

Published in final edited form as:

*J Neurosci.* 2011 February 2; 31(5): . doi:10.1523/JNEUROSCI.2610-10.2011.

## Tau protein is required for amyloid $\beta$ -induced impairment of hippocampal long-term potentiation

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### Abstract

Amyloid beta (A $\beta$ ) and tau protein are both implicated in memory impairment in mild cognitive impairment (MCI) and early Alzheimer's disease (AD), but whether and how they interact is unknown. Consequently, here we asked if tau protein is required for the robust phenomenon of A $\beta$ -induced impairment of hippocampal long-term potentiation (LTP), a widely accepted cellular model of memory. We used wild-type mice and mice with a genetic knockout of tau protein and recorded field potentials in an acute slice preparation. We demonstrate that the absence of tau protein prevents A $\beta$ -induced impairment of LTP. Moreover, we show that A $\beta$  increases tau phosphorylation and that a specific inhibitor of the tau kinase, glycogen synthase kinase 3 (GSK-3), blocks the increased tau phosphorylation induced by A $\beta$  and prevents A $\beta$ -induced impairment of LTP in wild-type mice. Together, these findings show that tau protein is required for A $\beta$  to impair synaptic plasticity in the hippocampus and suggest that the A $\beta$ -induced impairment of LTP is mediated by tau phosphorylation. We conclude that preventing the interaction between A $\beta$  and tau could be a promising strategy for treating cognitive impairment in MCI and early AD.

### Keywords

Alzheimer's disease; beta amyloid; tau protein; LTP; hippocampus; mouse

### Introduction

Amyloid beta (A $\beta$ ) and tau protein both have well-established roles in Alzheimer's disease (AD), forming the two hallmark pathologies visible in post-mortem AD brains as amyloid plaques and neurofibrillary tangles (NFTs), respectively (Hardy and Selkoe, 2002; Small and Duff, 2008). A $\beta$  plays a central role in disease pathogenesis, being implicated in the

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Author contributions: O.A.S., J.R.L., J.D., C.E.J.A., E.M.T. and M.V.-C. conducted experiments; H.N.D., M.P.V. and F.D. generated and maintained the *Tau*<sup>-/-</sup> mouse strain; O.A.S., J.R.L. and M.V.-C. analyzed the data. R.W.-M., O.P. and M.V.-C. designed the experiments. O.A.S., O.P. and M.V.-C. wrote the manuscript. All authors discussed the project and approved the final version of the manuscript.

synaptic dysfunction that is considered a major cellular mechanism underlying the cognitive deficits in patients with mild cognitive impairment (MCI) and early AD (Selkoe, 2002). Plaque formation is preceded by elevated levels of soluble, low-N oligomers of A $\beta$ , and evidence suggests it is these that are responsible for synaptic dysfunction (Lue et al., 1999; Hardy and Selkoe, 2002). Acute exposure to synthetic or naturally secreted A $\beta$  has been shown to impair hippocampal long-term potentiation (LTP) (Cullen et al., 1997; Lambert et al., 1998; Walsh et al., 2002; Wang et al., 2004; Townsend et al., 2006; Wei et al., 2010), a widely accepted cellular model of learning and memory (Bliss and Collingridge, 1993).

Tau protein may also play a crucial role in the cognitive decline in MCI and early AD. In the medial temporal lobe, the first area to be affected in AD, NFTs are elevated and their numbers in the hippocampal CA1 correlate with memory decline (Markesbery et al., 2006). Nevertheless, it has been suggested that tau intermediates are more important for the cognitive decline than the tangles themselves (Santacruz et al., 2005).

Thus, A $\beta$  and tau are both fundamental in the early stages of AD, but whether, and how, these two proteins interact is not yet established. Evidence for an important interaction includes the protection of tau-depleted neurons from A $\beta$ -induced neurodegeneration in culture (Rapoport et al., 2002). Moreover, the cognitive deficits that are seen in human amyloid precursor protein (hAPP)-overexpressing mice (Hsiao et al., 1996) are absent in hAPP-overexpressing mice with a genetic knockout of tau protein (Roberson et al., 2007). Therefore, to further investigate a possible interaction between A $\beta$  and tau and so help elucidate the mechanism underlying the cognitive decline in MCI and early AD, we asked whether tau is required for the A $\beta$ -mediated impairment of LTP in the hippocampus, one of the earliest regions affected in AD (Braak and Braak, 1991). For this, we used both wild-type mice and mice with a genetic knockout of tau protein (*Tau*<sup>-/-</sup> mice) (Dawson et al., 2001).

