(3):459-73.

2. Huang J, Friedland RP, Auchus AP. Diffusion tensor imaging of normal-appearing white matter in mild cognitive impairment and early Alzheimer disease: preliminary evidence of axonal degeneration in the temporal lobe. AJNR Am J Neuroradiol. 2007;28(10):1943-8.

3. Sun X, Salat D, Upchurch K, Deason R, Kowall N, Budson A, et al. Destruction of white matter integrity in patients with mild cognitive impairment and Alzheimer disease. J Investig Med. 2014;62(7):927-33.

4. Kanaan NM, Morfini GA, LaPointe NE, Pigino GF, Patterson KR, Song Y, et al. Pathogenic forms of tau inhibit kinesin-dependent axonal transport through a mechanism involving activation of axonal phosphotransferases. J Neurosci. 2011;31(27):9858-68.

5. Mudher A, Shepherd D, Newman TA, Mildren P, Jukes JP, Squire A, et al. GSK-3beta inhibition reverses axonal transport defects and behavioural phenotypes in Drosophila. Mol Psychiatry. 2004;9(5):522-30.

6. Kim J, Choi IY, Michaelis ML, Lee P. Quantitative in vivo measurement of early axonal transport deficits in a triple transgenic mouse model of Alzheimer's disease using manganese-enhanced MRI. Neuroimage. 2011;56(3):1286-92.

7. Cowan CM, Bossing T, Page A, Shepherd D, Mudher A. Soluble hyper-phosphorylated tau causes microtubule breakdown and functionally compromises normal tau in vivo. Acta Neuropathol. 2010;120(5):593-604.

8. Lin WL, Lewis J, Yen SH, Hutton M, Dickson DW. Ultrastructural neuronal pathology in transgenic mice expressing mutant (P301L) human tau. J Neurocytol. 2003;32(9):1091-105.

9. Probst A, Gotz J, Wiederhold KH, Tolnay M, Mistl C, Jaton AL, et al. Axonopathy and amyotrophy in mice transgenic for human four-repeat tau protein. Acta Neuropathol. 2000;99(5):469-81.

10. Sahara N, Perez PD, Lin WL, Dickson DW, Ren Y, Zeng H, et al. Age-related decline in white matter integrity in a mouse model of tauopathy: an in vivo diffusion tensor magnetic resonance imaging study. Neurobiol Aging. 2014;35(6):1364-74.

11. Waller A. Experiments on the section of the glossopharyngeal and hypoglossal nerves of the frog, and observations of the alterations produced thereby in the structure of their primitive fibres. Phil Trans Royal Soc London 1850;140::423-9.

12. Tang-Schomer MD, Johnson VE, Baas PW, Stewart W, Smith DH. Partial interruption of axonal transport due to microtubule breakage accounts for the formation of periodic varicosities after traumatic axonal injury. Exp Neurol. 2012;233(1):364-72.

13. Beirowski B, Nogradi A, Babetto E, Garcia-Alias G, Coleman MP. Mechanisms of axonal spheroid formation in central nervous system Wallerian degeneration. J Neuropathol Exp Neurol. 2010;69(5):455-72.

461 14. Shemesh OA, Erez H, Ginzburg I, Spira ME. Tau-induced traffic jams reflect
462 organelles accumulation at points of microtubule polar mismatching. Traffic. 2008;9(4):458-
463 71.

464 15. Park JY, Jang SY, Shin YK, Koh H, Suh DJ, Shinji T, et al. Mitochondrial swelling and
465 microtubule depolymerization are associated with energy depletion in axon degeneration.
466 Neuroscience. 2013;238:258-69.

467 16. Trimmer PA, Swerdlow RH, Parks JK, Keeney P, Bennett JP, Jr., Miller SW, et al.
468 Abnormal mitochondrial morphology in sporadic Parkinson's and Alzheimer's disease cybrid
469 cell lines. Exp Neurol. 2000;162(1):37-50.

470 17. Wirths O, Weis J, Kayed R, Saido TC, Bayer TA. Age-dependent axonal degeneration
471 in an Alzheimer mouse model. Neurobiol Aging. 2007;28(11):1689-99.

472 18. Lunn ER, Perry VH, Brown MC, Rosen H, Gordon S. Absence of Wallerian
473 Degeneration does not Hinder Regeneration in Peripheral Nerve. Eur J Neurosci. 1989;1(1):27-
474 33.

