INTRODUCTION

Although Alzheimer's disease (AD) was first described over 100 years ago, the neurodegenerative mechanisms underpinning this disease remain unclear. Thus, disease-modifying therapies still elude us and treatment is restricted to provision of symptomatic relief. One avenue of research investigates how hyper-phosphorylated tau, a microtubule-associated protein forming the tangle pathology in AD, causes neuronal dysfunction and degeneration. Hyper-phosphorylated tau cannot effectively bind and stabilise microtubules. It is hypothesised that this compromises cytoskeletal integrity within affected neurons in AD, disrupting axonal transport and synaptic function and manifesting in clinical symptoms. Compromised and reduced numbers of microtubules are reported in post-mortem AD brains. Furthermore, a direct correlation between hyper-phosphorylated tau-mediated axonal transport disruption and behavioural impairment is demonstrated in Drosophila (htau0N3R) and rodent (tau-T44 tg mice) models of AD. Supporting this, tangle pathology closely correlates with cognitive decline in AD patients. Hence, tau-based therapies are being considered for AD. Current strategies include drugs to reduce tau phosphorylation and aggregation. Recently, the use of microtubule-stabilising drugs to compensate for tau loss-of-function has been explored. Paclitaxel, a microtubule-stabilising drug, reduces axonopathy and improves behaviour in a rodent model of tauopathy. Although this supports the idea of by-passing abnormal tau and compensates for tau loss-of-function thus protecting against neuronal dysfunction, the development of a new generation of microtubule-stabilising agents exhibiting low toxicity and high BBB permeability is yet to be demonstrated in vivo.

MATERIALS AND METHODS

Stocks and drug treatment

Expression of htau0N3R (Bloomington Stock Centre, Bloomington, IN, USA; stock no. 181), htau0N4R (provided by Dr E Skoulakis; Alexander Fleming Institute, Athens, Greece) and amyloid beta-42 (Aβ42) (Ab13522, Ab42) (provided by Dr D Crowther; University of Cambridge, Cambridge, UK) was directed to Drosophila melanogaster motor neurons using either the motor neuron-specific drivers D42-Gal4, or the D42-Gal4 driver recombined with green fluorescent protein (GFP)-tagged neuropeptide-Y (D42-GAL4.UAS-NPY-GFP) (UAS-NPY-GFP provided by Dr I Robinson, University of Cambridge). Pan-neural expression was achieved with the Elav-Gal4 driver (Bloomington Stock Centre; stock no. 458). NAP (Peptide Protein Research, Fareham, UK) was delivered to basic fly food at concentrations of 50 ng ml⁻¹, 500 ng ml⁻¹, 2.5 μg ml⁻¹ or 25 μg ml⁻¹. Larval locomotion and electron microscopy analysis were conducted as previously described.

In vivo axonal transport analysis

Treatment groups were subjected to timed lays on apple juice agar plates. F1 eggs were transferred to basic or NAP food. htau0N4R larvae were assessed
at L3 wandering stage (day 5) at 25 °C. Htau⁰N⁴R and Aβ₄₂-HTau⁰N⁴R larvae were reared at 29 °C. For the NAP rescue experiment, early L3 larvae were removed from food plates at day 4 and either subjected to axonal transport analysis or moved onto a basic food plate or a NAP food plate for 24 h. Axonal transport was analysed as previously described.¹²³⁴

Immunohistochemistry

Dissected L3 larvae were immunostained as previously described.³ Body walls were incubated in goat anti-heroserdase peroxidase conjugated to fluorescein isothiocyanate (1:1000; ICN/Cappel, Solon, OH, USA) and mouse anti-tubulin (1:500; E7-Developmental Hybridoma Bank, Iowa City, IA, USA) antibodies and with Alexa Fluor 594 donkey anti-mouse (1:200; Invitrogen, Paisley, UK) secondary antibody. Muscle 4 from abdominal segments A3–A7 was imaged.³

Microtubule-binding assay

Three heads of 1 to 3-day-old flies, or 10 larval (L3) ventral cords were pooled and homogenised in 30 and 40 μl, respectively, of microtubule-binding assay buffer as previously described.³

Immunoprecipitation assay

Ten heads of 1 to 3-day-old flies were homogenised in 500 μl of microtubule-binding assay buffer and interactions between tau and dtau were assessed as described previously.³

Western blotting

Adult heads or dissected L3 larval ventral cords were homogenised, resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Blots were probed with anti-human tau (1:15,000, Dako, Cambridge, UK), anti-dtau (1:500, Professor St Johnston, University of Cambridge), anti-phospho-tau: PHF-1 (1:2000, Dr Peter Davies, Albert Einstein College of Medicine, Bronx, NY, USA), AT180 (1:100, Biosciences, Nottingham, UK), AT8 (1:800, Biosciences), tau-1 (1:2000, Millipore, Watford Herts, UK), p25262 (1:1000, Invitrogen), anti-tubulin (1:200) and anti-kiinesin (1:5000, Cytoskeleton, Denver, CO, USA). Signal was detected using fluorescein conjugated secondary antibodies.