In order to investigate a possible molecular pathway for this interaction, we tested whether glycogen synthase kinase 3 (GSK-3) is involved in the A $\beta$ -induced impairment of LTP. GSK-3 is a serine/threonine kinase known to phosphorylate tau protein (Ishiguro et al., 1993), and to play a role in both synaptic plasticity (Hooper et al., 2007) and A $\beta$ -induced and tau-mediated neurodegeneration (Tackenberg and Brandt, 2009). If GSK-3 is involved in an interaction between A $\beta$  and tau, inhibition of GSK-3 could be a promising therapeutic strategy for MCI and early AD.

## Methods

### Mice

Animal care and experimental procedures were conducted in accordance with UK Home Office regulations under the Animals (Scientific Procedures) Act of 1986. Animals had access to food and water *ad libitum*. Holding facilities were maintained at a temperature of approximately 22 °C, humidity of 60-70%, and with a 12-hour light/dark cycle. We used 4 to 6 month old *Tau*<sup>-/-</sup> mice on a C57BL6-J background (Dawson et al., 2001) and age-matched C57BL6-J mice purchased from Charles River Laboratories, Margate, UK. All mice were housed in the same animal facility under the same conditions for at least 2 weeks before experiments commenced. Mice of both sexes were used for the rodent A $\beta$  experiment; for all other experiments only males were used.

### Slice preparation

Parasagittal hippocampal slices (400  $\mu$ m) were prepared after decapitation under deep isoflurane-induced anaesthesia. After dissection in ice cold artificial cerebrospinal fluid (ACSF) containing (mM): NaCl 126; KCl 3; NaH<sub>2</sub>PO<sub>4</sub> 1.25; MgSO<sub>4</sub> 2; CaCl<sub>2</sub> 2; NaHCO<sub>3</sub>

25; glucose 10; pH 7.2-7.4; bubbled with carbogen gas (95% O<sub>2</sub>, 5% CO<sub>2</sub>), slices were maintained at room temperature (22-25 °C) in a submerged-style holding chamber for at least one hour and then incubated in drug/control solutions. For recording, slices were transferred to an interface-style recording chamber maintained at 33-35 °C superfused with ACSF at a rate of 2 mL/min, and recording started at least 15 minutes after the slices were transferred.

## Pharmacology

Rodent A<sub>1-42</sub> (rA<sub>1-42</sub>) was dissolved in ACSF to a concentration of 10 μM and frozen in aliquots. Single aliquots were defrosted and sonicated for 11 minutes before final dilution to 500 nM for slice incubation. Human A<sub>1-42</sub> (hA<sub>1-42</sub>) was freshly prepared on the day of experiment. It was initially dissolved in ACSF to a concentration of 5 μM. Aliquots were then sonicated for 11 minutes before final dilution to 220 nM in ACSF.

Hippocampal slices were incubated in a submerged-style holding chamber in ACSF with or without A<sub>1-42</sub> or A<sub>42-1</sub> for one to three hours before recording. Perfusion with half concentration of the drug continued after slices were transferred to the interface chamber.

AR-A014418 was dissolved in DMSO at 100 mM concentration and stored in frozen aliquots. For slice incubation, single aliquots of AR-A014418 were defrosted and diluted to a final concentration of 1 μM in ACSF. Slices were incubated for 30-45 minutes in AR-A014418 before the addition of A<sub>1-42</sub> or control ACSF.

In some experiments, to block GABA<sub>A</sub> receptor-mediated inhibition, gabazine (SR 95531) was bath applied at 100 nM concentration to slices from which the CA3 had been removed.

hA<sub>1-42</sub>, hA<sub>42-1</sub>, rA<sub>1-42</sub> and gabazine were purchased from Tocris, Bristol, UK; AR-A014418 was obtained from Sigma Aldrich.

## Electrophysiological protocols

Extracellular field recordings from CA1 were made with an Axoclamp-2A amplifier in bridge mode and data acquired with an Instrutech ITC-16 A/D board (Instrutech Corp., Port Washington, NY) using Igor Pro software (WaveMetrics, Lake Oswego, OR, USA). Borosilicate glass recording electrodes were filled with ACSF. Recording and stimulation electrodes were positioned in the stratum radiatum of CA1. Synaptic efficacy was monitored by stimulating the Schaffer collaterals at 0.2 Hz (50 μs, 20-60 μA), with a monopolar tungsten electrode connected to a stimulus isolator unit (ISO-flex, A.M.P.I. Jerusalem). Stimulation strength was set to elicit a fEPSP of half-maximal amplitude. fEPSP slopes were monitored for a baseline period of at least 15 minutes. If synaptic transmission was stable (< 15% change in fEPSP slopes over 15 minutes), a single high-frequency stimulus train was delivered (100 Hz for 1 s). To measure paired-pulse ratio both before and after LTP induction, two 50 μs pulses with an inter-pulse interval of 40 ms were given at a low stimulation strength.