475 19. Conforti L, Gilley J, Coleman MP. Wallerian degeneration: an emerging axon death
476 pathway linking injury and disease. Nat Rev Neurosci. 2014;15(6):394-409.

477 20. Ding C, Hammarlund M. Mechanisms of injury-induced axon degeneration. Curr Opin
478 Neurobiol. 2019;57:171-8.

479 21. Llobet Rosell A, Neukomm LJ. Axon death signalling in Wallerian degeneration
480 among species and in disease. Open Biol. 2019;9(8):190118.

481 22. Coleman MP, Hoke A. Programmed axon degeneration: from mouse to mechanism to
482 medicine. Nat Rev Neurosci. 2020;21(4):183-96.

483 23. Kaneko S, Wang J, Kaneko M, Yiu G, Hurrell JM, Chitnis T, et al. Protecting axonal
484 degeneration by increasing nicotinamide adenine dinucleotide levels in experimental
485 autoimmune encephalomyelitis models. J Neurosci. 2006;26(38):9794-804.

486 24. Hasbani DM, O'Malley KL. Wld(S) mice are protected against the Parkinsonian
487 mimetic MPTP. Exp Neurol. 2006;202(1):93-9.

488 25. Sajadi A, Schneider BL, Aebischer P. Wlds-mediated protection of dopaminergic fibers
489 in an animal model of Parkinson disease. Curr Biol. 2004;14(4):326-30.

490 26. Meyer zu Horste G, Miesbach TA, Muller JI, Fledrich R, Stassart RM, Kieseier BC, et
491 al. The Wlds transgene reduces axon loss in a Charcot-Marie-Tooth disease 1A rat model and
492 nicotinamide delays post-traumatic axonal degeneration. Neurobiol Dis. 2011;42(1):1-8.

493 27. Samsam M, Mi W, Wessig C, Zielasek J, Toyka KV, Coleman MP, et al. The Wlds
494 mutation delays robust loss of motor and sensory axons in a genetic model for myelin-related
495 axonopathy. J Neurosci. 2003;23(7):2833-9.

496 28. Wang MS, Davis AA, Culver DG, Glass JD. WldS mice are resistant to paclitaxel
497 (taxol) neuropathy. Ann Neurol. 2002;52(4):442-7.

498 29. Fang Y, Soares L, Teng X, Geary M, Bonini NM. A novel Drosophila model of nerve
499 injury reveals an essential role of Nmnat in maintaining axonal integrity. Curr Biol.
500 2012;22(7):590-5.

501 30. Neukomm LJ, Burdett TC, Seeds AM, Hampel S, Coutinho-Budd JC, Farley JE, et al.
502 Axon Death Pathways Converge on Axundead to Promote Functional and Structural Axon
503 Disassembly. Neuron. 2017;95(1):78-91 e5.

504 31. Neukomm LJ, Burdett TC, Gonzalez MA, Zuchner S, Freeman MR. Rapid in vivo
505 forward genetic approach for identifying axon death genes in Drosophila. Proc Natl Acad Sci
506 U S A. 2014;111(27):9965-70.

507 32. Osterloh JM, Yang J, Rooney TM, Fox AN, Adalbert R, Powell EH, et al. dSarm/Sarm1
508 is required for activation of an injury-induced axon death pathway. Science.
509 2012;337(6093):481-4.

510 33. Paglione M, Rosell AL, Chatton JY, Neukomm LJ. Morphological and Functional
511 Evaluation of Axons and their Synapses during Axon Death in Drosophila melanogaster. J Vis
512 Exp. 2020(157).

513 34. Xiong X, Hao Y, Sun K, Li J, Li X, Mishra B, et al. The Highwire ubiquitin ligase
514 promotes axonal degeneration by tuning levels of Nmnat protein. PLoS biology.
515 2012;10(12):e1001440.

516 35. Nishimura I, Yang Y, Lu B. PAR-1 kinase plays an initiator role in a temporally ordered
517 phosphorylation process that confers tau toxicity in Drosophila. Cell. 2004;116(5):671-82.

518 36. Papanikolopoulou K, Roussou IG, Gouzi JY, Samiotaki M, Panayotou G, Turin L, et
519 al. Drosophila Tau Negatively Regulates Translation and Olfactory Long-Term Memory, But
520 Facilitates Footshock Habituation and Cytoskeletal Homeostasis. J Neurosci.
521 2019;39(42):8315-29.

