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ORIGINAL ARTICLE

Leptin prevents aberrant targeting of tau to hippocampal synapses via PI 3 kinase driven inhibition of GSK3β

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Abstract

Amyloid-β (Aβ) and hyper-phosphorylated tau are key hallmarks of Alzheimer's disease (AD), with an accumulation of both proteins linked to hippocampal synaptic dysfunction. Recent evidence indicates that Aβ drives mis-localisation of tau from axons to synapses, resulting in AMPA receptor (AMPAR) internalisation and impaired excitatory synaptic function. These tau-driven synaptic impairments are thought to underlie the cognitive deficits in AD. Consequently, limiting the synapto-toxic effects of tau may prevent AD-related cognitive deficits. Increasing evidence links leptin dysfunction with higher AD risk, and numerous studies have identified neuroprotective properties of leptin in AD models of Aβ-induced toxicity. However, it is unclear if leptin protects against tau-related synaptic dysfunction. Here we show that $A\beta_{1\text{-}42}$ significantly increases dendritic and synaptic levels of tau and p-tau in hippocampal neurons, and these effects were blocked by leptin. In accordance with GSK-3β being involved in tau phosphorylation, the protective effects of leptin involve PI 3-kinase (PI3K) activation and inhibition of GSK-3β. $A\beta_{1\text{-}42}$-driven synaptic targeting of tau was associated with the removal of GluA1-containing AMPARs from synapses, which was also inhibited by leptin-driven inhibition of GSK-3β. Direct application of oligomeric tau to hippocampal neurons caused internalisation of GluA1-containing AMPARs and this effect was blocked by prior application of leptin. Similarly, leptin prevented the ability of tau to block induction of activity-dependent long-term potentiation (LTP) at hippocampal SC-CA1 synapses. These findings increase our understanding of the neuroprotective actions of leptin in the early pre-clinical stages of AD and further validate the leptin system as a therapeutic target in AD.

KEYWORDS
Alzheimer's disease, amyloid, GSK3β, leptin, synapse, tau

Abbreviations: AD, Alzheimer's disease; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; Aβ, amyloid beta; ANOVA, analysis of variance; APP, amyloid precursor protein; DMSO, dimethyl sulphoxide; fEPSP, field excitatory postsynaptic potential; GSK-3β, glycogen synthase kinase-3 beta,; HEPES, (4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid); HFS, high-frequency stimulation; LepR, leptin receptor; LTD, long-term depression; LTP, long-term potentiation; MAP, microtubule associated protein; NFT, neurofibrillary tangles; NMDA, N-methyl-D-aspartate; PI3K, phosphoinositide 3-kinase; PFA, paraformaldehyde; p-tau, phosphorylated tau; RRID, research resource Identifier; SC, Schaffer collateral; SEM, standard error of the mean; TA, temporoammonic.

1 | INTRODUCTION

The hippocampus is a key brain area involved in learning and memory and is one of the first sites of neurodegeneration in Alzheimer's disease (AD). Poor diet and sedentary lifestyle are established risk factors in AD, and dysfunctions in metabolic hormonal systems, like leptin, are implicated in the development of AD (Cunnane et al., 2020; Stranahan & Mattson, 2011). Leptin is an adipose-derived hormone that controls energy expenditure by activating hypothalamic nuclei. However, leptin also possesses cognitive-enhancing properties, attributed to its hippocampal actions (Irving & Harvey, 2021). Thus, leptin enhances performance in hippocampal-dependent memory tasks (Doherty et al., 2022; Malekizadeh et al., 2017; Oomura et al., 2006), whereas leptin-insensitive animals (*fa/fa* rats, *db/db* mice) display impaired memory (Li et al., 2002). Additionally, leptin regulates the cellular events underlying learning and memory, as it enhances long-term potentiation (LTP; Shanley et al., 2001; Wayner et al., 2004), and alters excitatory synaptic strength at hippocampal Schaffer-collateral (SC)-CA1 and temporoammonic (TA)-CA1 synapses by regulating α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor (AMPAR) trafficking (Luo et al., 2015; McGregor et al., 2018; Moult et al., 2010). Moreover, leptin rapidly increases spine and synapse number as well as enhancing neurogenesis in hippocampal neurons (Dhar, Wayman, et al., 2014; Dhar, Zhu, et al., 2014; Garza et al., 2008; O'Malley et al., 2007).

Increasing evidence links altered leptin levels with AD, as AD risk is increased when leptin levels fall out-with the normal physiological range (Lieb et al., 2009; McGregor & Harvey, 2018). Circulating leptin levels are also lower than normal in AD patients and in rodent models of AD (Power et al., 2001; Bigalke et al., 2011). These findings have fuelled the possibility that the leptin system is a novel therapeutic target to treat AD.

Amyloid β (Aβ)-containing plaques and neurofibrillary tangles (NFTs) containing hyper-phosphorylated tau (p-tau) are pathological hallmarks of AD. Prior to plaque and tangle formation, soluble Aβ oligomers can induce synaptic dysfunction, that ultimately leads to synapse loss and neurodegeneration. Both Aβ and tau oligomers block induction of hippocampal LTP, resulting in impaired learning and memory (Fá et al., 2016; Lasagna-Reeves et al., 2012; Shankar et al., 2008; Walsh et al., 2002). Acute exposure to Aβ also promotes AMPAR internalisation and facilitates the induction of long-term depression (LTD; Shankar et al., 2008; Li et al., 2009), whereas chronic Aβ treatment leads to neuronal cell death (Martins et al., 2013). Recent evidence indicates that leptin protects against these aberrant effects of Aβ on synaptic function and neuronal viability (Doherty et al., 2013, 2022; Malekizadeh et al., 2017), as well as reducing Aβ production and burden (Fewlass et al., 2004; Marwarha et al., 2010).

The microtubule associated protein (MAP) tau, is a key mediator of Aβ-induced toxicity, as reductions in tau protein protect against memory impairments and neurodegeneration in AD models (Ittner et al., 2010; Leroy et al., 2012; Roberson et al., 2007). Tau is primarily located in distal axons where it regulates microtubule assembly and stabilisation (Drechsel et al., 1992), with limited tau migration to somato-dendritic regions (Ittner & Ittner, 2018). Aβ oligomers increase tau phosphorylation (Jin et al., 2011), by activating GSK-3β, the serine/threonine kinase that regulates tau phosphorylation (Jo et al., 2011; Wagner et al., 1996). This in turn causes aberrant targeting of p-tau to synapses, which drives internalisation of AMPARs and N-methyl-D-aspartate (NMDA) receptors (NMDARs; Hoover et al., 2010; Miller et al., 2014) and impairs hippocampal synaptic function. Although leptin reduces tau phosphorylation via phosphoinositide 3-kinase (PI3K)-dependent inhibition of glycogen synthase kinase-3 beta, (GSK-3β; Greco et al., 2009), it is unclear if leptin influences AD-related aberrant targeting of tau to synapses.

Here, we provide the first compelling evidence that leptin prevents $A\beta_{1\text{-}42}$-induced targeting of tau and p-tau to hippocampal synapses via a process involving PI3K-driven inhibition of GSK-3β. $A\beta_{1\text{-}42}$-driven synaptic targeting of tau was associated with the removal of GluA1-containing AMPARs from synapses, and this effect was also prevented by leptin-dependent inhibition of GSK-3β. In accordance with increased synaptic expression of tau causing synaptic impairments, direct application of tau caused a significant reduction in surface AMPAR expression in hippocampal neurons, which was blocked by leptin. Furthermore, prior treatment with leptin reversed tau inhibition of activity-dependent long-term potentiation (LTP) at hippocampal Schaffer collateral (SC)-CA1 synapses. These findings have important implications for the neuroprotective role of leptin in neurodegenerative disease.

2 | EXPERIMENTAL PROCEDURES

2.1 | Hippocampal culture preparation

Sprague Dawley rats (research resource Identifier (RRID): RGD5508397) were housed in enriched cages, with 2 cages attached via a plastic tube. Animals had ad libitum access to food and water and were maintained under a 12 h light/dark cycle and in an environmentally controlled room (22–23°C). Each cage contained a breeding pair of adult (3–8 months) rats (obtained from Charles River, UK), and the number of pups varied depending on the litter. A total of 28 male (450–510 g) and 28 female (250–310 g) parental rats were used for breeding and 212 rat pups (0–3 days; both sexes) were used for preparation of hippocampal cultures.