## Protein extraction and Western blot analysis

Slices were prepared as described above from wild-type and *Tau*<sup>-/-</sup> mice and incubated in ACSF with or without 220 nM hA<sub>1-42</sub> for two hours. A subset of slices were pre-incubated in 1 μM AR-A014418 for 30 minutes. The hippocampus was dissected from each slice and immediately frozen at -80 °C. Total protein was extracted by homogenising hippocampal slices in RIPA buffer (Sigma Aldrich, Poole, UK), supplemented with phosphatase inhibitors (PhosSTOP, Roche, Burgess Hill, UK) and protease inhibitors (Complete Mini Protease Inhibitor Cocktail, Roche), followed by brief microcentrifugation. Protein

concentrations were determined using the Bradford method. 2  $\mu$ g total protein was separated on 3-8% Tris Acetate polyacrylamide gels (Invitrogen, Paisley, UK) and transferred to polyvinylidene fluoride membrane. Membranes were blocked in phosphate buffered saline containing 0.1% Tween 20 and 10% milk and probed with primary antibody. Membranes were probed initially with an anti-phospho tau primary antibody (AT8; 1:1000 Thermo Scientific, Cramington, UK), before stripping in Restore buffer (Pierce) and reprobing with a primary antibody to detect total tau (Tau5; 1:1000; Fitzgerald, Acton, MA, US). Primary antibody binding was detected using an anti-mouse HRP-conjugated secondary antibody (Biorad; Hemel Hempstead, UK) and ECL Plus reagent (GE Healthcare; Chalfont St Giles, UK), apposed to photographic film (CL-Xposure film, Thermo Scientific). Films were digitized and optical densities determined using ImageJ (v1.43u; National Institutes of Health). In order to control for between-membrane variation, all samples from an individual mouse were run on a single gel, and optical densities were expressed as ratios of phospho/total tau normalized to the respective ACSF-only condition. Values presented are the mean of duplicate Western blots.

### Data analysis

Changes in synaptic efficacy were estimated using the mean fEPSP slopes (measured from the middle third of the rising slope of the fEPSP) 30 to 45 minutes after high-frequency stimulation normalized to the mean fEPSP slope during the last 5 minutes of baseline recording. Paired-pulse ratio was expressed as the mean ratio of the amplitude of the second fEPSP to the amplitude of the first fEPSP (average of five paired pulses). Data were analyzed using Igor Pro, Matlab and SPSS, and are given as mean  $\pm$  s.e.m. Statistical significance was assessed using the Student's *t*-test or one-way ANOVA followed by *post hoc* analysis with Bonferroni corrections when applicable. Unless otherwise stated, numbers (*N*) refer to the number of slices, obtained from at least six animals. Experiments in Figure 1 were performed blind such that the experimenter was not aware of genotype. In a subset (approximately half) of experiments in Figure 2, the experimenter was unaware of drug and genotype. As the average and variability of blind and non-blind experiments were similar, these data were pooled.

## Results

### LTP in A $\beta$ <sub>1-42</sub>-exposed slices from *Tau*<sup>-/-</sup> mice

First, we wanted to confirm that A<sub>1-42</sub> inhibits LTP in hippocampal slices from wild-type mice. We monitored fEPSPs evoked by extracellular stimulation of the Schaffer collateral pathway and induced LTP using high-frequency stimulation (100 Hz for 1 s). In slices pre-treated with 500 nM rodent A<sub>1-42</sub> (rA<sub>1-42</sub>) for 1-3 hrs, LTP was almost completely blocked (control: 153  $\pm$  12 %, *N* = 19; rA<sub>1-42</sub>: 112  $\pm$  11 %, *N* = 21, *t*-test *P* < 0.05; Fig. 1a,c). Similarly, human A<sub>1-42</sub> (hA<sub>1-42</sub>) also strongly impaired LTP (control: 137  $\pm$  5 %, *N* = 11; hA<sub>1-42</sub>: 115  $\pm$  5 %, *N* = 9, *t*-test *P* < 0.01; Fig. 2a,c), consistent with previous reports (Walsh et al., 2002; Wang et al., 2004; Townsend et al., 2006). This was a specific effect of hA<sub>1-42</sub> because slices pre-treated with a control peptide, containing the hA peptide sequence in reverse order (hA<sub>42-1</sub>), showed a synaptic potentiation (137  $\pm$  7%, *N* = 13; Fig. 2a,c) equivalent to that in control slices (*t*-test *P* = 0.49). These results demonstrate that, in wild-type mice, acute application of A<sub>1-42</sub> impairs one or more of the cellular mechanisms necessary for LTP.

