Microtubule stabilising peptides rescue tau phenotypes in-vivo

Shmma Quraishe, Megan Sealey, Louise Cranfield & Amritpal Mudher

The microtubule cytoskeleton is a highly dynamic, filamentous network underpinning cellular structure and function. In Alzheimer's disease, the microtubule cytoskeleton is compromised, leading to neuronal dysfunction and eventually cell death. There are currently no disease-modifying therapies to slow down or halt disease progression. However, microtubule stabilisation is a promising therapeutic strategy that is being explored. We previously investigated the disease-modifying potential of a microtubule-stabilising peptide NAP (NAPVSIPQ) in a well-established Drosophila model of tauopathy characterised by microtubule breakdown and axonal transport deficits. NAP prevented as well as reversed these phenotypes even after they had become established. In this study, we investigate the neuroprotective capabilities of an analogous peptide SAL (SALLRSIPA). We found that SAL mimicked NAP's protective effects, by preventing axonal transport disruption and improving behavioural deficits, suggesting both NAP and SAL may act via a common mechanism. Both peptides contain a putative 'SIP' (Ser-Ile-Pro) domain that is important for interactions with microtubule end-binding proteins. Our data suggests this domain may be central to the microtubule stabilising function of both peptides and the mechanism by which they rescue phenotypes in this model of tauopathy. Our observations support microtubule stabilisation as a promising disease-modifying therapeutic strategy for tauopathies like Alzheimer's disease.

Alzheimer's disease (AD) is the commonest cause of dementia in the elderly. It is characterised by progressive cognitive decline associated with neuronal dysfunction and death. Extracellular plaques made up of Aβ peptide and intraneuronal filaments/tangles composed of abnormal, highly phosphorylated forms of tau, neuropathologically define AD. Though this disease was first described over 100 years ago, disease-modifying therapies are still elusive and AD is on the rise. It is estimated that 65.7 million people worldwide will be living with dementia by 2030. Clearly, there is an urgent, unmet need for disease-modifying therapies to treat AD.

Tau is a microtubule binding protein that is important for the assembly, maintenance and stability of microtubules (MT). Hyper-phosphorylation of tau, as found in AD, decreases its affinity for tubulin, compromising its ability to stabilise the MTs and thus disrupting cytoskeletal integrity and axonal transport. These phospho-tau mediated phenotypes are evident in many in-vivo models of tauopathy including our own Drosophila model in which a wild-type, highly phosphorylated isoform of human tau (htau0N3R) is expressed. Drosophila is an established model system for analysing the cellular and molecular mechanisms that underlie a variety of neurodegenerative diseases, particularly tau-associated diseases. Htau0N3R-expression in this model causes neuronal dysfunction, characterised by MT destabilisation, axonal transport disruption, synaptic defects and behavioural impairments. This model has been used to explore the effectiveness of disease-modifying interventions to either reduce tau phosphorylation or enhance MT stabilisation. Treatment with NAP (NAPVSIPQ also known as 'davunetide'), a small octapeptide derived from activity dependent neuroprotective protein (ADNP) effectively restores MT integrity and protects MT-dependent axonal transport in both rodent and Drosophila models of disease. Moreover, in the Drosophila model, NAP-mediated protection against htau0N3R phenotypes spans cellular and molecular dysfunction through to behavioural defects.