Statistics

Statistical analysis was carried out using Prism 5.0 (GraphPad, University of Southampton, Southampton, UK). T-test or one-way analysis of variance, with Bonferroni’s Multiple comparison post-hoc analysis.

RESULTS

NAP prevents htau⁰N⁴R-mediated disruption of locomotor behaviour

Expression of the highly phosphorylated 0N3R human tau isoform (htau⁰N⁴R) in motor neurons of Drosophila manifests in a number of distinct phenotypes including crawling defects in larvae.⁶ We first tested whether this phenotype could be prevented by NAP. Untreated htau⁰N⁴R larvae exhibited a restricted and non-continuous crawling behaviour (Figure 1b; Supplementary Video 1) compared with controls (Figure 1a; Supplementary Video 2). This defective crawling behaviour improved significantly following NAP treatment leading to smoother, more directed movement over a greater distance like controls (Figure 1c; Supplementary Video 3). Using the image-tracking software Ethovision, crawling parameters were quantified including body wall contractions, velocity and meander (turning rate per distance travelled). In all, 2.5 μg ml⁻¹ NAP significantly improved body wall contractions and velocity compared with untreated htau⁰N⁴R larvae (Figures 1d and e). Meander was also improved, but did not reach significance (P = 0.06) (Figure 1f). Crawling behaviour of controls treated with NAP was no different to untreated controls (Supplementary Figure 1).

NAP stabilises microtubules and improves axonal transport and neuromuscular junction (NMJ) morphology

As disruption of the microtubule cytoskeleton causes behavioural defects in this model, we investigated whether microtubule stabilisation was the mechanism by which NAP rescued the locomotor phenotype of htau⁰N⁴R larvae. The ultrastructural organisation of the microtubule cytoskeleton was examined in transverse sections of peripheral nerves by transmission electron microscopy. Controls displayed characteristic, 25 nm circular transverse microtubule profiles (black arrows Figures 2a–c). In contrast, htau⁰N⁴R larvae had noticeably fewer such microtubule profiles and displayed many disorganised and mis-aligned microtubules (black arrowheads Figures 2d–f). A number of axons relatively devoid of microtubules were also evident in htau⁰N⁴R larvae (asterisks Figure 2f). NAP re-stabilised the disrupted and disorganised microtubules as evidenced by the appearance of more intact transverse microtubule profiles in treated htau⁰N⁴R larvae (black arrows Figures 2g–i). Quantification of the number of intact microtubule profiles per axon confirmed that there were significantly fewer microtubule profiles in htau⁰N⁴R axons (5.32 ± 0.28) compared with controls (8.10 ± 0.20). NAP increased this number back to control levels (8.07 ± 0.30; Figure 2j). The range of intact microtubules per individual axon in each condition is illustrated in Figure 2k. Almost half the axons from controls contained large numbers of microtubules (>5), with very few axons containing no microtubules (1%). However, the majority (>65%) of htau⁰N⁴R axons contained either none (30%) or very few (<5) microtubules (35%). In contrast, the peripheral nerves of NAP-treated htau⁰N⁴R larvae had a similar microtubule profile to controls. There was an increase in the number of axons containing large microtubules (>5) intact transverse microtubule profiles (58%) compared with untreated htau⁰N⁴R (35%), and a reduction in the number of axons with no microtubules (7%; Figure 2k). NAP treatment did not change the number of microtubule profiles in controls (untreated controls had 9.22 ± 0.42 intact microtubule (MT) profiles whereas NAP-treated controls had 9.98 ± 0.45 intact MT profiles; Supplementary Figure 2).