To investigate a possible interaction between A and tau, we tested the effect of A<sub>1-42</sub> on LTP in *Tau*<sup>-/-</sup> mice (Dawson et al., 2001). Slices incubated in control solution showed normal levels of LTP and, remarkably, slices pre-incubated in rA<sub>1-42</sub> showed LTP of similar magnitude (control: 157  $\pm$  11 %, *N* = 11; rA<sub>1-42</sub>: 145  $\pm$  12 %, *N* = 15, *t*-test *P* =

0.51; Fig. 1b,c). Equivalent results were obtained using hA<sub>1-42</sub> (control: 138 ± 5%, *N* = 15; hA<sub>1-42</sub>: 138 ± 7%, *N* = 9, *t*-test *P* = 0.48; Fig. 2b,c). Univariate ANOVA revealed a main effect of treatment, as well as a genotype\*treatment interaction both for rodent and human A<sub>1-42</sub> (rA<sub>1-42</sub>  $F_{1,65} = 3.23$  *P* < 0.05; hA<sub>1-42</sub>  $F_{1,40} = 5.03$ , *P* < 0.05) with main effects of treatment, due to a significant effect of A<sub>1-42</sub> in wild-type slices.

To investigate whether this result could be due to different basic synaptic properties between wild-type and *Tau*<sup>-/-</sup> mice, we measured the synaptic input-output relationship and paired-pulse ratio (PPR). We found no difference in synaptic input-output relationships between wild-type and *Tau*<sup>-/-</sup> mice, indicating equivalent basal synaptic transmission (Supplementary Fig. 1a). Moreover, we observed no difference in the PPR (Supplementary Fig. 1b) between wild-type and *Tau*<sup>-/-</sup> mice [wild-type PPR before LTP, 1.67 ± 0.05; PPR after LTP, 1.68 ± 0.10, *N* = 12; *Tau*<sup>-/-</sup> PPR before LTP, 1.63 ± 0.11, PPR after LTP, 1.72 ± 0.06, *N* = 10. RM ANOVA revealed no effect of genotype (between-subjects factor  $F_{1,17} = 0.323$ ; *P* = 0.58) on PP value before or after LTP induction (within-subjects factor  $F_{1,17} = 0.55$ ; *P* = 0.47); Supplementary Fig. 1]. There was also no significant difference in post-tetanic potentiation (PTP) between wild-type and *Tau*<sup>-/-</sup> mice (wild-type PTP, 193 ± 9%, *N* = 28 mice; *Tau*<sup>-/-</sup> PTP, 211 ± 10%, *N* = 18 mice, *t*-test *P* = 0.18; Fig. 1a,b and 2a,b). To confirm that the results were not due to differences in GABAergic inhibition, experiments were repeated in the presence of 100 nM gabazine in slices with the CA3 removed, with equivalent results (Supplementary Fig. 2). Altogether, these results suggest that differences in basic synaptic properties are unlikely to account for the lack of A<sub>1-42</sub>-induced impairment of LTP in *Tau*<sup>-/-</sup> mice.

Given that NFTs comprising hyperphosphorylated tau are a prominent AD pathology alongside A<sub>1-42</sub> plaques, we asked whether the A<sub>1-42</sub>-induced impairment of LTP is associated with tau phosphorylation and requires activity of glycogen synthase kinase 3 (GSK-3), a serine/threonine kinase known to phosphorylate tau protein (Ishiguro et al., 1993). We used AR-A014418, which is a highly specific GSK-3 inhibitor that does not significantly inhibit closely related kinases such as cdk2 or cdk5 (Bhat et al., 2003). First, to test whether hA<sub>1-42</sub> increases tau phosphorylation and AR-A014418 inhibits tau phosphorylation under our experimental conditions, we used Western blot analysis of hippocampal tissue from slices incubated in ACSF with or without 220 nM hA<sub>1-42</sub>. In a subset of experiments, slices were pre-incubated in 1 μM AR-A014418 to inhibit GSK-3 activity. A significant effect of treatment on the ratio of phosphorylated to total tau was found (ANOVA  $F_{2,22} = 3.99$  *P* < 0.05; Fig. 3). Incubation with hA<sub>1-42</sub> significantly increased tau phosphorylation in hippocampal tissue compared to control slices (AT8/Tau5 ratio = 1.53 ± 0.26, *N* = 10, one-sample *t*-test *P* < 0.05), and the phosphorylation ratio in slices pre-incubated with AR-A014418 was significantly lower than those incubated with hA<sub>1-42</sub> alone (AT8/Tau5 ratio = 0.72 ± 0.14, *N* = 9, two-sample *t*-test *P* < 0.01). Slices exposed to 1 μM AR-A014418 alone showed equivalent tau phosphorylation to that observed in control conditions (AT8/Tau5 ratio = 0.96 ± 0.16, *N* = 4). Next we tested whether this concentration of AR-A014418 could block the hA<sub>1-42</sub>-induced impairment of LTP. When hippocampal slices from wild-type mice were pre-exposed to 1 μM AR-A014418 for 30 minutes prior to incubation with hA<sub>1-42</sub>, we observed LTP of a magnitude equivalent to that in control slices (control: 134 ± 6%, *N* = 9; hA<sub>1-42</sub>: 135 ± 5%, *N* = 9, *t*-test *P* = 0.58; Fig. 4a,b) and in slices exposed to AR-A014418 alone (134 ± 6%, *N* = 9, *t*-test *P* = 0.51 compared to control; Fig. 4a,b). This recovery of LTP was attributable to AR-A014418, since LTP was not observed in slices pre-exposed to vehicle (0.01% DMSO) for 30 minutes prior to incubation with hA<sub>1-42</sub> (Supplementary Fig. 3a,b), and vehicle alone did not affect LTP (Supplementary Fig. 3a,b). The finding that AR-A014418 completely blocked the hA<sub>1-42</sub>-induced augmented tau phosphorylation as well as impairment of LTP suggests that