522 37. Ma X, Zhu Y, Lu J, Xie J, Li C, Shin WS, et al. Nicotinamide mononucleotide
523 adenylyltransferase uses its NAD(+) substrate-binding site to chaperone phosphorylated Tau.
524 Elife. 2020;9.

525 38. Ali YO, Allen HM, Yu L, Li-Kroeger D, Bakhshizadehmahmoudi D, Hatcher A, et al.
526 NMNAT2:HSP90 Complex Mediates Proteostasis in Proteinopathies. PLoS Biol.
527 2016;14(6):e1002472.

528 39. Ali YO, Ruan K, Zhai RG. NMNAT suppresses tau-induced neurodegeneration by
529 promoting clearance of hyperphosphorylated tau oligomers in a Drosophila model of
530 tauopathy. Hum Mol Genet. 2012;21(2):237-50.

531 40. Eric D Hoopfer 1 TM, Ryan J Watts, Oren Schuldiner, Dennis D M O'Leary, Liqun
532 Luo. Wlds protection distinguishes axon degeneration following injury from naturally
533 occurring developmental pruning. Neuron. 2006;15;50(6):883-95.

534 41. Sinadinos C, Cowan CM, Wyttenbach A, Mudher A. Increased throughput assays of
535 locomotor dysfunction in Drosophila larvae. J Neurosci Methods. 2012;203(2):325-34.

536 42. MacDonald JM, Beach MG, Porpiglia E, Sheehan AE, Watts RJ, Freeman MR. The
537 Drosophila cell corpse engulfment receptor Draper mediates glial clearance of severed axons.
538 Neuron. 2006;50(6):869-81.

539 43. Chee F, Mudher A, Newman TA, Cuttle M, Lovestone S, Shepherd D. Overexpression
540 of tau results in defective synaptic transmission in Drosophila neuromuscular junctions.
541 Biochem Soc Trans. 2006;34(Pt 1):88-90.

542 44. Sealey MA, Vourkou E, Cowan CM, Bossing T, Quraishe S, Grammenoudi S, et al.
543 Distinct phenotypes of three-repeat and four-repeat human tau in a transgenic model of
544 tauopathy. Neurobiol Dis. 2017;105:74-83.

545 45. Fischer LR, Culver DG, Davis AA, Tennant P, Wang M, Coleman M, et al. The WldS
546 gene modestly prolongs survival in the SOD1G93A fALS mouse. Neurobiol Dis. 2005;19(1-
547 2):293-300.

548 46. Gultner S, Laue M, Riemer C, Heise I, Baier M. Prion disease development in slow
549 Wallerian degeneration (Wld(S)) mice. Neurosci Lett. 2009;456(2):93-8.

550 47. Vande Velde C, Garcia ML, Yin X, Trapp BD, Cleveland DW. The neuroprotective
551 factor Wlds does not attenuate mutant SOD1-mediated motor neuron disease. Neuromolecular
552 Med. 2004;5(3):193-203.

553 48. Edgar JM, McLaughlin M, Yool D, Zhang SC, Fowler JH, Montague P, et al.
554 Oligodendroglial modulation of fast axonal transport in a mouse model of hereditary spastic
555 paraplegia. J Cell Biol. 2004;166(1):121-31.

556 49. Gillingwater TH, Haley JE, Ribchester RR, Horsburgh K. Neuroprotection after
557 transient global cerebral ischemia in Wld(s) mutant mice. J Cereb Blood Flow Metab.
558 2004;24(1):62-6.

559 50. Antenor-Dorsey JA, O'Malley KL. WldS but not Nmnat1 protects dopaminergic
560 neurites from MPP+ neurotoxicity. Mol Neurodegener. 2012;7:5.

561 51. Adalbert R, Nogradi A, Babetto E, Janeckova L, Walker SA, Kerschensteiner M, et al.
562 Severely dystrophic axons at amyloid plaques remain continuous and connected to viable cell
563 bodies. Brain. 2009;132(Pt 2):402-16.

564 52. Stokin GB, Lillo C, Falzone TL, Brusch RG, Rockenstein E, Mount SL, et al.
565 Axonopathy and transport deficits early in the pathogenesis of Alzheimer's disease. Science.
566 2005;307(5713):1282-8.

567 53. Tsai J, Grutzendler J, Duff K, Gan WB. Fibrillar amyloid deposition leads to local
568 synaptic abnormalities and breakage of neuronal branches. Nat Neurosci. 2004;7(11):1181-3.