We next investigated whether the restoration of microtubule integrity in NAP-treated htau⁰N⁴R larvae improves axonal transport as we know that axonal transport is disrupted in this model as a consequence of microtubule breakdown.⁶ Expression of vesicular neupeptide-Y-GFP (vGFP) in motor neurons of larvae allows this htau⁰N⁴R-mediated phenotype to be visualised in vivo, in real time, in living intact larvae. A homogeneous distribution of vGFP as a consequence of microtubule breakdown.⁶ Expression of vesicular neupeptide-Y-GFP (vGFP) in motor neurons of larvae allows this htau⁰N⁴R-mediated phenotype to be visualised in vivo, in real time, in living intact larvae. A homogeneous distribution of vGFP indicating efficient axonal transport was evident in the peripheral nerves of NAP-treated and untreated controls (Figures 3b.i and a.i). As expected, in htau⁰N⁴R larvae the distribution of vGFP was dramatically different, with the appearance of large vGFP accumulates within axons, illustrating profound axonal transport disruptions (Figure 3c.i). However, in NAP-treated htau⁰N⁴R larvae axonal transport appeared normal, with very few vGFP accumulates within axons (Figure 3b.ii–iv). Quantification of the total area occupied by vGFP demonstrates this result (Figure 3e). A 10-fold higher dose of NAP (25 μg ml⁻¹) did not lead to any further improvement (Figure 3e). NAP also significantly reduced axonal transport defects in animals expressing comparable levels of another tau isoform (htau⁰N⁴R alone or htau⁰N⁴R and Aβ₄₂-HTau⁰N⁴R), illustrating that NAP can rescue defects induced by both 3 and 4 repeat tau isoforms (Figure 3f and Supplementary Figure 3).

Synaptic function and NMJ morphology are also compromised in our model.⁵ To ascertain whether the protective effect of NAP extends to the synapse, the NMJ on muscle 4 was visualised using an antibody against a neuronal surface antigen (anti-heroserdase peroxidase). Anti-tubulin antibody was also used to visualise the microtubule cytoskeleton within the NMJ terminals. The NMJ morphology in controls was characterised by a bead-like distribution of pre-synaptic boutons (Figures 3a.ii–iv). The tubulin cytoskeleton extended into the distal ends of the NMJ (Figure 3a.iii). Neither the morphology nor the tubulin cytoskeleton of these controls was altered by NAP (Figure 3b.ii–iv). In contrast, htau⁰N⁴R larvae NMJs exhibited profound

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morphological aberrations, including thinning of inter-bouton axon segments and appearance of 'mini' satellite boutons (arrowheads Figure 3c.ii). The cytoskeleton was also disrupted, with a complete absence of tubulin immunoreactivity in the most distal regions (asterisk Figure 3c.iii). NMJs of NAP-treated htau0N3R larvae also exhibited morphological aberrations similar to those of untreated htau0N3R larvae, but with a greater number of 'mini' satellite boutons (arrowheads Figure 3d.ii). However, the tubulin cytoskeleton in these animals extended into the most distal ends of the NMJ similar to that of the controls (asterisks Figure 3d.iii). The area encompassed by this NMJ was significantly smaller in htau0N3R larvae compared with controls. NAP treatment significantly increased this (Figure 3g).

Figure 1. NAPVSIPQ (NAP) improves the locomotor phenotype in htau0N3R-expressing Drosophila. Oral administration of 2.5 μg ml−1 NAP improves the locomotor performance of htau0N3R wandering third instar larvae. (a–c) Traces of paths taken by L3 larvae in 2-min free crawling on 100 cm² plates are shown for OreR controls (a), htau0N3R untreated larvae (b) and htau0N3R larvae treated with NAP (c). Crawling performance was quantified using a tracking-software Ethovision. In all, 2.5 μg ml−1 NAP improved crawling performance on three different measures: body wall contractions per minute (d), velocity (e) and meander (f). Scale bar: 2 cm. Error bars represent mean ± s.e.m. (d–f); one-way analysis of variance, post-hoc Bonferroni’s multiple comparison test, ***P < 0.001 (body wall contractions); ****P < 0.0001 (velocity); P = 0.06 (meander). n = 16–20.

Tau phosphorylation is not altered by NAP

Hyper-phosphorylation of htau in our model reduces its (and the endogenous Drosophila tau (dtau))’s microtubule-binding ability thus compromising cytoskeletal integrity.¹ We therefore asked whether NAP was preventing the emergence of the htau0N3R-mediated phenotypes by reducing tau phosphorylation. We examined a number of phospho-tau epitopes associated with AD in both NAP-treated and untreated htau0N3R larvae and adults. NAP did not alter tau phosphorylation at any of the sites examined (Figures 4a–d and Supplementary Figures 4A–E). Although there was a trend for reduced tau phosphorylation at thr231/235 (AT180) following NAP treatment, this was not statistically significant (P = 0.3409; Figure 4b). As phospho-tau levels were unaltered, we next explored the possibility that NAP was protecting the htau0N3R larva by increasing endogenous dtau and/or by reducing toxic htau. Neither the total htau or dtau levels were altered by NAP in both larvae and adults, suggesting that NAP does not block or enhance degradation of these proteins (Figures 4e and f; Supplementary Figures 4F and G). Interestingly, although dtau levels were unaltered, we next explored the possibility that NAP was protecting the htau0N3R larva by increasing endogenous dtau and/or by reducing toxic htau. Neither the total htau or dtau levels were altered by NAP in both larvae and adults, suggesting that NAP does not block or enhance degradation of these proteins (Figures 4e and f; Supplementary Figures 4F and G). Interestingly, although dtau levels were unaltered, NAP treatment significantly reduced the percentage of dtau bound to microtubules (MTs) following NAP treatment in both htau0N3R-expressing larvae and adults (Figures 4g and h). However, htau binding to MTs was not similarly increased (Figures 4i and j). This subcellular redistribution of dtau to the MTs was not attributable to the reduction of pathological htau0N3R/dtau interaction that we have previously reported³ (Figure 4k).
NAP not only prevents the emergence of tau phenotype, it also rescues it once established.