activation of GSK-3 and its phosphorylation of tau are required for the effect of A<sub>1-42</sub> on synaptic plasticity.

## Discussion

Two principal findings emerge from this work. Firstly, in *Tau*<sup>-/-</sup> mice, neither the rodent nor human version of A<sub>1-42</sub> impaired hippocampal LTP, in contrast to the robust impairment of LTP they caused in slices from wild-type mice. Secondly, a specific inhibitor of the tau kinase GSK-3 prevented both the augmented tau phosphorylation and impairment of LTP that were otherwise seen following A<sub>1-42</sub> treatment in wild-type mice.

Since cognitive decline in early AD is associated with pathology in the medial temporal lobe (Braak and Braak, 1991), we investigated LTP at the hippocampal CA3-CA1 synapse, a widely accepted cellular model for learning and memory. Our confirmation that A<sub>1-42</sub> impaired LTP in slices from wild-type mice is in agreement with a wealth of previous reports (Cullen et al., 1997; Lambert et al., 1998; Chen et al., 2002; Walsh et al., 2002; Wang et al., 2004; Townsend et al., 2006) showing that soluble A oligomers have an inhibitory effect on LTP and synaptic function. It also suggests that, under our experimental conditions, A assembled into the oligomeric complexes thought to be the synaptotoxic form of the peptide. We show that the magnitude of LTP is not reduced in slices from *Tau*<sup>-/-</sup> mice exposed to either human or rodent A<sub>1-42</sub>. This suggests that tau protein is one element required for the synaptotoxic effects of A<sub>1-42</sub>, consistent with the finding that tau-depleted neurons do not degenerate in the presence of A (Rapoport et al., 2002). The similar synaptic input-output relationships and unchanged paired-pulse ratios with LTP in wild-type and *Tau*<sup>-/-</sup> mice that we observed suggests that this result is not caused by different presynaptic properties in *Tau*<sup>-/-</sup> mice, but that the absence of tau uncouples A from its downstream effects that impair the postsynaptic mechanisms required for LTP. In support of this postsynaptic interpretation, a dendritic role for tau in mediating A toxicity was recently reported (Ittner et al., 2010). Nevertheless, this does not exclude a contribution of presynaptic mechanisms, for example A-induced deficits in axonal transport are prevented in *Tau*<sup>-/-</sup> neurons (Vossel et al., 2010).

It is a priority to establish the molecular pathways that underlie the A<sub>1-42</sub>-induced impairment of LTP. Our finding that the specific GSK-3 inhibitor AR-A014418 can prevent impairment of LTP by hA<sub>1-42</sub> in wild-type slices suggests that GSK-3, via its subsequent phosphorylation of tau (Ishiguro et al., 1993), is a key component in the pathway by which A exerts its pathogenic downstream effects on LTP, similar to A-mediated neurodegeneration (Tackenberg and Brandt, 2009). In support of this, we demonstrate that inhibition of GSK-3 prevents the augmented tau phosphorylation seen following incubation in A alone. However, while our results show that A-induced impairment of LTP is associated with increased levels of tau phosphorylation, and that a specific inhibitor of GSK-3 blocks both increased phosphorylation and impairment of LTP, they do not provide conclusive evidence that phosphorylated tau protein mediates this impairment. The results also do not exclude the involvement of other kinases previously implicated in the A-induced impairment of LTP, including c-Jun N-terminal kinase, cdk5, p38 MAPK (Wang et al., 2004) and PKA (Vitolo et al., 2002). Nevertheless, the complete recovery of LTP magnitude in the presence of AR-A014418 is striking.