NAP is reported to modulate MT dynamics in a fashion similar to MT plus-end tracking proteins (+TIPs). +TIPS target the dynamic ends of MTs, catalysing immediate changes in MT stability, directionality and growth. However, the majority of +TIPs do not interact directly with the MT plus-end and/or MT lattice. Instead, this interaction occurs through end-binding proteins (EBs), which recognise and bind conserved Ser-x-Ile-Pro (SxIP) polypeptide motifs within +TIPs. In addition to the classical 'SxIP' motif, 'SIP' and 'IP' sequences are also reported to mediate EB interaction with its binding partners. NAP contains a 'SIP' motif...
within its amino acid sequence (NAPVSIPQ). It interacts in-vitro\(^1\) with both EB1, a key regulator of MT dynamics and polymerisation\(^2\),\(^3\), and EB3 a central component in dendritic spine formation\(^4\). Silencing of either EB1 or EB3 abolishes NAP's protective activity in PC12 cells. Furthermore, silencing of EB3 in primary cortical neurons inhibits NAP-mediated dendritic spine formation\(^5\). A novel NAP analogue, SKIP, is reported to bind NAP and enhance axonal transport in ADNP-deficient mice\(^6\). It is therefore conceivable that NAP restores MT integrity and function in our Drosophila model of tauopathy by interacting with EBs via its SIP domain. The data presented here tests this hypothesis by exploring the MT stabilising potential of another analogous peptide called SAL (SALLRSIPA also termed ADNF-9), which also contains a SIP domain. SAL is derived from the glial precursor protein, activity dependent neurotrophic factor (ADNF). It exhibits similar neuroprotective capabilities to NAP in numerous animal and cell models of injury and disease\(^7\)\(^-\)\(^11\).

In this study we investigated whether SAL, like NAP could also protect against htau\(^0\)N3R-mediated neuronal dysfunction in our Drosophila model of tauopathy. The phenotypes that arise in this model occur as a direct or indirect consequence of MT breakdown. This model was therefore ideally suited to test SAL's ability to modulate MT integrity and thus determine the importance of the SIP domain in MT stabilising therapeutic approaches.

**Results**

**SAL prevents htau\(^0\)N3R-mediated locomotor impairment.** Expression of htau\(^0\)N3R within motor neurons of Drosophila manifests in a number of distinct phenotypes including crawling defects in larvae\(^12\). Larvae expressing htau\(^0\)N3R exhibit a restricted and non-continuous crawling behaviour indicative of impaired neuronal function\(^12\)\(^-\)\(^15\). Using the image-tracking software Ethovision, crawling parameters were quantified including velocity, meander (turning rate per distance travelled) and angular velocity (turning rate per time elapsed). Meander and angular velocity are presented on a negative measurement scale in Ethovision. As previously shown\(^14\), 2.5\(\mu\)g/ml NAP treatment significantly improved velocity (Fig. 1a, red bar, \(p = 0.0003, n = 14\)) compared to untreated htau\(^0\)N3R-expressing larvae (Fig. 1a, black bar, \(n = 39\)). Meander and angular velocity were also significantly improved in NAP-treated larvae (Fig. 1b, red bar, \(p = 0.017, n = 12\) and Fig. 1c, red bar, \(p = 0.0426, n = 12\)), respectively) compared to untreated htau\(^0\)N3R-expressing larvae (Fig. 1b and c, black bar, \(n = 44\) and 39, respectively). Larvae treated with SAL displayed a dose dependent improvement in all parameters tested. Treatment with 1.25\(\mu\)g/ml and 2.5\(\mu\)g/ml of SAL (Fig. 1a, pale green bar, \(n = 16\) and light green bar, \(n = 39\)), respectively) did not improve velocity compared to untreated htau\(^0\)N3R-expressing larvae (Fig. 1a, black bar, \(n = 39\)). However, 5\(\mu\)g/ml SAL (Fig. 1a, green bar, \(p = 0.0001, n = 29\)) and 10\(\mu\)g/ml SAL (Fig. 1a, dark green bar, \(p = < 0.0001, n = 40\)) significantly improved velocity compared to untreated htau\(^0\)N3R larvae (Fig. 1a, black bar, \(n = 39\)). Meander of htau\(^0\)N3R-expressing larvae treated with 1.25\(\mu\)g/ml and 2.5\(\mu\)g/ml of SAL (Fig. 1b, pale green bar, \(n = 17\) and light green bar, \(n = 40\)), respectively) did not improve compared to untreated htau\(^0\)N3R-expressing larvae (Fig. 1b, black bar, \(n = 44\)). However, 5\(\mu\)g/ml SAL (Fig. 1b, green bar, \(p = 0.0061, n = 31\)) and 10\(\mu\)g/ml SAL (Fig. 1b, dark green bar, \(p = 0.0022, n = 49\)) significantly improved meander compared to untreated htau\(^0\)N3R larvae (Fig. 1b, black bar, \(n = 44\)). Similar to the results for the previous crawling parameters assessed, angular velocity of htau\(^0\)N3R-expressing larvae treated with 1.25\(\mu\)g/ml and 2.5\(\mu\)g/ml of SAL (Fig. 1c, pale green bar, \(n = 17\) and light green bar, \(n = 38\)), respectively) did not improve compared to untreated htau\(^0\)N3R-expressing larvae (Fig. 1c, black bar, \(n = 39\)). However, as also demonstrated for velocity and meander, 5\(\mu\)g/ml SAL (Fig. 1c, green bar, \(p = 0.0044, n = 31\)) and 10\(\mu\)g/ml SAL (Fig. 1c, dark green bar, \(p = 0.0165, n = 46\)) significantly improved angular velocity compared to untreated htau\(^0\)N3R larvae (Fig. 1c, black bar, \(n = 39\)). Treatment with 2.5\(\mu\)g/ml and 10\(\mu\)g/ml SAL did not alter the crawling performance of controls compared to untreated controls (Supplementary Figure 1b–d).