The results thus far show that NAP prevents the emergence of hyper-phosphorylated tau-mediated neuronal and behavioural phenotypes. To investigate whether NAP can alter tau phenotypes if administered after they are already established, we devised a 'rescue protocol'. In this protocol, htau0N3R larvae were reared on normal food for 4 days and then transferred to food containing NAP for 1 day. Axonal transport was assessed at day 4 before NAP treatment was begun and then again at day 5, after only 1 day of NAP treatment. Axonal transport was disrupted in untreated htau0N3R larvae even at day 4 (light grey bar Figure 5a). However, NAP treatment for 1 day significantly reduced this axonal transport disruption (white bar Figure 5a). Importantly, this rescue of axonal transport deficit following only 1 day of NAP treatment was the same as if it were given continuously for 5 days (striped bar Figure 5a).

Figure 2. NAPVSIPQ (NAP) protects against microtubule destabilisation in htau0N3R Drosophila. Representative electron micrographs showing transverse sections of peripheral nerves in L3 larvae. Axon profiles in control larvae show regularly spaced correctly aligned transverse microtubules (black arrows in a–c). In axons of htau0N3R larvae, the microtubules were disrupted, with fewer correctly aligned transverse microtubules (black arrows in d–f) and more disorganised microtubules visible in the same axon profiles (black arrowheads in d–f). Axons relatively devoid of microtubules were also observed (asterisks in f). However, in htau0N3R larvae fed 2.5 μg ml⁻¹ NAP, there was a complete rescue of microtubule integrity (g–i). Scale bar 500 nm. Control axons contained on average 8.10 ± 0.20 correctly aligned microtubule profiles per cross-section; larvae-expressing htau0N3R contained only 5.32 ± 0.28, and those expressing htau0N3R but reared on NAP contained 8.07 ± 0.30 profiles (j). Average numbers of correctly aligned visible microtubule profiles in axons of control and htau0N3R NAP treated animals were significantly greater than those of untreated htau0N3R animals. Axons exhibited a range of intact correctly aligned transverse microtubules (k); the majority of control axons contained large numbers of microtubules (>5), and very few axons contained no microtubules. However, the majority of htau0N3R axons contain either none or very few microtubules (<5). Htau0N3R larvae reared on NAP had a greater number of axons containing large numbers of intact transverse microtubules (>5) and fewer axons with no microtubules (k). (Error bars represent mean ± s.e.m., **P < 0.01, as determined by unpaired Student's two-tailed t-test, n = 5). NS, not significant.
Furthermore, the rescue persisted even if htau\textsuperscript{ON3R} larvae reared on NAP for 4 days were taken off the drug and reared on normal food for a further 1 day (dark grey bar Figure 5a). Overall, the deficits observed at day 5 in all htau\textsuperscript{ON3R} larvae reared on these variations of NAP treatment was significantly less than that observed in untreated htau\textsuperscript{ON3R} larvae reared on normal food for 5 days (black bar Figure 5a). Interestingly, if NAP treatment was not begun at any point, the axonal transport disruption became progressively worse as the animals aged from day 4 to day 5 (first grey bar vs black bar in Figure 5a).

To confirm the low toxicity of NAP\textsuperscript{16,19,22} we assessed pupariation/eclosion rates of control and htau\textsuperscript{ON3R} larvae with and without NAP treatment. NAP did not affect pupariation or eclosion of either lines (Supplementary Figure 5).
DISCUSSION
We demonstrate that compensating for tau loss-of-function by stabilising microtubules is a disease-modifying therapeutic strategy that should be considered for the treatment of tauopathies.

We show that treatment with the microtubule-stabilising peptide NAP significantly improves all phenotypes caused by expression of highly phosphorylated tau in vivo. This includes reinstatement of cytoskeletal integrity, prevention of axonal transport and synaptic...