Previous studies have shown a correlation between behavior and synaptic plasticity, with both learning and LTP being impaired in the same animal model of AD (Chapman et al., 1999; Stéphan et al., 2001). Combined with the behavioral findings of Roberson et al. (2007), the present data are the first to link LTP and behavior through tau protein. This both strengthens the link between synaptic plasticity and memory, and suggests a mechanism

underlying the cognitive dysfunction in early AD. Nevertheless, our data were obtained with acute application of synthetic A $\beta$  peptides, whereas the lack of cognitive deficits was found in hAPP-overexpressing and tau knockout mice (Roberson et al., 2007); there may be subtle differences between chronic *in vivo* and acute *in vitro* exposure. For example, 1-2 days APP overexpression or A $\beta$  exposure can reduce spine density, whereas one hour exposure to A $\beta$  peptides alters the structural plasticity of individual spines (Wei et al., 2010).

Overall, the findings presented here indicate that the absence of tau prevents the synaptic dysfunction induced by A $\beta$ <sub>1-42</sub>, and support previous suggestions that tau acts downstream of A $\beta$  (Hardy and Selkoe, 2002), although parallel pathways remain possible (Small and Duff, 2008). These results, using acute exposure to soluble A $\beta$ <sub>1-42</sub>, may be relevant to early AD, when synaptic dysfunction is present but before extensive plaque formation has occurred. However, later in the disease process, the absence of functional tau protein may render the brain more vulnerable to A $\beta$  (Dawson et al., 2010). Nevertheless, the molecular cascades being uncovered suggest that GSK-3 might be a potential drug target for treating synaptic dysfunction, and hence improving cognitive function, during early AD.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

This work was supported by the Wellcome Trust and an equipment grant from the Alzheimer's Research Trust (funded by Doris Field CT). O.A.S. holds a Wellcome Trust OXION studentship. E.M.T is a Royal Society Research Fellow. F.D. held a Wellcome Trust Studentship in Neuroscience. R.W.-M. held a Wellcome Trust Research Career Development Fellowship. M.V.-C. holds a Wellcome Trust OXION Training Fellowship.