Data were analysed by unpaired Student's two-tailed t-test.

**SAL prevents htau\(^0\)N3R-mediated disruption of axonal transport.** In this model, axonal transport is disrupted because of MT breakdown\(^6\)\(^-\)\(^11\). This can be visualised in-vivo and in real time through the expression of vesicular neuropeptide-Y-GFP (vGFP) in the motor neurons of living intact larvae\(^11\). Efficient axonal transport in untreated wild-type (wt) control larvae was evident by a homogeneous distribution of vGFP in peripheral nerves (Fig. 2a, n = 10). In htau\(^0\)N3R-expressing larvae, large vGFP accumulates were distributed within the axons of peripheral nerves, illustrating profound axonal transport disruptions (Fig. 2b and e, black bar, \(p < 0.0001, n = 9\)). As previously demonstrated, 2.5\(\mu\)g/ml of NAP prevented axonal transport deficits in htau\(^0\)N3R-expressing larvae, thus restoring MT integrity (Fig. 2c and e, red bar, \(p < 0.0001, n = 5\))\(^15\). Given that SAL improved crawling behaviour at higher doses (5\(\mu\)g/ml and 10\(\mu\)g/ml) compared to the more efficacious NAP (2.5\(\mu\)g/ml), we assessed axonal transport deficits in htau\(^0\)N3R-expressing larvae treated with 10\(\mu\)g/ml of SAL. We found that 10\(\mu\)g/ml of SAL prevented axonal transport deficits as effectively as 2.5\(\mu\)g/ml of NAP (Fig. 2d and e, dark green bar, \(p < 0.0001, n = 9\)). Quantification of the total axonal area occupied by vGFP accumulates confirmed these results (Fig. 2e). Data were analysed by one-way ANOVA with Bonferroni's correction.