569 54. Shemesh OA, Spira ME. Hallmark cellular pathology of Alzheimer's disease induced
570 by mutant human tau expression in cultured Aplysia neurons. Acta Neuropathol.
571 2010;120(2):209-22.

572 55. Ubhi KK, Shaibah H, Newman TA, Shepherd D, Mudher A. A comparison of the
573 neuronal dysfunction caused by Drosophila tau and human tau in a Drosophila model of
574 tauopathies. Invert Neurosci. 2007;7(3):165-71.

575 56. Chatterjee S, Sang TK, Lawless GM, Jackson GR. Dissociation of tau toxicity and
576 phosphorylation: role of GSK-3beta, MARK and Cdk5 in a Drosophila model. Hum Mol
577 Genet. 2009;18(1):164-77.

578 57. Passarella D, Goedert M. Beta-sheet assembly of Tau and neurodegeneration in
579 Drosophila melanogaster. Neurobiol Aging. 2018;72:98-105.

580 58. Jackson GR, Wiedau-Pazos M, Sang TK, Wagle N, Brown CA, Massachi S, et al.
581 Human wild-type tau interacts with wingless pathway components and produces
582 neurofibrillary pathology in Drosophila. Neuron. 2002;34(4):509-19.

583 59. Fang EF. Mitophagy and NAD(+) inhibit Alzheimer disease. Autophagy.
584 2019;15(6):1112-4.

585 60. Conforti L, Tarlton A, Mack TG, Mi W, Buckmaster EA, Wagner D, et al. A
586 Ufd2/D4Cole1e chimeric protein and overexpression of Rbp7 in the slow Wallerian
587 degeneration (WldS) mouse. Proc Natl Acad Sci U S A. 2000;97(21):11377-82.

588 61. Mack TG, Reiner M, Beirowski B, Mi W, Emanuelli M, Wagner D, et al. Wallerian
589 degeneration of injured axons and synapses is delayed by a Ube4b/Nmnat chimeric gene. Nat
590 Neurosci. 2001;4(12):1199-206.

591 62. Avery MA, Sheehan AE, Kerr KS, Wang J, Freeman MR. Wld S requires Nmnat1
592 enzymatic activity and N16-VCP interactions to suppress Wallerian degeneration. J Cell Biol.
593 2009;184(4):501-13.

594 63. Beirowski B, Babetto E, Gilley J, Mazzola F, Conforti L, Janeckova L, et al. Non-
595 nuclear Wld(S) determines its neuroprotective efficacy for axons and synapses in vivo. J
596 Neurosci. 2009;29(3):653-68.

597 64. Gilley J, Coleman MP. Endogenous Nmnat2 is an essential survival factor for
598 maintenance of healthy axons. PLoS Biol. 2010;8(1):e1000300.

599 65. Farley JE, Burdett TC, Barria R, Neukomm LJ, Kenna KP, Landers JE, et al.
600 Transcription factor Pebbled/RREB1 regulates injury-induced axon degeneration. Proc Natl
601 Acad Sci U S A. 2018;115(6):1358-63.

602 66. Avery MA, Rooney TM, Pandya JD, Wishart TM, Gillingwater TH, Geddes JW, et al.
603 WldS prevents axon degeneration through increased mitochondrial flux and enhanced
604 mitochondrial Ca2+ buffering. Curr Biol. 2012;22(7):596-600.

605 67. Press C, Milbrandt J. Nmnat delays axonal degeneration caused by mitochondrial and
606 oxidative stress. J Neurosci. 2008;28(19):4861-71.

607 68. Cieri D, Vicario M, Vallese F, D'Orsi B, Berto P, Grinzato A, et al. Tau localises within
608 mitochondrial sub-compartments and its caspase cleavage affects ER-mitochondria
609 interactions and cellular Ca(2+) handling. Biochim Biophys Acta Mol Basis Dis.
610 2018;1864(10):3247-56.
611

612

613

614

615

616 ## Figure legends

617

618 Fig 1. Tau-mediated axonal swellings are not evident in hTau^{0N3R};Wld^{S} axons in which Wld^{S}
619 pathway is activated. a) Axonal swellings (arrowheads) in naïve htau^{0N3R};Wld^{S} axons where
620 Wld^{S} pathway has not been activated. b) These swellings do not appear in hTau^{0N3R};Wld^{S}
621 axons where Wld^{S} has been activated, at any time point post-activation (pa). b) Quantification
622 of coverage of axonal swellings. Values are presented as the mean ± SEM. n=6-10.
623 P<0.0001.