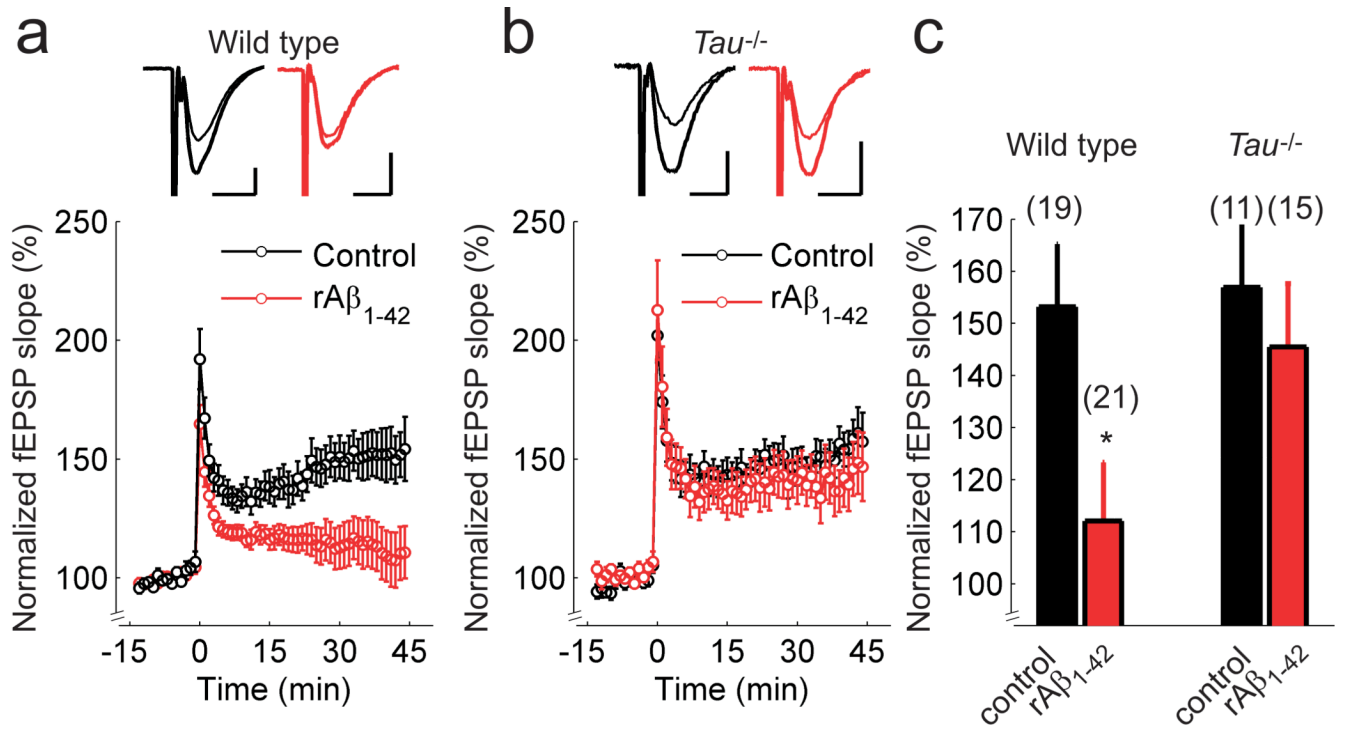
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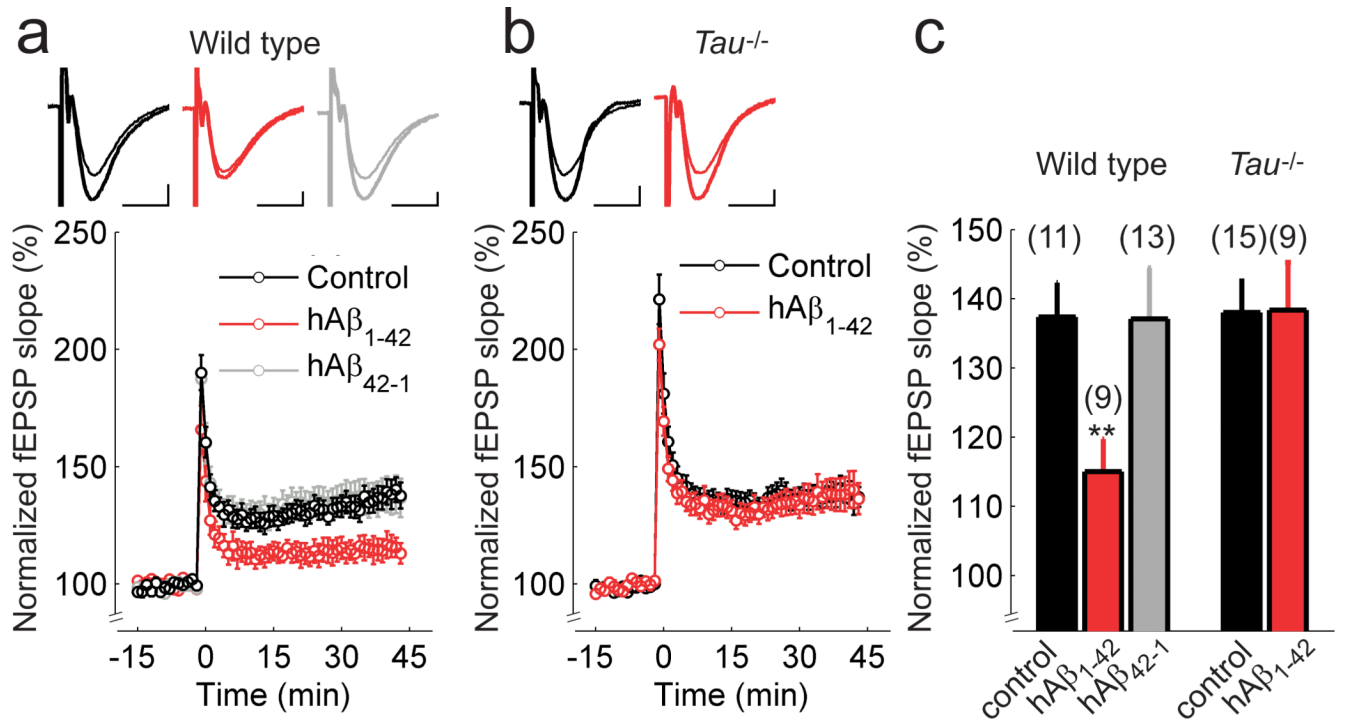


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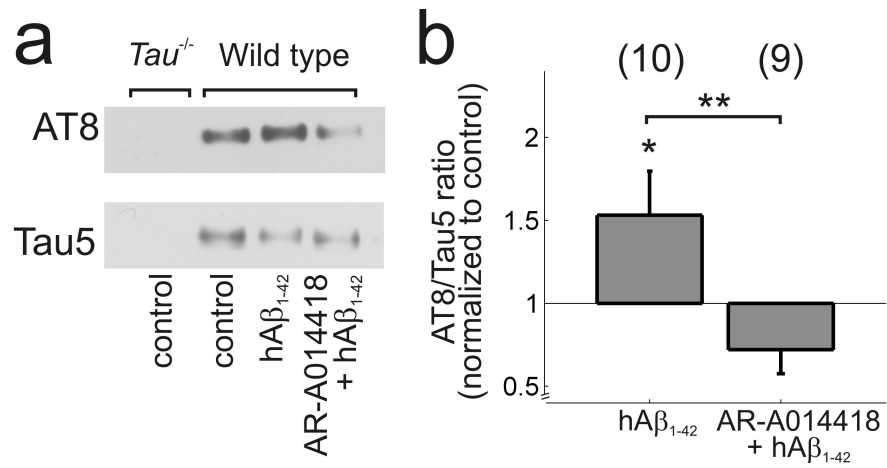
**Figure 1. Rodent A<sub>1-42</sub> does not reduce LTP in slices of *Tau*<sup>-/-</sup> mice**