**SAL does not alter tau phosphorylation.** The MT binding and stabilising ability of htau is reduced by hyper-phosphorylation\(^6\),\(^-\)\(^9\), leading to compromised cytoskeletal integrity. Reduction of tau phosphorylation through genetic and chemical manipulation is known to improve htau\(^0\)N3R-mediated phenotypes\(^6\)\(^-\)\(^11\). However, we previously reported that NAP does not alter the phosphorylation status of htau\(^0\)N3R and was conferring protection by another mechanism\(^14\). We therefore investigated whether SAL was preventing the emergence of the htau\(^0\)N3R-mediated phenotypes by reducing tau phosphorylation or whether it too, was bypassing the pathogenic phospho-htau\(^0\)N3R protein. We examined a number of phospho-tau epitopes associated with AD in both htau\(^0\)N3R-treated and untreated htau\(^0\)N3R-expressing 1–3 day old flies. Each phospho-tau antigen (intensity of signal, pixels/mm\(^2\)) was normalised to total tau levels on the same blot. This enabled assessment of changes in the phosphorylation status of tau, independent of changes in total tau levels. The AT180 monoclonal antibody detects phosphorylation of tau at Thr231. This site is important for MT binding, and phosphorylation, as occurs in AD.
at this site, inhibits binding of tau to MTs. HtauON3R-expressing flies treated with SAL at 5 or 10 μg/ml did not show changes in tau phosphorylation at the phospho-tau epitope detected by the AT180 antibody compared to untreated htauON3R larvae (Fig. 3a, n = 5). The AT8 monoclonal antibody detects phosphorylation of tau at Ser202/Thr205. HtauON3R-expressing flies treated with SAL at 5 or 10 μg/ml did not show changes in tau phosphorylation at the phospho-tau epitope detected by the AT8 antibody compared to untreated htauON3R larvae (Fig. 3b, n = 5). Another phospho-tau epitope, Ser396/Ser404, that is abnormally phosphorylated in AD can be detected by the PHF-1 antibody. Similar to AT180 and AT8, htauON3R-expressing flies treated with SAL at 5 or 10 μg/ml did not show changes in tau phosphorylation at the phospho-tau epitope detected by the PHF-1 antibody.
neurons (used in this study (D42- and Elav-Gal4) are not exclusively post-mitotic and motor-neuron specific49,50. D42-Gal4
ysis as they do not express htau0N3R (Supplementary Figure 1a). Wide view panels of all the blots are presented in control for all samples (Fig. 3d,e and Supplementary Figure 2). We did not include wt control animals in this anal-
SAL was able to rescue htau0N3R phenotypes without altering phosphorylation at key disease-associated epitopes.
In this study, we demonstrate that the neuroprotective peptide SAL (SALLRSIPA) provides protection against
neuronal dysfunction11. We previously demonstrated by EM that cytoskeletal integrity in wt (OreR) controls was
also protects against htau0N3R-mediated phenotypes as also demonstrated for an analogous neuroprotective peptide NAP
SAL and NAP – two neuroprotective peptides. SAL and NAP are short peptides derived from two
secreted astroglial parent proteins, ADNF and ADNP (respectively)15,38,39. NAP and SAL were identified as the
essential regions of their respective parent proteins for conferring neuroprotection39. In subsequent studies, SAL
and NAP were found to protect against a variety of cellular insults including neurotoxic drugs such as NMDA40
and ethanol28,41. They were also found to be protective in models of Alzheimer’s disease27,42,43, diabetic neuropa-
y44, amylolateral sclerosis and ADNP induced tauopathy10,17,45. The molecular mechanism underpinning NAP's
neuroprotective ability is thought to occur by MT stabilisation46, which counters axonal transport defects14,17. It is
conceivable that like NAP, SAL also has MT stabilising effects as it has been shown to displace NAP in an
in-vitro neuronal system24. These results are reminiscent of those observed with NAP-treatment in this model of tauopathy11. SAL is reported to exhibit a similar neuroprotective profile compared to NAP15,37. However, several studies have also shown that NAP is more efficacious than SAL15,29. This is consistent with our observations in the present study.