Primary hippocampal cultures were prepared as previously described (Clements & Harvey, 2020). Briefly, neonatal Sprague Dawley rats (0–3 days old) were killed by cervical dislocation in accordance with Schedule 1 of the UK Animals Scientific Procedures Act, 1986 (Home Office project licence: P9198AAB7). The brains of three to four neonates were pooled and used for the preparation of each neuronal culture. Hippocampi were removed and washed in (4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid) (HEPES)-buffered saline comprising (in mM) 135 NaCl (Cat. no. S9888, Sigma), 5 KCl (Cat. no. P3911, Sigma), 1 $CaCl_2$ (Cat. no. C1016, Sigma), 1 $MgCl_2$ (Cat. no. M8266; Sigma), 10 HEPES (Cat. no. H3375, Sigma), and 25 D-glucose (Cat. no. G8270, Sigma, pH 7.4). Hippocampi were treated

with papain (1.5 mg/mL, Cat. no. P4762, Sigma-Aldrich) for 20 min at 37°C and dissociated cells were plated onto sterile coverslips (35 mm diameter; Greiner Bio-One Ltd.) coated with poly-D-lysine (20 μg/mL; Cat. no. P7886, Sigma, 1–2 h). Cultures were maintained in Neurobasal-A medium (Cat. No. 21103049, Thermofisher Scientific) in a humidified atmosphere of 95% O_2 and 5% CO_2 for up to 3 weeks.

2.2 | Immunocytochemistry

Immunocytochemistry was carried out on primary hippocampal cultures between 7 and 15 days in vitro (DIV). Neurons were washed in HBS containing glycine (10 μM) and incubated under various conditions at room temperature for 1 h, or 20 min for exogenous oligomeric tau experiments. Control neurons in each experiment were treated with either HBS alone or HBS containing reverse inactive $A\beta_{42\text{-}1}$ (1 μM). Neurons were fixed with 4% paraformaldehyde (PFA) for 5 min and then permeabilised with 0.1% Triton X-100 for 5 min. Neurons were incubated with a rabbit anti-tau primary antibody (Cat. no. 13–6400, Genscript, 1:200) for 30 min at room temperature. Neurons were then treated with an anti-rabbit Alexa 555-conjugated secondary antibody (Cat. no. A78954, Invitrogen, 1:250) for 30 min in the dark to allow visualisation of tau-positive immunostaining. For experiments investigating the localisation of phosphorylated tau (p-tau), neurons were incubated with a rabbit anti-p-tau (Ser396) primary antibody (Cat. No. 9632, Cell Signalling Technology, 1:500) for 30 min at room temperature followed by 30 min incubation in the dark with an anti-rabbit Alexa 555-conjugated secondary antibody (Cat. no. A78954, Invitrogen, 1:500).

To investigate the synaptic localisation of tau or p-tau, neurons were labelled for PSD-95, a protein enriched at synapses. Following incubation with tau/p-tau primary and secondary antibodies, neurons were treated with a mouse anti-PSD-95 primary antibody (Cat. no. MA1-046, Thermo Fisher Scientific, 1:500) followed by incubation with an anti-mouse Alexa 488-conjugated secondary antibody (Cat. No. A-11001, Invitrogen, 1:500) to allow % co-localisation of tau/p-tau and PSD-95 to be determined.

To investigate the axonal localisation of p-tau, neurons were dual labelled for GAP-43, a protein enriched at axons, and endogenous p-tau. Following incubation with a mouse anti-p-tau (Ser396) primary antibody (Cat. No. 9632, Cell Signalling Technology, 1:500) and anti-mouse Alexa 488-conjugated secondary antibody (Cat. No. A-11001, Invitrogen, 1:500), neurons were treated with a rabbit anti-GAP-43 primary antibody (Chemicon International, 1:500) followed by incubation with an anti-rabbit Alexa 555-conjugated secondary antibody (Cat. no. A78954, Invitrogen, 1:500) to allow % co-localisation of p-tau and GAP-43 to be determined.

For experiments investigating expression of tau and p-tau at dendrites, neurons were dual labelled with a rabbit anti-tau primary antibody (Cat. no. 13-6400, Genscript, 1:200) and a mouse anti-p-tau (Ser396) primary antibody (Cat. No. 9632, Cell Signalling Technology, 1:500). Neurons were then labelled with an anti-rabbit Alexa 555-conjugated secondary antibody (Cat. no. A78954, Invitrogen, 1:250) and anti-mouse Alexa 488-conjugated secondary antibody (Cat. No. A-11001, Invitrogen, 1:500), respectively.

For experiments investigating the surface GluA1 expression, live neurons were incubated with a primary antibody against the N-terminal region of the GluA1 subunit of the AMPA receptor (1:100) for 30 min at 4°C. The GluA1 primary is an in-house sheep antibody against the synthetic peptide (RTSDSRDHTRVDWKR) corresponding to 253–267 residues of GluA1 (Moult et al., 2010). Neurons were then fixed with 4% PFA (Cat. No. J19943.K2, Thermofisher) and incubated with a donkey anti-sheep Alexa 488-conjugated secondary antibody (Cat. No. A-11015. Invitrogen, 1:250) for 30 min in the dark.

2.3 | Microscopy

Image acquisition was carried out using either confocal (Zeiss LSM 510; Leica SP5) or fluorescence microscopes (DeltaVision Elite). For confocal imaging, 488- and 543-nm laser lines were used to excite the Alexa 488 and 555 fluorophores, respectively. Images were obtained in a single-tracking mode or multi-tracking mode for dual labelling experiments using a 15-s scan speed. For DeltaVision imaging, images were acquired using a 40×/1.40 NA U Plan S Apochromat objective at 1×1 binning and a CoolSNAP HQ or HQ2 camera (Photometrics). Intensity of staining in all images was determined offline using Lasersharp software (Carl Zeiss). Analysis lines (50 μm) were drawn along randomly selected dendritic regions and mean fluorescence intensity was calculated for each dendrite (Clements & Harvey, 2020; McGregor et al., 2018). For synaptic co-localisation experiments, tau or p-tau immunolabelling was compared with dendritic PSD-95 labelling. The total number of synapses was determined by counting the number of PSD-95 puncta within a 50 μm section of dendrite. The number of tau or p-tau puncta that co-localised with PSD-95-positive sites were counted and expressed as a percentage of the total number of PSD-95-positive sites (Clements et al., 2023; Clements & Harvey, 2020). A similar mechanism of co-localisation analysis was performed for experiments involving p-tau and GAP-43 immunolabelling.

2.4 | Hippocampal slice preparation and electrophysiology

10 Sprague Dawley rats (P12-24; 5 male: 5 female) were used for electrophysiological experiments. Parasagittal hippocampal slices (350 μm) were prepared from Sprague–Dawley rats as before (Clements & Harvey, 2020). Animals were killed by cervical dislocation in accordance with Schedule 1 of the UK Animals (Scientific Procedures) Act 1986 (Home Office project licence: P9198AAB7). Brains were rapidly dissected and removed before submerging in ice-cold artificial CSF (aCSF; bubbled with 95% O_2 and 5% CO_2) containing the following (in mM): 124 NaCl (Cat. no. S9888, Sigma UK), 3 KCl (Cat. No. P3911, Sigma UK), 26 $NaHCO_3$ (Cat. No. S6014, Sigma UK), 1.25 NaH_2PO_4 (Cat. No. S8282, Sigma UK), 2 $CaCl_2$ (Cat. No. C1016,

Sigma UK), 1 $MgSO_4$ (Cat. No. M7506, Sigma UK), and 10 D-glucose (Cat. no. G8270, Sigma UK). Once prepared, slices were allowed to recover at room temperature in oxygenated aCSF for at least 60 min before use.

Standard extracellular recordings were used to monitor evoked field excitatory postsynaptic potentials (fEPSPs) from stratum radiatum. Parasagittal slices were submerged in a chamber perfused with aCSF at 2.5 mL/min at room temperature. Recording pipettes (3–5 MΩ) containing aCSF were placed into the stratum radiatum to record the Schaffer collateral (SC) input to CA1 synapses. The SC-CA1 pathway was stimulated at 0.033 Hz using a bipolar stimulating electrode. The slope of the fEPSP was measured and expressed relative to the pre-conditioning baseline. The maximum peak amplitude was determined, and baseline was recorded at a stimulus intensity that evoked peak amplitude at 50% of the maximum. Activity-dependent LTP was induced by delivery of high frequency stimulation (HFS; 100 Hz for 1 s). Synaptic field potentials were low-pass filtered at 2 kHz and digitally sampled at 10 kHz. Data were monitored online and analysed offline using the WinLTP 2.20 programme (RRID:SCR 008590; Anderson and Collingridge, 2007). Excitatory postsynaptic potential slope was measured, and baseline recordings were only considered stable when the slope values did not differ more than 15% for at least a 20 min period. The operator was not blinded to recording of the data.

2.5 | Data and statistical analysis

For immunocytochemical and electrophysiology studies, all statistical analysis was carried out using Sigmaplot 14.0/14.5 and SPSS Process V28. All data were assessed for normality using Shapiro–Wilk tests. No analysis for outliers was used and all statistical tests were two-tailed and used $p<0.05$ significance threshold. No statistical methods were used to pre-determine sample size.