Figure 4. For caption please refer page 840.
NAP (davunetide) rescues neuronal dysfunction in tauopathy

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Dysfunction and improvement of behavioural defects. Intriguingly, although hyper-phosphorylated tau is considered to be the toxic species in tauopathies, NAP rescued all phospho-tau phenotypes without altering phosphorylation at key disease-associated epitopes. Furthermore, NAP treatment is sufficient to rescue neuronal dysfunction even after it has already become established. Thus, NAP prevents, but more importantly, rescues phospho-tau-mediated neuronal dysfunction in vivo.

NAP stabilises microtubules

Microtubule integrity is critical for intracellular transport of materials and cytoskeletal dysfunction contributes to degeneration in many neurodegenerative conditions. Our previous findings illustrate that microtubule destabilisation caused by tau loss-of-function is a key mechanism by which highly phosphorylated tau mediates neuronal dysfunction. In other models too, tau loss-of-function and subsequent trafficking impairments cause neurodegeneration. The data presented here further underlines this and demonstrates that microtubule stabilisation can effectively protect against this.

Taxol-based microtubule-stabilising drugs are considered for treating AD but their widespread toxicity and poor BBB penetrability precludes their use. To overcome this, a newer generation of drugs like small peptides (NAP) and small molecules (Epothilones, for example, EpoD) have been formulated. Both NAP and EpoD interact with MTs in vitro, reduce axonopathy and improve behaviour with minimal toxicity in rodent models of tauopathy. In agreement with these studies, we also report an improvement in behavioural deficits of htau expressing larvae following treatment with NAP. However, we further extend these findings and demonstrate for the first time that re-instatement of the structure and function of microtubules is the mechanism by which NAP confers neuroprotection in vivo. Furthermore, our study pioneers evaluations of live axonal transport directly visualised in a living intact animal treated with NAP, or indeed any other microtubule-stabilising agent. Corroborating studies in rodents using Mn+ + enhanced magnetic resonance imaging are underway.

We also observed that NAP restored the tubulin cytoskeleton within NMJs, and the resultant impact on synaptic function will have contributed to the behavioural improvements seen following NAP treatment. Furthermore, NAP has been shown to increase expression of the synaptic protein synaptophysin in neuronal cultures and in vivo, which is associated with neuroprotection. However, the NMJs of NAP-treated htau expressing animals had a greater number of 'mini' satellite boutons, which may reflect compensatory sprouting to maintain neuronal function. Indeed NAP has been shown to induce neurite outgrowth in vitro, and this would support our observations.

The molecular mechanism by which NAP stabilises MTs is not known. We hypothesise that NAP directly associates with MTs and modulates their dynamics. In support for this, NAP directly colocalises with tubulin and alters MT dynamics in cell culture. Our data also suggest that disoriented/disaligned MTs (such as those present in the htau-expressing larvae) are NAP's most likely substrate, which is why it does not alter cytoskeletal integrity (and therefore neuronal function or behaviour) in controls. This is in complete agreement with recent studies showing a more robust effect on MT polymerisation in the compromised state.

NAP by-passes hyper-phosphorylated tau

Clinical research in AD has largely focused on anti-amyloid therapies, but the growing consensus now is that truly effective disease-modifying therapies will need to target tau as well. Thus, tau-based therapeutic targets are eagerly anticipated. Agents that reduce tau phosphorylation and aggregation are currently being tested in clinical trials. Interestingly, we find that microtubule stabilisation rescues neuronal dysfunction by by-passing hyper-phosphorylated tau altogether and directly compensating for its detrimental effects. Similar results were reported in a rodent model of tauopathy in which EpoD improves cognitive performance without altering tau phosphorylation in young mice. However, in older mice with pre-existing tau pathology, EpoD reduces hyper-phosphorylated tau. Consistent with this, intranasal administration of NAP for 3 months reduces tau phosphorylated at AT8 and AT180 sites in triple transgenic AD mice. In contrast to these findings, we did not observe any significant changes in levels of either total tau or in phosphorylation of tau at the sites we examined following NAP treatment. This suggests that NAP is not affecting tau degradation or phosphorylation pathways. It is possible that in our model, where very early pre-tangle stages of disease are modelled and neuronal dysfunction before death is assessed, NAP-mediated protection occurs primarily due to microtubule stabilisation. However, it is conceivable that after prolonged periods of NAP treatment, as is the case in the studies described above, the restoration of microtubule integrity reinstates some of the homeostatic mechanisms (including some which impact tau-phosphorylation) that may be lost in the presence of hyper-phosphorylated tau. Our finding that NAP increases the percentage of dtau bound to MTs, which we previously showed is compromised by hyper-phosphorylated htau, supports this argument. We speculate that this occurs because stabilisation of MTs by NAP increases the MT surface area available for dtau binding, thus further improving MT integrity. As the level of dtau and the pathological dtau/htau interaction is unaltered (this is not surprising given that this interaction is dependent on the phosphorylation state of htau, which is unchanged by NAP), it is likely that dtau from the soluble cytosolic pool is recruited back to the MTs. Indeed dtau levels were reciprocally reduced in the supernatant fraction of NAP-treated animals.