Discussion
In this study, we demonstrate that the neuroprotective peptide SAL (SALLRSIPA) provides protection against htau0N3R-mediated phenotypes as also demonstrated for an analogou

**Figure 2. SAL prevents axonal transport deficits in htau0N3R-expressing Drosophila larvae.** Wild-type, control untreated larvae exhibited a homogeneous distribution of vGFP fluorescence in peripheral nerves indicative of an efficient axonal transport system (a). Htau0N3R larvae exhibited accumulation of vGFP, indicative of disrupted axonal transport (b). 2.5μg/ml NAP prevented accumulation of vGFP in htau0N3R larval motor neurons (c). 10μg/ml SAL also prevented accumulation of vGFP in htau0N3R larval motor neurons (d). The total area of axons (within a defined region) encompassed by vGFP accumulates was greater in htau0N3R larvae compared to controls. 2.5μg/ml NAP and 10μg/ml SAL reduced the area covered by vesicular accumulates back to control levels (e). Data were analysed by one-way ANOVA with Bonferroni’s correction. Error bars represent mean ± S.E.M., P**** < 0.0001; n = wt (10), htau0N3R (9), 2.5μg/ml NAP (5), 10μg/ml SAL (9). Scale bar: 10μm.
Figure 3. SAL does not alter total htau\textsuperscript{\textbf{0N3R}} levels or phosphorylation at a number of sites relevant to AD. For each phospho-tau antigen, intensity of signal (pixels/mm\textsuperscript{2}) was normalised to total tau levels. There was no significant change in the levels of the phospho-tau epitopes detected by AT180 (a), AT8 (b), and PHF-1 (c) after treatment with 5 \(\mu\)g/ml SAL (green bars) and 10 \(\mu\)g/ml SAL (dark green bars) SAL. Total dtau levels were not altered by SAL treatment (d). Representative blots are shown (a-d). All lanes were run on the same gel. Data were analysed by one-way ANOVA with Bonferroni’s correction. Error bars represent mean ± S.E.M; \(n = htau^{\text{\textbf{0N3R}}}(5), 5 \mu\text{g/ml SAL}(5), 10 \mu\text{g/ml SAL}(5).\)
their impact on transgenic control lines with a UAS background (e.g. UAS-LacZ). However, our primary aim in this study was to determine if SAL-treatment significantly affected htau0N3R-phenotypes arising because of MT destabilisation. As such, treated and untreated Drosophila expressing htau0N3R were reared and tested alongside each other to minimise any titration and genetic artefacts. Interestingly, we found that axonal transport deficits could be prevented and efficient axonal transport maintained in htau0N3R-expressing animals at a comparable level to robust wt OreR control larvae.

Mode of action of SAL and NAP. Hyper-phosphorylated tau is considered to be the toxic species in tauopathies. It is believed to cause degeneration both due to loss of MT-binding function and accumulation of toxic tau aggregates51,52. Tau-centric disease-modifying strategies rescue tau phenotypes by reducing tau phosphorylation11,53, increasing MT stabilisation14,46–54 or reducing tau aggregation55. Our data imply neuroprotective effects independent of reductions in tau phosphorylation, but whether these peptides impact on tau aggregation remains to be determined. Interestingly, we have previously shown that inhibition of GSK-3β rescues tau phenotypes and restores MT integrity, by reducing tau phosphorylation with a consequent increase in tau protein levels and insoluble granular tau oligomers56. In the present study, we did not observe any significant changes in total htau0N3R protein and phosphorylation levels after treatment with SAL and as demonstrated for NAP14. Previous findings from our lab, as well as other studies conducted in rodent and cell culture models of tauopathy, strongly suggest that NAP neuroprotects by fortification of MTs14,15,16,57.