In immunocytochemical studies, repeat experiments were carried out on at least 3 different cultures, as previous published studies indicate that a minimum of three separate cultures are required for immunocytochemical experiments (Alexander et al., 2018). Data were obtained from at least 3 dendrites from a minimum of 4 randomly selected neurons for each treatment. Within a given experiment, all conditions, including illumination intensity, exposure, and photomultiplier gains, were kept constant. Within each experiment, data were normalised relative to mean fluorescence intensity in control neurons to allow comparison across multiple experiments. All data are expressed as mean ± SEM, and statistical analyses were performed using one-way ANOVA followed by Tukey's post hoc test for comparisons between multiple groups. The *n* value represents the number of analysed dendrites across all experiments.

In electrophysiological studies, excitatory postsynaptic potential slope was measured offline using the WinLTP 2.20 programme (Anderson and Collingridge, 2007) and expressed as a percentage relative to baseline. All data are represented as mean ± SEM. The magnitude of LTP was determined 50–60 min after HFS. Comparisons between baseline and LTP within an experiment were made using repeated-measures ANOVA. Comparisons of magnitude of LTP between different experiments were made using Student's paired *t*-test or one-way ANOVA with Tukey's post hoc test for comparisons between 2 groups and multiple groups, respectively. *The n* value represents an individual slice taken from separate animals, and the number of animals used for each experiment was determined following previously published studies which analysed similar parameters (Clements & Harvey, 2020; Malekizadeh et al., 2017). All electrophysiological experiments were carried out with $n \geq 5$ for each pooled dataset, indicating that studies are performed on at least 5 slices.

3 | RESULTS

3.1 | Leptin prevents amyloid β induced mis-localisation of tau to dendrites and synapses

Previous studies indicate that Aβ promotes translocation of tau from axons to dendritic spines, where it disrupts excitatory synaptic transmission (Miller et al., 2014). Thus, to mirror tau targeting to synapses, cultured hippocampal neurons were treated with $A\beta_{1\text{-}42}$ (1 μM; 1 h) and dendritic levels of tau monitored using immunocytochemistry. Application of $A\beta_{1\text{-}42}$ significantly increased dendritic tau immunostaining (to 1.60 ± 0.08 of control (HBS); $n=36$ dendrites; $n=3$ cultures; $F_{[2105]}=19.9$; $p<0.001$; Figure 1a,b). In contrast, treatment with the inactive reverse $A\beta_{42\text{-}1}$ (1 μM; 1 h) had no significant effect on dendritic tau levels (1.23 ± 0.05; $n=36$ dendrites; $n=3$ cultures; $F_{[2105]}=19.9$; $p=0.06$; Figure 1a,b).

Numerous studies have identified neuroprotective effects of leptin (Russo et al., 2004; Guo et al., 2008; Davis et al., 2014), with leptin preventing the aberrant actions of Aβ (Doherty et al., 2013;

FIGURE 1 Leptin prevents $A\beta_{1\text{-}42}$-induced mis-localisation of tau to dendrites and synapses. (a) Representative images of endogenous tau labelling in culture hippocampal neurons (7–12 DIV; scale bar = 10 μm). In this and subsequent figures, the white boxes indicate magnified dendritic regions with the corresponding dendrite depicted below. (b) Pooled data showing $A\beta_{1\text{-}42}$ treatment increased dendritic tau labelling relative to control, whereas treatment with inactive reverse $A\beta_{42\text{-}1}$ had no significant effect. (c) Representative images of endogenous tau and PSD-95 labelling in cultured hippocampal neurons (7–13 DIV; scale bar = 10 μm). (d) Pooled data showing $A\beta_{1\text{-}42}$ treatment increased dendritic tau intensity relative to control and leptin prevented this effect. (e) Pooled data showing leptin treatment prevented $A\beta_{1\text{-}42}$-induced increase in co-localisation of tau and PSD-95. (f) Pooled data of relative PSD-95 intensity across all treatments. Data in (a) and (b) represent mean ± SEM of 36 dendrites from 3 separate cultures, whereas (c–f) are mean ± SEM of 48 dendrites from 4 separate cultures. In this and subsequent figures, *, **, and *** represent $p<0.05$; $p<0.01$; $p<0.001$, respectively as determined by one-way ANOVA and Tukey's post hoc analysis.

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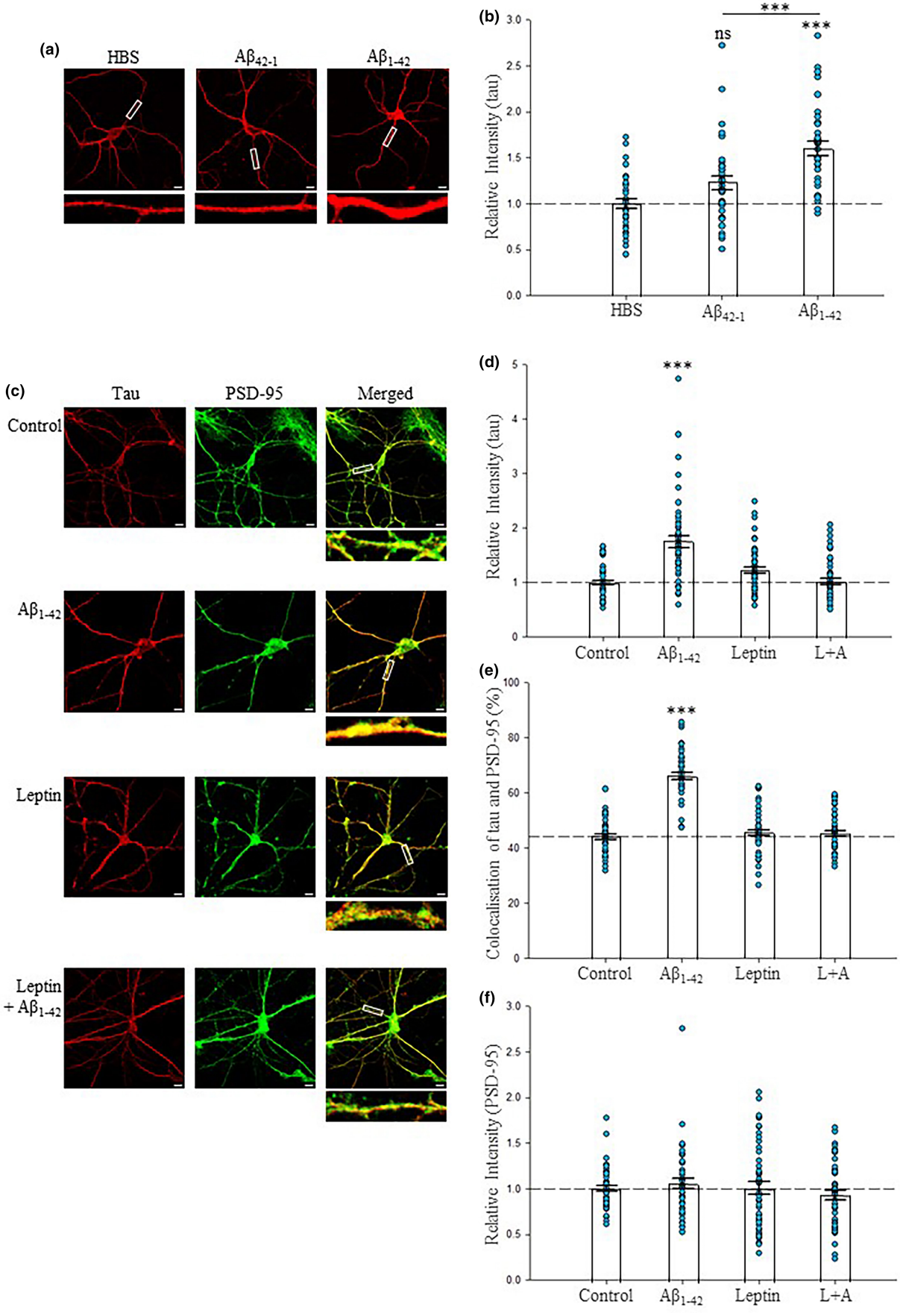


Martins et al., 2013). To examine if leptin influences $A\beta_{1\text{-}42}$-driven translocation of tau to dendrites, cultured hippocampal neurons were treated with leptin in the presence of $A\beta_{1\text{-}42}$. Treatment with leptin alone (10 nM; 1 h) had no effect on dendritic tau immunolabelling per se (1.23 ± 0.06; $n = 48$ dendrites; $n = 4$ cultures; $F_{[3188]} = 22.0$; $p = 0.14$; Figure 1c,d). However, leptin prevented $A\beta_{1\text{-}42}$-induced mis-localisation of tau such that dendritic tau labelling in neurons treated with leptin plus $A\beta_{1\text{-}42}$ (1.02 ± 0.06; $n = 48$ dendrites; $n = 4$ cultures; $F_{[3188]} = 22.0$; $p = 1.00$; Figure 1c,d) was not significantly different from control. These data indicate that leptin prevents aberrant targeting of tau to dendrites.