NAP rescues as well as prevents tau-mediated dysfunction

An important consideration for therapies aiming to target dysfunctional cellular processes is the extent of damage that will have already occurred by the time clinical symptoms manifest. We therefore examined NAP's capacity to reinstate microtubule...
integrity after it had already become compromised and found that treatment after onset of dysfunction was just as efficacious as prophylactic treatment before onset. This is supported by the findings of others that NAP is protective when administered immediately after injury\textsuperscript{18,41,42} and also when given chronically after the onset of dysfunction in rodent models of AD.\textsuperscript{20,22,43} However, these studies have not investigated the effect of NAP treatment on MT integrity and function. Ours is the first study to investigate NAP’s effects on both MT integrity (electron microscopy) and function (axonal transport) after the onset of dysfunction in vivo.

Furthermore NAP-mediated neuroprotection was long lasting, both in our model and in other models of neuronal injury.\textsuperscript{18,44} These results and ours are intriguing and suggest that, as speculated by Sudo and Bass,\textsuperscript{38} NAP may stabilise the microtubule lattice in such a way that its protective effect is retained, possibly like an ‘imprint memory’, even after its removal.

CONCLUSION

The mechanism(s) by which abnormally hyper-phosphorylated and aggregated tau cause neuronal dysfunction and neurodegeneration in tauopathies like AD is not entirely clear. The general consensus is that disease-associated aberrations (such as hyper-phosphorylation in AD and mutation in familial tauopathies), which reduce tau binding to microtubules cause cytoskeletal destabilisation leading to disruption of axonal transport before tau aggregation. Thus, microtubule stabilisation and restoration of axonal transport is probably the most promising disease-modifying therapeutic target to counter abnormal tau-mediated neuronal dysfunction. Our results support this view by demonstrating that a microtubule-stabilising drug can effectively protect against all hyper-phosphorylated tau-mediated phenotypes spanning from cellular and molecular dysfunction to behavioural defects in vivo. Microtubule-stabilising drugs such as NAP that penetrate the BBB and are non-toxic therefore hold great promise for the treatment of all tauopathies. Encouragingly phase II clinical trials showed promising results with improvements in cognitive scores, evidence of BBB permeability and good tolerability in patients with amnestic mild cognitive impairment.\textsuperscript{45} Therefore, NAP has been investigated in phase II/III clinical trials for the treatment of Progressive Supranuclear Palsy, a ‘pure’ severe tauopathy\textsuperscript{45} and the results of this are currently being evaluated. Understanding the mode of action of NAP will pave the way for similar disease-modifying therapies in AD and related tauopathies that have thus far been untreatable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1. Bonda DJ, Lee HP, Lee HG, Friedlich AL, Perry G, Zhu X et al. Novel therapeutics for Alzheimer’s disease: an update. Curr Open Drug Discov Dev 2010; 13: 235–246.
2. Alonso AC, Zaidi T, Grundke-Iqbal I, Iqbal K. Role of abnormally phosphorylated tau in the breakdown of microtubules in Alzheimer disease. Proc Natl Acad Sci USA 1994; 91: 5562–5566.
3. Lovestone S, Reynolds CH. The phosphorylation of tau: a critical stage in neurodevelopment and neurodegenerative processes. Neuroscience 1997; 78: 309–324.
4. 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: 593–604.
5. Chee FC, Mudher A, Cuttle MF, Newman TA, MacKay D, Lovestone S et al. Over-expression of tau results in defective synaptic transmission in Drosophila neuromuscular junctions. Neurobiol Dis 2005; 20: 918–928.
6. 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: 522–530.
7. Terry RD. The pathogenesis of Alzheimer disease: an alternative to the amyloid hypothesis. J Neuropathol Exp Neurol 1996; 55: 1023–1025.
8. Cash AD, Aliev G, Siedlak SL, Nunomura A, Fujioka H, Zhu X et al. Microtubule reduction in Alzheimer’s disease and aging is independent of tau filament formation. Am J Pathol 2003; 162: 1623–1627.
NAP (davunetide) rescues neuronal dysfunction in tauopathy
S Quraishe et al

9 Ishihara T, Hong M, Zhang B, Nakagawa Y, Lee MK, Trojanowski JQ et al. Age-dependent emergence and progression of a tauopathy in transgenic mice overexpressing the shortest human tau isoform. *Neuron* 1999; 24: 751–762.

10 Arriagada PV, Growdon JH, Hedley-Whyte ET, Hynman BT. Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. *Neurology* 1992; 42(3 Pt 1): 631–639.