In this study, we assessed the neuroprotective capabilities of both NAP and SAL peptides individually. SAL showed dose-dependent neuroprotective effects, consistent with our previous observations for NAP44. Other studies have also reported dose-dependent neuroprotective effects for both peptides57. In a rat model of cholinotoxicity, NAP was more efficacious compared to SAL in choline-repleted conditions25. NAP has also been shown to be more effective than SAL in providing long term protection against loss of spatial memory in apolipoprotein-E-deficient mice15 and AF64A-treated animals29. A combinatorial peptide approach would also have been interesting but is beyond the scope of the current study. However, previous studies have investigated the protective effects of combining both NAP and SAL. A few studies have shown that both peptides are more efficacious together, than either alone58–61. These peptides do not exhibit stereo-selectivity59. The more stable, all D-amino acid SAL (D-SAL) showed efficacy in vivo and in vitro models of disease50,51. In a model of fetal alcohol syndrome (FAS), administration of both D-NAP and D-SAL reduced fetal demise, however, no significant differences between combination and individual drug treatments were seen. In the same study, apolipoprotein E knockout mice treated with both D-NAP and D-SAL showed improved performance in the Morris water maze60. In another study, NAP alone was effective in preventing alcohol-induced fetal death, whereas SAL at the same dose was not protective. However, a combinatorial treatment with NAP and SAL was more effective in preventing growth restriction due to prenatal alcohol treatment61. These studies suggest a dose-dependent, synergistic effect rather than an additive effect. The differences reported in the literature and the differences in efficacious dose that we too observe may be attributable to the non-homologous amino acids either side of the SIP motif in the two peptides.

Interestingly, both peptides are derived from parent proteins that are secreted by glial cells in response to vasoactive intestinal peptide (VIP)52–55. VIP is expressed under conditions of stress and one of the early events that occurs during stress or insult mediated injury is a dynamic reorganisation of the cytoskeleton62. Both NAP and SAL (NAPSVIPQ and SALLRSIPA) contain a SIP motif55. The ‘SIP’ motif within NAP has been implicated in protection against ethanol and tetrodotoxin toxicity in cortical neurons40. Substitution of proline (P) with alanine (A) shows dose-dependent neuroprotective effects, consistent with our previous observations for NAP14. Other studies have also reported dose-dependent neuroprotective effects for both peptides57. In a rat model of cholinotoxicity, NAP was more efficacious compared to SAL in choline-repleted conditions25. NAP has also been shown to be more effective than SAL in providing long term protection against loss of spatial memory in apolipoprotein-E-deficient mice15 and AF64A-treated animals29. A combinatorial peptide approach would also have been interesting but is beyond the scope of the current study. However, previous studies have investigated the protective effects of combining both NAP and SAL. A few studies have shown that both peptides are more efficacious together, than either alone58–61. These peptides do not exhibit stereo-selectivity59. The more stable, all D-amino acid SAL (D-SAL) showed efficacy in vivo and in vitro models of disease50,51. In a model of fetal alcohol syndrome (FAS), administration of both D-NAP and D-SAL reduced fetal demise, however, no significant differences between combination and individual drug treatments were seen. In the same study, apolipoprotein E knockout mice treated with both D-NAP and D-SAL showed improved performance in the Morris water maze60. In another study, NAP alone was effective in preventing alcohol-induced fetal death, whereas SAL at the same dose was not protective. However, a combinatorial treatment with NAP and SAL was more effective in preventing growth restriction due to prenatal alcohol treatment61. These studies suggest a dose-dependent, synergistic effect rather than an additive effect. The differences reported in the literature and the differences in efficacious dose that we too observe may be attributable to the non-homologous amino acids either side of the SIP motif in the two peptides.

Methods

Drosophila genotypes and drug treatments. Transgenic expression of htau0N3R (y1 w1118; P[UAS-MAPTA]59 A: Bloomington Stock Centre, stock no. 181) was directed to Drosophila melanogaster motor neurons using either D42-Ga4, or the D42-Gal4 driver fused to vesicular GFP-tagged neuropeptide-Y (D42-GAL4.UAS-NPYvGFP) as previously described11. Pan-neural expression was established with the Elav-Gal4 driver. Female virgin flies homozygous for the D42 or Elav driver were crossed to male flies homozygous for htau0N3R under the UAS promoter (+; +; UAS-htau0N3R), or with Oregon-R (OreR) wt, control males. Stocks and transgenic crosses were maintained at 23°C on a 12 h light/dark cycle. Flies were raised on basic food consisting of malt extract, maize meal, soya flour, agar, granulated sugar, yeast, and propionic acid. NAP (NAPSVIPQ) and SAL (SALLRSIPA) (L-isomers synthesised by Peptide Protein Research Ltd, UK) were delivered to basic fly food at a final concentration of 2.5 µg/ml (NAP) and 1.25 µg/ml, 2.5 µg/ml, 5 µg/ml or 10 µg/ml (SAL). Late L3-stage larvae were selected for by size and wandering behaviour.