Previous studies indicate that Aβ also promotes targeting of tau to hippocampal synapses (Miller et al., 2014). Thus, to determine if $A\beta_{1\text{-}42}$ influences the synaptic expression of tau, dual labelling experiments were performed and tau localisation was compared to the excitatory synaptic marker, PSD-95. In control neurons the degree of co-localisation between tau and PSD-95 was 44.1 ± 1.0% ($n = 48$ dendrites; $n = 4$ cultures; Figure 1c,e), indicating that ca. 44% of synapses contained tau. Application of $A\beta_{1\text{-}42}$ significantly increased synaptic tau levels as the % co-localisation of tau and PSD-95 increased to 66.2 ± 1.4% ($n = 48$ dendrites; $n = 4$ cultures; $F_{[3188]} = 87.4$; $p < 0.001$; Figure 1c,e) compared to control. Leptin had no effect on synaptic tau levels per se (45.6 ± 1.1% co-localisation; $n = 48$ dendrites; $n = 4$ cultures; $F_{[3188]} = 87.4$; $p = 0.77$; Figure 1c,e); however, leptin prevented $A\beta_{1\text{-}42}$-induced targeting of tau to synapses, as the % co-localisation between tau and PSD-95 was not significantly different from control in leptin plus $A\beta_{1\text{-}42}$ treated neurons (45.2 ± 1.1% co-localisation; $n = 48$ dendrites; $n = 4$ cultures; $F_{[3188]} = 87.4$; $p = 0.89$; Figure 1c,e). The increase in dendritic and synaptic tau levels induced by $A\beta_{1\text{-}42}$ likely occurs prior to synapse loss, as PSD-95 immunolabelling was not significantly different between treatments ($n = 48$ dendrites; $n = 4$ cultures; $F_{[3188]} = 0.96$; $p = 0.41$; Figure 1c,f). In agreement with previous studies (Miller et al., 2014), these data indicate that $A\beta_{1\text{-}42}$ drives movement of tau to hippocampal synapses. Moreover, we find that the ability of $A\beta_{1\text{-}42}$ to drive tau into hippocampal synapses is inhibited by leptin.

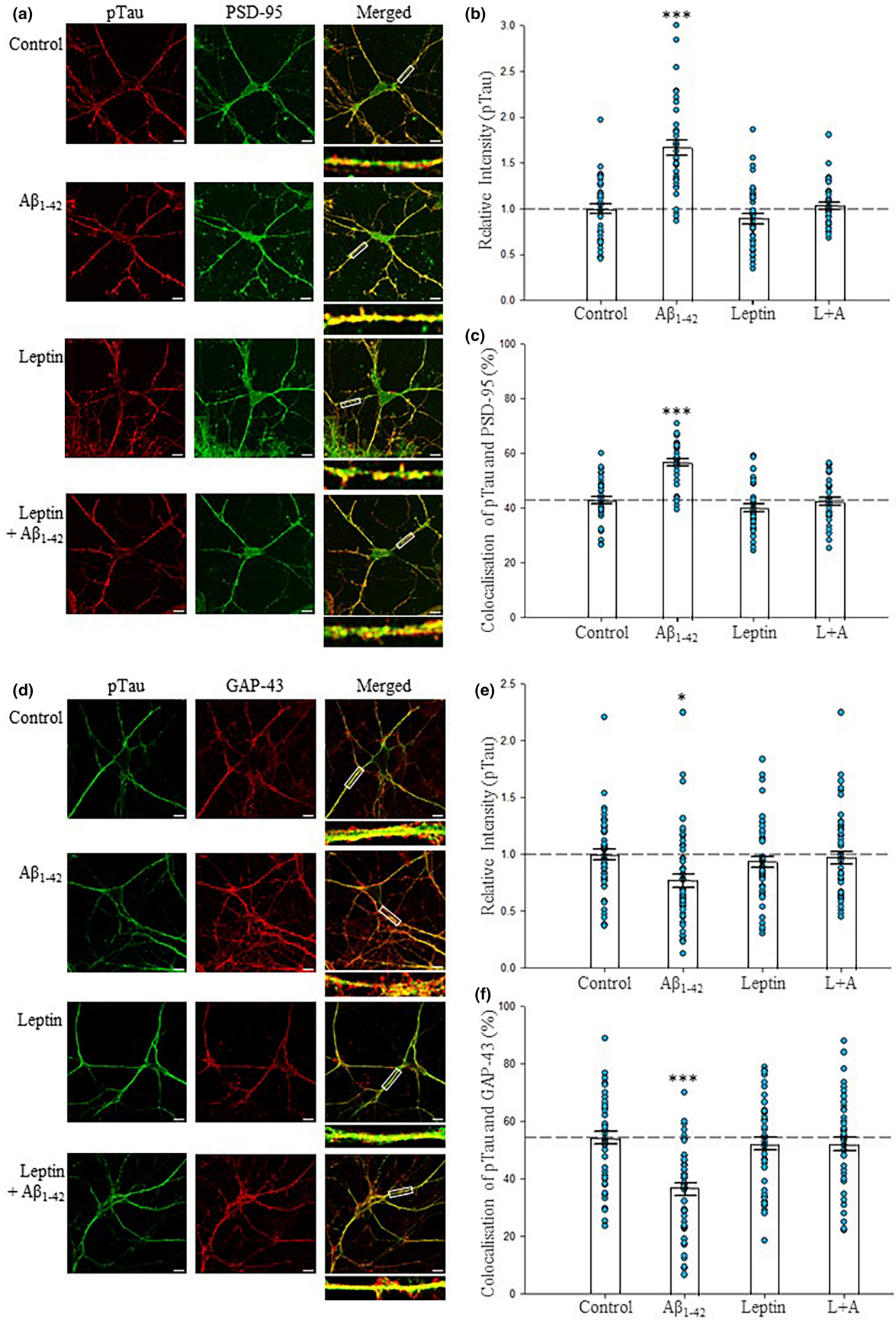
3.2 | Leptin prevents Aβ-driven mis-localisation of p-tau to dendrites and synapses

Phosphorylation of tau is a significant pathology in AD, but it is also a key process involved in tau-related synapto-toxicity, as synaptic targeting of tau is dependent on phosphorylation of tau (Hoover et al., 2010; Miller et al., 2014). In line with these studies, $A\beta_{1\text{-}42}$ treatment significantly increased dendritic p-tau immunolabelling relative to control (1.67 ± 0.09; $n = 36$ dendrites; $n = 3$ cultures; $F_{[3140]} = 32.2$; $p < 0.001$; Figure 2a,b). Leptin treatment alone had no effect on dendritic p-tau labelling per se (0.89 ± 0.06; $n = 36$ dendrites; $n = 3$ cultures; $F_{[3140]} = 32.2$; $p = 0.59$; Figure 2a,b); however, leptin prevented the effects of $A\beta_{1\text{-}42}$ such that dendritic p-tau labelling was not significantly different from control, in neurons treated with leptin and $A\beta_{1\text{-}42}$ (1.03 ± 0.04; $n = 36$ dendrites; $n = 3$ cultures; $F_{[3140]} = 32.2$; $p = 0.99$; Figure 2a,b).

To verify that $A\beta_{1\text{-}42}$ also increased the synaptic expression of p-tau, dual labelling approaches were used to compare the % co-localisation of p-tau and PSD-95. In control neurons, the degree of co-localisation between p-tau and PSD-95 was 42.9 ± 1.4% ($n = 36$ dendrites; $n = 3$ cultures), indicating that ca. 43% of excitatory synapses contained p-tau. Treatment with $A\beta_{1\text{-}42}$ significantly increased % co-localisation (to 56.7 ± 1.4%; $n = 36$ dendrites; $n = 3$ cultures; $F_{[3140]} = 28.7$; $p < 0.001$; Figure 2a,c), indicating increased synaptic levels of p-tau. Treatment with leptin had no effect on % co-localisation per se (40.1 ± 1.5%; $n = 36$ dendrites; $n = 3$ cultures; $F_{[3140]} = 28.7$; $p = 0.49$; Figure 2a,c). However, leptin prevented the aberrant effects of $A\beta_{1\text{-}42}$ as the % co-localisation between p-tau and PSD-95 was not significantly different from control after leptin addition (42.4 ± 1.4%; $n = 36$ dendrites; $n = 3$ cultures; $F_{[3140]} = 28.7$; $p = 0.99$; Figure 2a,c). Targeting of p-tau to synapses likely occurs prior to synapse loss as PSD-95-positive labelling ($n = 36$ dendrites; $n = 3$ cultures; $F_{[3140]} = 0.90$; $p = 0.44$; data not shown) and the average number of synapses per dendrite ($n = 36$ dendrites; $n = 3$ cultures; $F_{[3140]} = 0.69$; $p = 0.56$; data not shown) did not differ significantly between treatments. These data indicate that $A\beta_{1\text{-}42}$ promotes targeting of p-tau to synapses, and this effect is prevented by leptin.