(a, b) Hippocampal Schaffer collateral-CA1 LTP in wild-type (a) and *Tau*<sup>-/-</sup> mice (b) in control ACSF (black), or after incubation with rA<sub>1-42</sub> (red). The insets show superimposed example traces before and 40 min after high-frequency stimulation for each condition. Scale bars: 5 ms, 200 μV. (c) Summary of results 40-45 min after high-frequency stimulation. Error bars are s.e.m. \* *P* < 0.05. The numbers of slices are shown in parentheses.



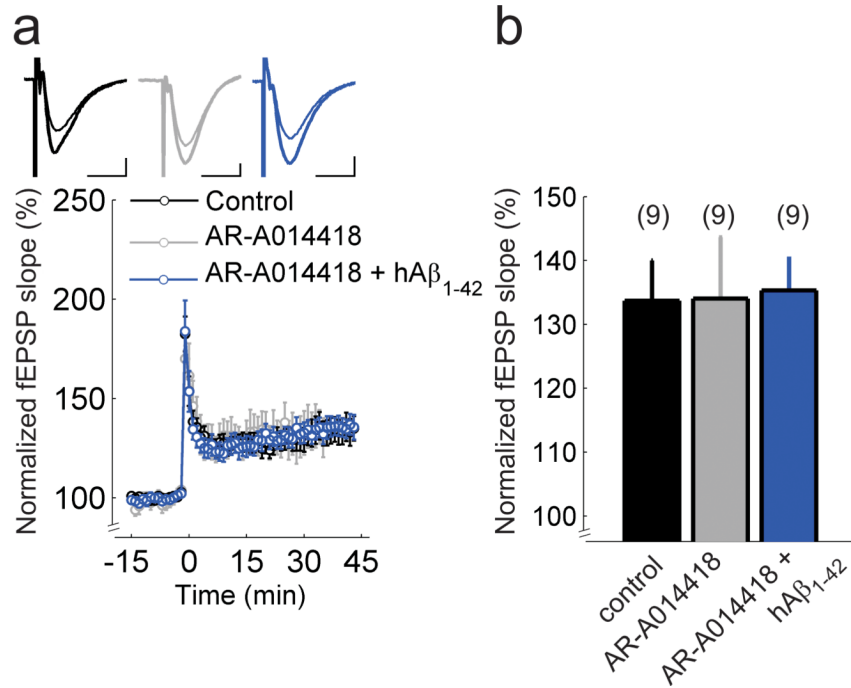
**Figure 2. Human A<sub>1-42</sub> does not reduce LTP in slices of *Tau*<sup>-/-</sup> mice**

(a, b) Hippocampal Schaffer collateral-CA1 LTP in wild-type (a) and *Tau*<sup>-/-</sup> mice (b) in control ACSF (black), or after incubation with hA<sub>1-42</sub> (red) or the control peptide hA<sub>42-1</sub> (gray). The insets show superimposed example traces before and 40 min after high-frequency stimulation for each condition. Scale bars: 5 ms, 200 μV. (c) Summary of results 40-45 min after high-frequency stimulation. Error bars are s.e.m. \*\*  $P < 0.01$ . The numbers of slices are shown in parentheses.



**Figure 3. A specific inhibitor of GSK-3 reduces phosphorylation of tau**

(a) Example Western blot showing immunoreactivity to Tau5 (total Tau) and AT8 (phosphorylated Tau). (b) Pooled data for AT8/Tau5 ratios normalized to immunoreactivity observed in control conditions in each mouse. \*  $P < 0.05$ ; \*\*  $P < 0.01$ . The numbers of mice are shown in parentheses.



**Figure 4. The specific GSK-3 inhibitor AR-A014418 prevents impairment of LTP by human A $\beta_{1-42}$  in wild-type slices**

(a) Hippocampal Schaffer collateral-CA1 LTP in wild-type mice in control ACSF (black), or after incubation with AR-A014418 alone (gray) or AR-A014418 followed by incubation with hA $\beta_{1-42}$  (blue). The insets show superimposed example traces before and 35 min after high-frequency stimulation for each condition. Scale bars: 5 ms, 200  $\mu$ V. (b) Summary of results 30-35 min after high-frequency stimulation. Error bars are s.e.m. The numbers of slices are shown in parentheses.