624

625 Fig 2. Tau-mediated axonal swellings are halted from progressing upon activation of the
626 Wld^{S} pathway. a) Quantification of axonal swellings reveals that in naïve hTau^{0N3R};Wld^{S}
627 axons, where Wld^{S} pathway has not been activated, the level of swellings increases
628 significantly at 4 wks and 6 wks (n=7-8). b) In hTau^{0N3R};Wld^{S} axons where Wld^{S} has been
629 activated the area covered by axonal swellings does not increase significantly over time post
630 Wld^{S} pathway activation (pa) (n=8-16). Values are presented as the mean ± SEM.
631 ***P<0.001, ****P<0.0001.

632

633 Fig 3. Co-expression of Wld^{S} with hTau^{0N3R} does not delay tau-mediated axonal
634 degeneration. At 1 week after eclosion all genotypes display normal olfactory receptor
635 neuron (ORN) morphology (a). At 3 weeks after ecclosion, axonal swellings are apparent in
636 htau^{0N3R} expressing ORNs with similar morphology observed in hTau^{0N3R};Wld^{S} ORNs. b)
637 Quantification of swelling coverage indicates that co-expression of Wld^{S} does not delay the
638 onset nor slow the progression of tau-mediated axonal degeneration. Values are presented as

639 the mean ± SEM. n=6-11, ****P<0.0001. Wld^{S}, slow Wallerian degeneration; hTau^{0N3R},
640 0N3R human tau isoform; n.s., not significant.
641
642 Fig 4. Wld^{S} does not improve tau-mediated axonal dysfunction. a) Expression of hTau^{0N3R}
643 results in the appearance of vesicular aggregates, which are also apparent in hTau^{0N3R};Wld^{S}
644 axons. b) No significant improvement in aggregate coverage was observed in hTau^{0N3R};Wld^{S}
645 axons compared with hTau^{0N3R} axons (n=10 larvae). c) hTau^{0N3R} NMJs display aberrant
646 morphology, typified by thinning of the axon (arrowheads) and microsatellite boutons
647 (arrows), with this also observed in htau^{0N3R};Wld^{S} NMJs (scale bar = 25 μm). Co-expression
648 of Wld^{S} with hTau^{0N3R} did not rescue thinning of the inter-bouton axon (d) or the alterations
649 in bouton size (e) (n=4 larvae, 3-6 NMJs/larva). Analysis of locomotor behaviour indicated
650 that co-expression of hTau^{0N3R};Wld^{S} did not significantly improve the hTau^{0N3R}-mediated
651 alterations in f) meander – relative turning/distance travelled, g) angular velocity – relative
652 turning / time elapsed and h) velocity of larval crawling (n>17). Values are presented as the
653 mean ± SEM. **P<0.01; ****P<0.0001. Wld^{S}, slow Wallerian degeneration; htau^{0N3R}, 0N3R
654 human tau isoform; NMJs, neuromuscular junctions; Ctl, control.
655
656 Fig 5. Tau expression in naïve hTau^{0N3R};Wld^{S} expressing axons is not different to
657 hTau^{0N3R};Wld^{S} axons where Wld^{S} pathway has been activated . Visualisation of the
658 membrane bound CD8-GFP protein shows extensive membrane fragmentation indicative of
659 axonal degeneration in naïve hTau^{0N3R};Wld^{S} neurons at 6 weeks (green upper panels in A).
660 Human tau is found within both the axonal processes as well as axonal swellings as
661 visualised by a polyclonal anti-tau antibody (red upper panels in A). No such membrane
662 fragmentation is evident in 6 week old hTau^{0N3R};Wld^{S} expressing axons (green lower panel in
663 A) even 5 weeks after Wld^{S} pathway activation (pa) despite persisting human tau levels (red

664 lower panels in A). (B) Quantification shows no differences in levels of total human tau
665 though there is a (non-significant) trend for a reduction in tau phosphorylated at PHF-1
666 between naïve hTau^{0N3R};Wld^{S} expressing axons and hTau^{0N3R};Wld^{S} expressing axons where
667 Wld^{S} has been activated even 6 weeks post activation (pa). (n=5). Values are presented as the
668 mean ± SEM ($p>0.05$).
669
670
671
672
673