To determine if an increase in dendritic p-tau in response to $A\beta_{1\text{-}42}$ was concordant with movement of tau from axons, neurons were dual labelled with p-tau and GAP-43, an axonal marker. $A\beta_{1\text{-}42}$ treatment significantly decreased both the relative intensity of p-tau in axons (0.77 ± 0.06; $n = 48$ dendrites; $n = 4$ cultures; $F_{[3188]} = 3.7$; $p < 0.05$; Figure 2d,e) and the % co-localisation of p-tau and GAP-43 (Control = 54.3 ± 2.3%; $A\beta_{1\text{-}42} = 36.5 \pm 2.2\%$; $n = 48$ dendrites; $n = 4$ cultures; $F_{[3188]} = 13.8$; $p < 0.001$; Figure 2d,f). Leptin treatment alone had no significant effect on relative intensity of axonal p-tau (0.93 ± 0.05; $n = 48$ dendrites; $n = 4$ cultures; $F_{[3188]} = 3.7$; $p = 0.81$; Figure 2d,e) or % co-localisation of p-tau and GAP-43 (52.4 ± 2.2%; $n = 48$ dendrites; $n = 4$ cultures; $F_{[3188]} = 13.8$; $p = 0.92$; Figure 2d,f). However, when applied in combination, leptin

FIGURE 2 Leptin prevents $A\beta_{1\text{-}42}$-induced targeting of phosphorylated tau to dendrites and synapses. (a) Representative images of endogenous p-tau and PSD-95 labelling in cultured hippocampal neurons (7–14 DIV; scale bar = 10 μm). (b) Pooled data showing $A\beta_{1\text{-}42}$ treatment increased dendritic p-tau levels and that leptin treatment prevented this effect. (c) Pooled data of % co-localisation of p-tau and PSD-95. Treatment with leptin prevented $A\beta_{1\text{-}42}$-induced increase in synaptic p-tau levels. (d) Representative images of p-tau labelling in cultured hippocampal neurons (8–11 DIV; scale bar = 10 μM). (e) Pooled data showing that $A\beta_{1\text{-}42}$ treatment decreased axonal p-tau levels and this effect was inhibited by leptin. (f) Pooled data of % co-localisation of p-tau and the axonal marker, GAP-43. Treatment with leptin prevented $A\beta_{1\text{-}42}$-induced decrease in axonal p-tau levels. Data in (a–c) represent mean ± SEM of 36 dendrites from 3 separate cultures, whereas (d–f) are mean ± SEM of 48 dendrites from 4 separate cultures.



prevented the $A\beta_{1\text{-}42}$-driven decrease in relative intensity of axonal p-tau (0.97 ± 0.06; $n = 48$ dendrites; $n = 4$ cultures; $F_{[3188]} = 3.7$; $p = 0.98$; Figure 2d,e) and % co-localisation of p-tau and GAP-43 (52.2 ± 2.3%; $n = 48$ dendrites; $n = 4$ cultures; $F_{[3188]} = 13.8$; $p = 0.91$; Figure 2d,f). Relative intensity of GAP-43 did not vary significantly between treatments ($n = 48$ dendrites; $n = 4$ cultures; $F_{[3188]} = 0.07$; $p = 0.98$; data not shown), indicating that $A\beta_{1\text{-}42}$ treatment did not induce axonal degeneration. Together, these data indicate that $A\beta_{1\text{-}42}$ treatment promotes aberrant movement of p-tau from axons into synapses in hippocampal neurons. Leptin prevented this movement of tau, resulting in p-tau remaining in the axon, thereby exhibiting neuroprotective properties in this cellular model of tau dysfunction.

3.3 | Mis-localisation of p-tau underlies the $A\beta_{1\text{-}42}$-induced increase in total dendritic tau

To determine if the $A\beta_{1\text{-}42}$ -driven increase in dendritic tau expression is due to movement of p-tau, dual labelling was used to simultaneously compare total tau and p-tau (Ser396) in hippocampal neurons. As before, $A\beta_{1\text{-}42}$ treatment significantly increased total dendritic tau labelling compared to control (1.37 ± 0.06; $n = 60$ dendrites; $n = 5$ cultures; $F_{[3236]} = 19.1$; $p < 0.001$; Figure 3a,b), and this effect was accompanied by a significant increase in dendritic p-tau labelling (1.25 ± 0.05; $n = 60$ dendrites; $n = 5$ cultures; $F_{[3236]} = 20.3$; $p < 0.001$; Figure 3a,b); accounting for ca. 68% of the increase in total dendritic tau observed within the same neurons. Treatment with leptin prevented the $A\beta_{1\text{-}42}$-driven increase in both dendritic tau and p-tau levels, such dendritic tau labelling (0.98 ± 0.03; $n = 60$ dendrites; $n = 5$ cultures; $F_{[3236]} = 19.1$; $p = 0.99$; Figure 3a,b) was not significantly lower than control, and p-tau labelling (0.84 ± 0.04; $n = 60$ dendrites; $n = 5$ cultures; $F_{[3236]} = 20.3$; $p = 0.03$; Figure 3a,b), was significantly reduced after leptin treatment.

3.4 | Leptin prevents Aβ-induced mis-localisation of tau and p-tau via PI3K activation

PI 3 kinase (PI3K) is a key leptin receptor signalling cascade implicated in the neuro-protective effects of leptin, including its ability to prevent Aβ-induced internalisation of AMPARs (Doherty et al., 2013). To determine if PI3K contributes to leptin-mediated neuroprotection here, two chemically distinct inhibitors of PI3K were used, namely, wortmannin and LY294002. In accordance with our previous findings, $A\beta_{1\text{-}42}$ treatment significantly increased dendritic tau labelling compared to control (1.84 ± 0.12; $n = 36$ dendrites; $n = 3$ cultures; $F_{[6245]} = 19.0$; $p < 0.001$; Figure 3c,d), and leptin prevented this effect (1.18 ± 0.07; $n = 36$ dendrites; $n = 3$ cultures; $F_{[6245]} = 19.0$; $p = 0.73$; Figure 3c,d). However, LY294002 (10 μM; 1 h) blocked the protective effects of leptin as an increase in dendritic tau was observed in the combined presence of leptin, $A\beta_{1\text{-}42}$ and LY294002 (1.75 ± 0.09; $n = 36$ dendrites; $n = 3$ cultures; $F_{[6245]} = 19.0$; $p < 0.001$; Figure 3c,d). Similarly, treatment with wortmannin (50 nM; 1 h) inhibited the effects of leptin such that a significant increase in dendritic tau was observed in neurons treated with leptin, $A\beta_{1\text{-}42}$ and wortmannin (1.38 ± 0.06; $n = 36$ dendrites; $n = 3$ cultures; $F_{[6245]} = 12.1$; $p < 0.001$; not shown). Inhibition of PI3K also blocked the protective effects of leptin on p-tau targeting to synapses, such that in wortmannin treated neurons, leptin failed to prevent the increase in dendritic p-tau immunolabelling induced by $A\beta_{1\text{-}42}$ (1.50 ± 0.06; $n = 36$ dendrites; $n = 3$ cultures; $F_{[6245]} = 30.0$; $p < 0.001$; Figure 3d,e). These data indicate that PI3K activation underlies the ability of leptin to prevent $A\beta_{1\text{-}42}$-driven targeting of tau and p-tau to dendrites.

3.5 | The protective effects of leptin involve inhibition of GSK-3β

GSK-3β is an important kinase that phosphorylates tau, and Aβ enhances GSK-3β activity (Jo et al., 2011; Martins et al., 2013) resulting in hyperphosphorylation and subsequent trafficking of tau to synapses (Hoover et al., 2010; Miller et al., 2014). Leptin driven inhibition of GSK-3β contributes to the protective effects of leptin, including its ability to reduce tau phosphorylation (Greco et al., 2009). To determine if GSK-3β plays a role in this cellular model, two distinct inhibitors of GSK-3β were utilised, namely, SB216763 (a non-specific inhibitor of GSK-3) and TCS2002 (a selective GSK-3β inhibitor). Application of either TCS2002 ($n = 36$ dendrites; $n = 3$ cultures; $F_{[6245]} = 25.4$; $p = 0.43$; Figure 4a,b) or SB216763 ($n = 36$ dendrites; $n = 3$ cultures; $F_{[6245]} = 18.4$; $p = 1.00$; Figure 4d,e) alone had no effect on dendritic tau labelling per se. As before, $A\beta_{1\text{-}42}$ treatment significantly increased dendritic tau labelling compared to control (1.76 ± 0.09; $n = 36$ dendrites; $n = 3$ cultures; $F_{[6245]} = 25.4$; $p < 0.001$; Figure 4a,b). However, in the presence of TCS2002 (100 nM; 1 h), $A\beta_{1\text{-}42}$ had no effect as dendritic tau labelling was not significantly different from control (0.98 ± 0.06; $n = 36$ dendrites; $n = 3$ cultures; $F_{[6245]} = 25.4$; $p = 1.00$; Figure 4a,b), suggesting that inhibition of GSK-3β mimics the protective effects

FIGURE 3 Leptin prevents dendritic targeting of tau and p-tau via PI 3 kinase activation. (a) Representative images of endogenous total tau (red), and p-tau (green) labelling in cultured hippocampal neurons (10–15 DIV; scale bar = 10 μm). (b) Pooled data showing relative changes in tau (blue) and p-tau (red) in control conditions, and after $A\beta_{1\text{-}42}$, leptin and leptin plus $A\beta_{1\text{-}42}$. The increase in dendritic p-tau underlies most of the increase in total tau in response to $A\beta_{1\text{-}42}$. Data represent mean ± SEM of 60 dendrites from 5 separate cultures. (c) Representative images of endogenous tau labelling in cultured hippocampal neurons (7–15 DIV; scale bar = 10 μm). (d) Pooled data of dendritic tau labelling. Inhibition of PI 3 kinase with LY294002 blocks leptin's ability to reduce tau targeting to dendrites. (e) Pooled data of dendritic p-tau labelling. Blockade of PI 3 kinase with wortmannin inhibits the ability of leptin to reduce targeting of p-tau to dendrites. All data represent mean ± SEM of 36 dendrites from 3 separate cultures.