11 Ballatore C, Brunden KR, Trojanowski JQ, Lee VM, Smith 3rd AB, Huryn DM. Modulation of protein-protein interactions as a therapeutic strategy for the treatment of neurodegenerative tauopathies. *Curr Top Medica lChem* 2011; 11: 317–330.

12 Gozes I. Tau pathology and future therapeutic s. *Curr Alzheimer Res* 2010; 7: 685–696.

13 Trojanowski JQ, Smith AB, Huryn D, Lee VM. Microtubule-stabilising drugs for therapy of Alzheimer's disease and other neurodegenerative disorders with axonal transport impairments. *Exp Opin Pharmacother* 2005; 6: 683–686.

14 Zhang B, Maiti A, Shively S, Lakhani F, McDonald-Jones G, Bruce J et al. Microtubule-binding drugs offset tau sequestration by stabilizing microtubules and reversing fast axonal transport deficits in a tauopathy model. *Proc Natl Acad Sci USA* 2005; 102: 227–231.

15 Bassan M, Zamostiano R, Davidson A, Pinhasov A, Giladi E, Perl O et al. Complete sequence of a novel protein containing a femtomolar-activity-dependent neuroprotective peptide. *J Neurochem* 1999; 72: 1283–1293.

16 Gozes I, Morimoto BH, Tiong J, Fox A, Sutherland K, Dangoor D et al. NAP: research and development of a peptide derived from activity-dependent neuroprotective protein (ADNP). *CNS Drug Rev* 2005; 11: 353–368.

17 Divinski I, Holtsner-Cochoav M, Vullih-Shultzman I, Steineng RA, Gozes I. Peptide neuroprotection through specific interaction with brain tubulin. *J Neurochem* 2006; 98: 973–984.

18 Leker RR, Teichner A, Grigoriadis N, Ovadia H, Brenmanen DE, Fridkin M et al. NAP, a femtomolar-acting peptide, protects the brain against ischemic injury by reducing apoptotic death. *Stroke*; *J Cereb Circ* 2002; 33: 1085–1092.

19 Gozes I, Steineng RA, Spier AD. NAP mechanisms of neuroprotection. *J Mol Neurosci* 2004; 24: 67–72.

20 Shiyavev N, Jouroukhin Y, Giladi E, Poliozyidou E, Grigoriadis NC, Rosenman H et al. NAP protects memory, increases soluble tau and reduces tau hyperphosphorylation in a tauopathy model. *Neurobiol Dis* 2009; 34: 381–388.

21 Matsuoka Y, Gray AJ, Hirata-Fukae C, Minami SS, Waterhouse EG, Mattson MP et al. Intranasal NAP administration reduces accumulation of amyloid peptide and tau hyperphosphorylation in a transgenic mouse model of Alzheimer’s disease at early pathological stage. *J Mol Neurosci* 2007; 31: 165–170.

22 Matsuoka Y, Jouroukhin Y, Gray AJ, Ma L, Hirata-Fukae C, Li HF et al. A neuronal microtubule-interacting agent, NAPVPSIG, reduces tau pathology and enhances cognitive function in a mouse model of Alzheimer’s disease. *J Pharmacol Exp Ther* 2008; 325: 146–153.

23 Sinadinos C, Cowan CM, Wynttchben A, Mudher A. Increased throughput assays of locomotor dysfunction in Drosophila larva. *J Neurosci Methods* 2012; 203: 325–334.

24 Sinadinos C, Burridge-King T, Soh D, Thompson LM, Marsh JL, Wynttchben A et al. Live axonal transport disruption by mutant huntingtin fragments in Drosophila motor neuron axons. *Neurobiol Dis* 2009; 34: 389–395.

25 Lei P, Aytou S, Finkelstein DI, Spoerri L, Ciccotosto GD, Wright DK et al. Tau deficiency induces parkinsonism with dementia by impairing MAP-mediated iron export. *Nat Med* 2012; 18: 291–295.

26 Michaelis ML, Dobrowsky RT, Li G. Tau neurofibrillary pathology and microtubule stability. *J Mol Neurosci* 2002; 19: 289–293.

27 Michaelis ML, Chen Y, Hill S, Reiff S, Georg G, Rice A et al. Amyloid peptide toxicity and microtubule-stabilising drugs. *J Mol Neurosci* 2002; 19: 101–105.

28 Brunden KR, Yao Y, Potuzak JS, Ferrer NI, Ballatore C, James MJ et al. The characterization of microtubule-stabilizing drugs as possible therapeutic agents for Alzheimer’s disease and related tauopathies. *Pharmacol Res* 2011; 63: 341–351.