Larval locomotion assay. Larval locomotion analysis was conducted using a semi-quantitative assay as previously described32. Briefly, crawling behaviour was analysed on 1% agarose plates dyed with 0.1% w/v Alcian blue (Hopkin and Williams, UK). L3 larvae were positioned in the centre of each plate and allowed to acclimatise for 2 min prior to testing. Open field activity was recorded for 2 min (trial 1). This was further repeated for 2
more trials. Wherever possible, genotypes and treatments were randomised between adjacent plates. Videos of larval locomotion were analysed in Ethovision 3.0 software (Noldus) to determine velocity, angular velocity and meander.

**In-vivo axonal transport analysis.** All treatment groups were subjected to a 3–4 hour timed lay on apple juice agar plates. F1 eggs were transferred to either basic, NAP or SAL treated food. Larvae were left to develop to L3 wandering stage (day 5). Axonal transport analysis was conducted as previously described\(^7\). Briefly, L3 larvae were anaesthetised in diethyl ether vapour for 15 min, immobilised on glass slides in 1% agarose ventral face up and mounted under coverslips. Peripheral nerves were analysed between the 2nd and 4th denticle bands. For total area acquisition, vGFP accumulates were imaged at x63 on an Axioplan2 Epifluorescence Microscope (Zeiss), and thresholded in Metamorph software (Molecular Devices, CA, USA).

**Western blotting.** 1–3 day old adult fly heads were homogenised in buffer (150 mM NaCl, 50 mM MES pH 6.8, 1% triton-X, protease inhibitor cocktail and 1% SDS). For phospho-tau epitope detection, the following cocktail of phosphatase and kinase inhibitors was also added: 30 mM sodium fluoride, 20 mM sodium pyrophosphate, 40 mM 2-glycerophosphate, 3.5 mM sodium orthovanadate and 10 µM staurosporin. Samples were spun for 2 mins at 3,000 g; the supernatant was removed and heated for 5 mins at 95°C in Laemmli buffer. Samples were subjected to standard 10% SDS-PAGE and transferred to Protran Nitrocellulose Membrane (Whatman, UK). Blots were probed with the following primary antibodies: anti-human tau (1:15,000, Dako, UK), anti-dtau (1:500, Abcam, UK) and anti-phospho-tau: PHF-1 (1:2000, gifted by Dr. Peter Davies, Albert Einstein College of Medicine; Bronx, NY), AT8 (1:800, Source, Biosciences, UK), AT180 (1:100, Source, Biosciences, UK), anti-actin (1:5000, Abcam, UK) and anti-phospho-tau: (Zeiss), and thresholded in Metamorph software (Molecular Devices, CA, USA).

**Statistics.** Statistical analysis was carried out in Prism 5.0 (GraphPad, University of Southampton, Southampton, UK), using a T-test or one-way ANOVA with a post hoc Bonferroni’s Multiple Comparison Test. All values are reported as means ± S.E.M. P < 0.05 was considered significant.

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Author Contributions

S.Q. carried out experiments; S.Q. and A.M. contributed to scientific design and wrote the manuscript; M.S. contributed to western blotting experiments and L.C. contributed to larval locomotion assays. All authors reviewed the manuscript.

Additional Information

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Corrigendum: Microtubule stabilising peptides rescue tau phenotypes in-vivo

Shmma Quraishe, Megan Sealey, Louise Cranfield & Amritpal Mudher

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