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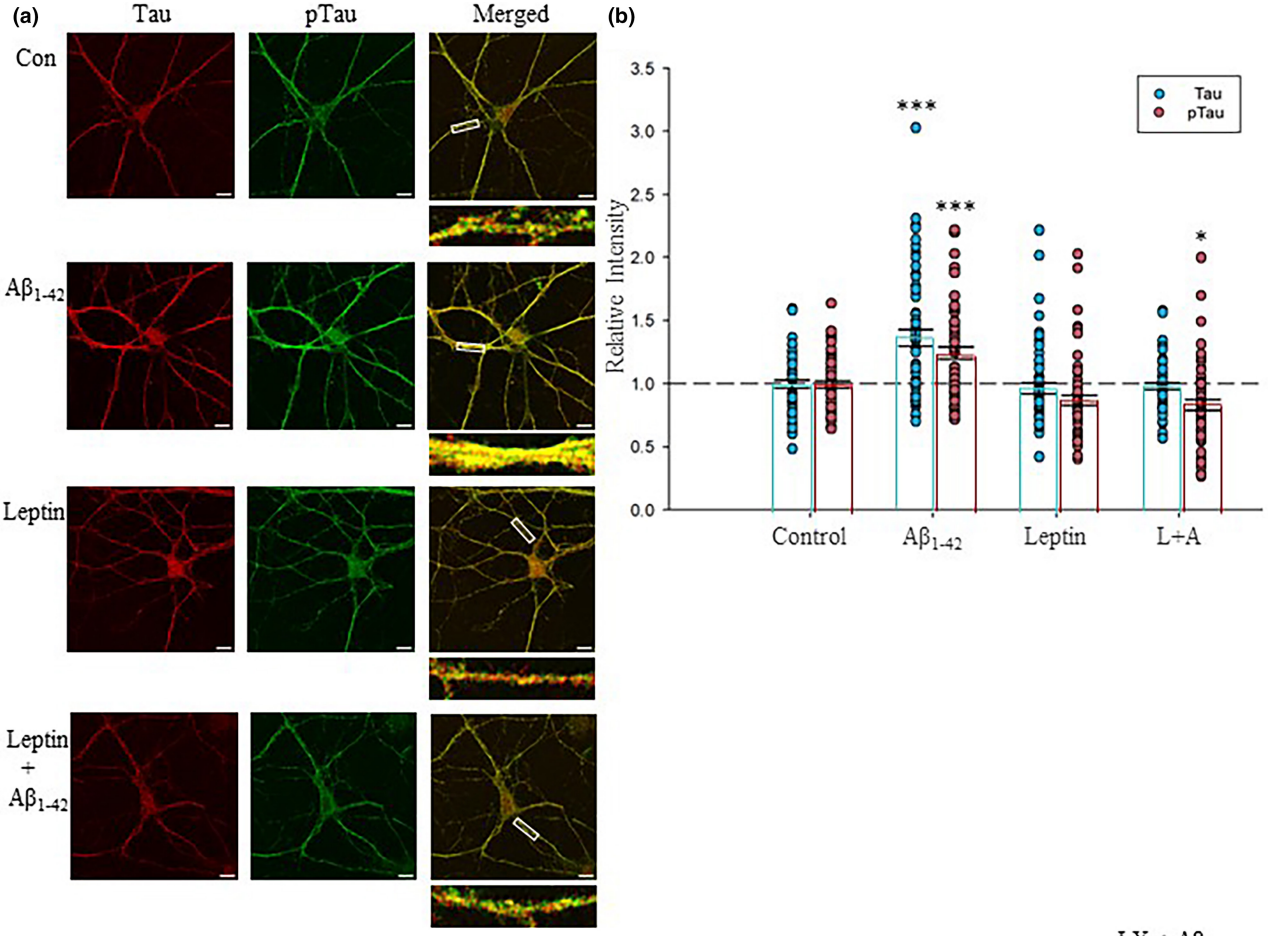

of leptin. TCS2002 also occluded the effects of leptin such that dendritic tau labelling in neurons treated with leptin, $A\beta_{1\text{-}42}$ and TCS2002 was not significantly different from control (0.99 ± 0.06; $n = 36$ dendrites; $n = 3$ cultures; $F_{[6245]} = 25.4$; $p = 1.00$; Figure 4a,b). In a similar manner, treatment with the non-selective GSK-3 inhibitor, SB216763 blocked the effects of $A\beta_{1\text{-}42}$ as dendritic tau immunolabelling was not significantly different from control, in the presence of SB216763 and $A\beta_{1\text{-}42}$ (1.09 ± 0.06; $n = 36$ dendrites; $n = 3$ cultures; $F_{[6245]} = 18.4$; $p = 0.93$; Figure 4d,e). Blockade of GSK3 also occluded the effects of leptin, as dendritic tau immunolabelling was not significantly different from control in neurons treated with SB216763, leptin, and $A\beta_{1\text{-}42}$ (0.98 ± 0.04; $n = 36$ dendrites; $n = 3$ cultures; $F_{[6245]} = 18.4$; $p = 1.00$; Figure 4d,e). Inhibition of GSK3β also mimicked and occluded the ability of leptin to prevent $A\beta_{1\text{-}42}$-driven increase in p-tau as dendritic p-tau labelling was not significantly different to control, in neurons treated with either $A\beta_{1\text{-}42}$ and TCS2002 (0.96 ± 0.04; $n = 48$ dendrites; $n = 4$ cultures; $F_{[6329]} = 32.7$; $p = 0.99$; Figure 4a,c) or a combination of leptin, $A\beta_{1\text{-}42}$ and TCS2002 (0.97 ± 0.04; $n = 48$ dendrites; $n = 4$ cultures; $F_{[6329]} = 32.7$; $p = 1.00$; Figure 4a,c). Together these data indicate that leptin prevents $A\beta_{1\text{-}42}$-driven trafficking of tau and p-tau to dendrites via a process involving inhibition of GSK-3β.

3.6 | Leptin prevents $A\beta_{1\text{-}42}$-driven AMPAR removal from synapses via PI3K-driven inhibition of GSK3β

Previous studies have shown that movement of tau to synapses results in AMPAR removal from hippocampal synapses (Miller et al., 2014; Teravskis et al., 2021). To verify if $A\beta_{1\text{-}42}$ promotes removal of synaptic AMPARs, immunocytochemical approaches were used to monitor the cell surface expression of the AMPA receptor subunit, GluA1 (Doherty et al., 2022; Moult et al., 2010). Incubation with $A\beta_{1\text{-}42}$, (1 μM, 1 h) had no effect on GluA1 surface expression per se as surface GluA1 immunolabelling was not significantly different to control (0.90 ± 0.02; $n = 48$ dendrites; $n = 4$ cultures; $F_{[7376]} = 13.4$; $p = 0.14$; Figure 5a,b). To assess whether $A\beta_{1\text{-}42}$ influences the synaptic localisation of GluA1, we compared surface GluA1 relative to the synaptic marker PSD-95. After treatment with $A\beta_{1\text{-}42}$, synaptic GluA1 expression was significantly decreased (to 46.70 ± 0.97% co-localisation; $n = 48$ dendrites; $n = 4$ cultures; $F_{[7376]} = 14.0$; $p < 0.001$, $n = 48$; Figure 5a,c) relative to control (53.15 ± 1.04% co-localisation; $n = 48$ dendrites; $n = 4$ cultures; Figure 5a,c), consistent with $A\beta_{1\text{-}42}$ promoting removal of GluA1-containing AMPA receptors from synapses. No significant changes in PSD-95 labelling were observed following treatment with $A\beta_{1\text{-}42}$ (0.90 ± 0.03; $n = 48$ dendrites; $n = 4$ cultures; $F_{[7376]} = 3.3$; $p = 0.47$; data not shown), indicating that the removal of GluA1-containing AMPA receptors from synapses occurs prior to synapse loss.