29 Brunden KR, Zhang B, Carroll J, Yao Y, Potuzak JS, Hogan AM et al. Epothilone D improves microtubule density, axonal integrity, and cognition in a transgenic mouse model of tauopathy. *J Neurosci* 2010; 30: 13861–13866.

30 Gozes I, Divinski I. NAP, a neuroprotective drug candidate in clinical trials, stimulates microtubule assembly in the living cell. *Curr Alzheimer Res* 2007; 4: 507–509.

31 Barten DM, Fanara P, Andorfer C, Hoque N, Wong P, Husted KH et al. Hyperdynamic microtubules, cognitive deficits, and pathology are improved in tau transgenic mice with low doses of the microtubule-stabilizing agent BMS-241027. *J Neurosci* 2012; 32: 7137–7145.

32 Davunetide (NAP) and D-NAP (AL-408) protect against tau hyperphosphorylation in a model of ALS: implications for axonal transport. *Neuroscience Meeting Planner. Society for Neuroscience: New Orleans, LA, USA, 2012, abstract 242.05/E23.

33 Smith-Swintsoky VL, Gozes I, Brenchman DE, D’Andrea MR, Plata-Salaman CR. Activity-dependent neurotrophic factor-9 and NAP promote neurite outgrowth in rat hippocampal and cortical cultures. *J Mol Neurosci* 2005; 25: 225–238.

34 Idan-Feldman A, Schir Y, Poliozyidou E, Touloumi Q, Lagoudaki R, Grigoriadics NI et al. Davunetide (NAP) as a preventative treatment for central nervous system complications in a diabetes rat model. *Neurol Di Bi 2011;* 34: 327–339.

35 Pascual M, Gueri C. The peptide NAP promotes neuronal growth and differentiation through extracellular signal-regulated protein kinase and Akt pathways, and protects neurons co-cultured with astrocytes damaged by ethanol. *J Neurochem* 2007; 103: 557–568.

36 Divinski I, Mittelman L, Gozes I. A femtomolar acting octapeptide interacts with tubulin and protects astrocytes against zinc intoxication. *J Biol Chem* 2004; 279: 28531–28538.

37 Oz S, Ishasho-Pachima Y, Gozes I. The ADNP derived peptide, NAP modulates the tubulin pool: implication for neurotrophic and neuroprotective activities. *PloS One* 2012; 7: e51458.

38 Sudo H, Baas PW. Strategies for diminishing katinan-based loss of microtubules in tauopathic neurodegenerative diseases. *Hum Mol Genet* 2011; 20: 763–778.

39 Mangialasche F, Solomon A, Winblad B, Mecacci P, Kivipelto M. Alzheimer’s disease: clinical trials and drug development. *Lancet Neurol* 2010; 9: 702–716.

40 Zhang B, Carroll J, Trojanowski JQ, Yao Y, Iba M, Potuzak JS et al. The microtubule-stabilizing agent, epothilone D, reduces axonal dysfunction, neurotoxicity, cognitive deficits, and Alzheimer-like pathology in an interventional study with aged tau transgenic mice. *J Neurosci* 2012; 32: 3601–3611.

41 Beni-Adani L, Gozes I, Cohen Y, Assaf Y, Steineng RA, Brenmanen DE et al. A peptide derived from activity-dependent neuroprotective protein (ADNP) ameliorates injury response in closed head injury in mice. *J Pharmacol Exp Ther* 2001; 296: 57–63.

42 Rotstein M, Bassan H, Kariw N, Speiser Z, Harel S, Gozes I. NAP enhances neurodevelopment of newborn apolipoprotein E-deficient mice subjected to hypoxia. *J Pharmacol Exp Ther* 2006; 319: 322–339.

43 Vullih-Shultzman I, Pinhasov A, Mandel S, Grigoriadis N, Touloumi Q, Pittel Z et al. Activity-dependent neuroprotective protein snippet NAP reduces tau hyperphosphorylation and enhances learning in a novel transgenic mouse model. *J Pharmacol Exp Ther* 2007; 323: 438–449.

44 Jehle T, Dimitru C, Auer S, Knth R, Vidal-Sanz M, Gozes I et al. The neuropeptide NAP provides neuroprotection against retinal ganglion cell damage after retinal ischemia and optic nerve crush. *Glaucoma’s archive for clinical and experimental ophthalmology – Albrecht von Graefes Archiv fur klinische und experimentelle Ophthalmologie* 2008; 246: 1255–1263.

45 Gold M, Lorenz S, Stewart AJ, Morimoto BH, Williams DR, Gozes I. Critical appraisal of the role of davunetide in the treatment of progressive supranuclear palsy. *Neuropsychiatric Disease Treatment* 2012; 8: 85–93.

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