We have shown previously that leptin prevents AMPA receptor internalisation after acute (20 min) exposure to $A\beta_{1\text{-}42}$ (Doherty et al 2010; Malekizadeh et al., 2017). As chronic $A\beta_{1\text{-}42}$ treatment also leads to synaptic removal of AMPA receptors, we examined if leptin protects against this aberrant effect of $A\beta_{1\text{-}42}$. Treatment with $A\beta_{1\text{-}42}$ caused a significant decrease in % co-localisation of GluA1 and PSD-95 (to 46.47 ± 0.76%; $n = 60$ dendrites; $n = 5$ cultures; $F_{[6413]} = 17.5$; $p < 0.001$; Figure 5d), compared to control (52.51 ± 0.97%; $n = 60$ dendrites; $n = 5$ cultures; Figure 5d), indicating synaptic removal of GluA1. Application of leptin alone had no effect on synaptic GluA1 expression compared to control (54.42 ± 1.03% co-localisation; $n = 60$ dendrites; $n = 5$ cultures; $F_{[6413]} = 17.5$; $p = 0.77$; Figure 5d). However, in leptin-treated neurons, the % co-localisation of GluA1 and PSD-95 was not significantly different from control, after chronic $A\beta_{1\text{-}42}$ exposure (53.61 ± 0.69% co-localisation; $n = 60$ dendrites; $n = 5$ cultures; $F_{[6413]} = 17.5$; $p = 0.98$; Figure 5d), indicating that leptin inhibits its $A\beta_{1\text{-}42}$-driven synaptic removal of GluA1-containing AMPARs. Surface GluA1 labelling did not vary significantly between treatments ($n = 60$ dendrites; $n = 5$ cultures; $F_{[6413]} = 1.8$; $p = 0.10$; data not shown) and the average number of synapses was not significantly different from control in any treatment group ($n = 60$ dendrites; $n = 5$ cultures; $F_{[6413]} = 3.9$; $p = 0.55$; data not shown), indicating that $A\beta_{1\text{-}42}$-driven removal of GluA1-containing AMPARs occurs specifically at synapses and prior to loss of synapses.

As PI3K signalling underlies leptin's ability to prevent the acute effects of $A\beta_{1\text{-}42}$ on AMPAR trafficking (Doherty et al., 2013; Malekizadeh et al., 2017), we determined if similar neuroprotective signalling pathways were involved in this study, using the PI3K inhibitor, wortmannin. Treatment with wortmannin alone had no effect on synaptic GluA1 expression (53.15 ± 0.85% co-localisation; $n = 60$ dendrites; $n = 5$ cultures; $F_{[6413]} = 17.5$; $p = 1.00$; Figure 5d). However, in wortmannin treated neurons, leptin failed to prevent the effects of $A\beta_{1\text{-}42}$ as a significant decrease in synaptic GluA1 expression was observed (to 46.48 ± 0.98% co-localisation; $n = 60$ dendrites; $n = 5$ cultures; $F_{[6413]} = 17.5$; $p < 0.001$; Figure 5d) compared to control. These data indicate that PI3K signalling is required for leptin to limit synaptic removal of GluA1-containing AMPARs after chronic treatment with $A\beta_{1\text{-}42}$.

FIGURE 4 Inhibition of GSK-3β underlies the effects of leptin. (a) Representative images of endogenous tau (top) and p-tau (bottom) labelling in cultured hippocampal neurons (7–8 DIV; scale bar = 10 μm). (b, c) Pooled data showing effects of TCS2002 treatment (GSK-3β inhibitor) on dendritic tau (b) or p-tau (c) labelling. TCS2002 treatment mimics and occludes the protective effects of leptin. (d) Representative images of endogenous tau in cultured hippocampal neurons (7–11 DIV; scale bar = 10 μM). (e) Pooled data showing the relative effects on dendritic tau labelling. SB216763 treatment mimics and occludes the protective effects of leptin. Data in (b) and (c) represent mean ± SEM of 36 dendrites from 3 separate cultures, whereas (e) represents mean ± SEM of 48 dendrites from 4 separate cultures.

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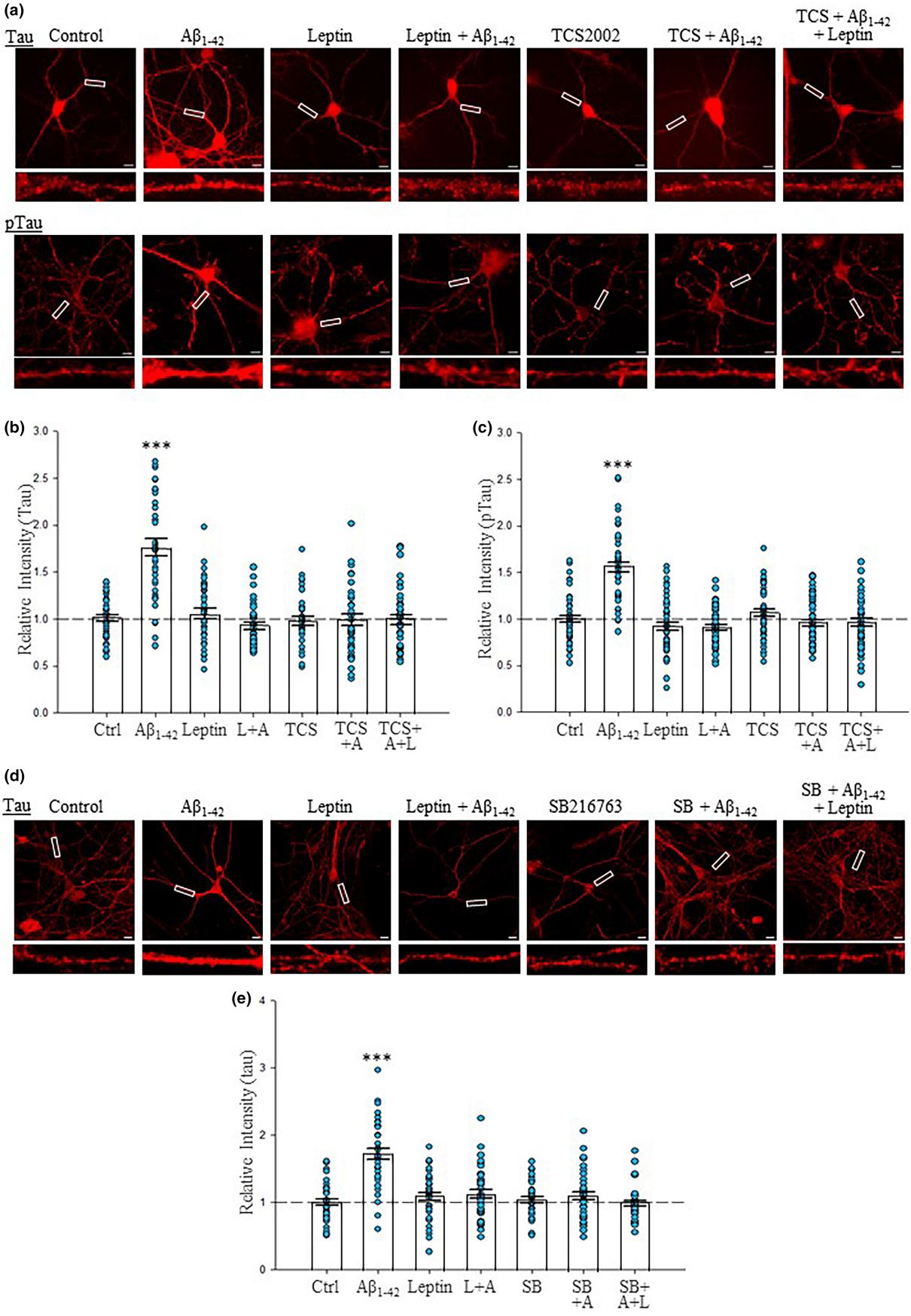

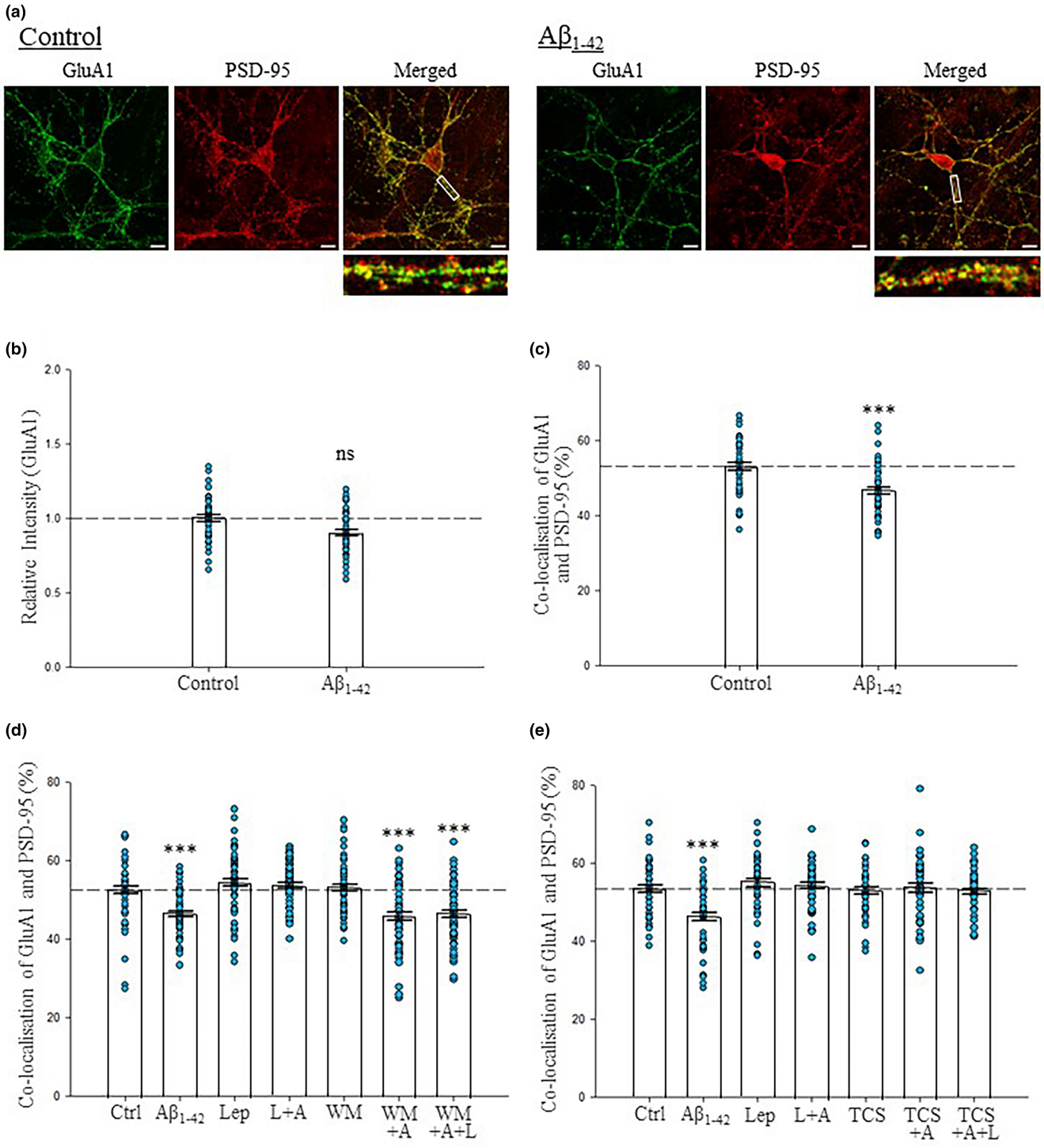

FIGURE 5 Leptin prevents $A\beta_{1\text{-}42}$-induced removal of GluA1-containing AMPARs from synapses via PI3K activation and inhibition of GSK-3β. (a) Representative images of surface GluA1 (green) and PSD-95 (red) labelling in cultured hippocampal neurons (9-14DIV; scale bar = 10 μm). (b) Pooled data showing $A\beta_{1\text{-}42}$ treatment for 60 min had no significant effect on surface GluA1 labelling. (c) Pooled data showing $A\beta_{1\text{-}42}$ treatment significantly decreased % co-localisation of surface GluA1 and PSD-95 compared to control. (d) Pooled data of % co-localisation of surface GluA1 and PSD-95. Treatment with wortmannin inhibited the ability of leptin to prevent $A\beta_{1\text{-}42}$-induced synaptic removal of GluA1. (e) Pooled data showing % co-localisation of surface GluA1 and PSD-95. Inhibition of GSK-3β with TCS2002 mimics and occludes the protective effects of leptin. Data in b, c, and e are mean ± SEM of 48 dendrites from 4 separate cultures, whereas D represents mean ± SEM of 60 dendrites from 5 separate cultures.

As inhibition of GSK3β occurs downstream of PI3K, the role of GSK3β was also examined, using the GSK3β inhibitor, TCS2002. As before, chronic application of $A\beta_{1\text{-}42}$ significantly decreased % co-localisation of GluA1 and PSD-95 (to 46.25 ± 1.12%; $n = 48$ dendrites; $n = 4$ cultures; $F_{[6329]} = 8.9$; $p < 0.001$; Figure 5e) compared to control (53.52 ± 0.95%; $n = 48$ dendrites; $n = 4$ cultures;

Figure 5e), and leptin prevented $A\beta_{1\text{-}42}$-driven removal of GluA1 from synapses as % co-localisation of GluA1 and PSD-95 was not significantly different from control (54.31 ± 0.89% co-localisation; $n = 48$ dendrites; $n = 4$ cultures; $F_{[6329]} = 8.9$; $p = 1.00$; Figure 5e). TCS2002 treatment alone had no effect on synaptic GluA1 expression (53.08 ± 0.93% co-localisation; $n = 48$ dendrites; $n = 4$ cultures; $F_{[6329]} = 8.9$; $p = 1.00$; Figure 5e). However, TCS2002 mimicked the effects of leptin, as % co-localisation of GluA1 and PSD-95 was not significantly different from control, in TCS2002 plus $A\beta_{1\text{-}42}$ treated neurons (53.77 ± 1.15% co-localisation; $n = 48$ dendrites; $n = 4$ cultures; $F_{[6329]} = 8.9$; $p = 1.00$; Figure 5e). Additionally, TCS2002 occluded the effects of leptin as % co-localisation of GluA1 and PSD-95 was not significantly different from control, in neurons treated with TCS2002, $A\beta_{1\text{-}42}$ and leptin (52.85 ± 0.70% co-localisation; $n = 48$ dendrites; $n = 4$ cultures; $F_{[6329]} = 8.9$; $p = 1.00$; Figure 5e). As inhibition of GSK-3β mirrors and occludes the effects of leptin, these data indicate that PI3K-driven inhibition of GSK3β underlies the ability of leptin to prevent $A\beta_{1\text{-}42}$-driven AMPAR removal from synapses.

3.7 | Leptin prevents tau-driven reduction in surface GluA1 expression, and block of hippocampal LTP

In accordance with previous studies (Hoover et al., 2010; Miller et al., 2014), our data suggest that increased synaptic levels of tau promote AMPAR removal from synapses. As oligomeric tau markedly impairs hippocampal synaptic function (Fá et al., 2016; Hu et al., 2018; Lasagna-Reeves et al., 2012; Ondrejcak et al., 2018; Puzzo et al., 2017), we examined if leptin protects against the direct effects of tau on AMPAR trafficking in hippocampal neurons. Application of tau (1 nM, 20 min) caused a significant decrease in surface GluA1 labelling compared to control (0.48 ± 0.02 of control; $n = 36$ dendrites; $n = 3$ cultures; $F_{[3140]} = 55.2$; $p < 0.001$; Figure 6a,b). Treatment with leptin alone (10 nM, 20 min) had no effect on surface GluA1 expression per se (0.98 ± 0.04 of control; $n = 36$ dendrites; $n = 3$ cultures; $F_{[3140]} = 55.2$; $p = 0.98$; Figure 6a,b). However, in leptin-treated neurons the ability of tau to decrease the surface GluA1 expression was inhibited as surface GluA1 labelling was not significantly different from control (0.97 ± 0.03; $n = 36$ dendrites; $n = 3$ cultures; $F_{[3140]} = 55.2$; $p = 0.94$; Figure 6a,b). These data indicate leptin prevents the ability of tau to internalise GluA1-containing AMPARs.

Recent studies indicate that tau oligomers also inhibit induction of hippocampal LTP leading to deficits in learning and memory (Fá et al., 2016; Lasagna-Reeves et al., 2012; Ondrejcak et al., 2018; Puzzo et al., 2017). Consequently, we examined if leptin influenced the effects of tau on activity dependent LTP at SC-CA1 synapses in juvenile hippocampal slices. In control slices, delivery of a HFS (100 Hz, 1 s) paradigm readily induced LTP as a significant increase in synaptic transmission was observed, which was maintained at least 1 h after HFS (133.4 ± 1.4% of baseline; $n = 5$ slices; $F_{[1,4]} = 96.6$; $p < 0.001$; Figure 6c,d). Application of 10 nM tau (20 min) had no effect on basal synaptic transmission (102.0 ± 0.6% of baseline; $n = 5$ slices; $F_{[2,8]} = 2.8$; $p = 0.12$; Figure 6c,d). However, addition of tau prior to HFS, blocked the induction of LTP as synaptic transmission was not significantly different from control (106.2 ± 1.5% of baseline; $n = 5$ slices; $F_{[2,8]} = 2.78$; $p = 0.12$; Figure 6c,d), and the magnitude of synaptic transmission 1 h after HFS, was significantly lower in tau-treated slices compared to control LTP ($n = 5$ slices; $F_{[2,12]} = 182.9$; $p < 0.001$; Figure 6c,d). These data indicate that acute treatment with tau blocks the induction of hippocampal LTP at SC-CA1 synapses.

We then examined if leptin protects against the aberrant effects of tau on hippocampal synaptic plasticity. In control slices, HFS resulted in a significant increase in synaptic transmission (to 137.2 ± 1.1% of baseline; $n = 5$ slices; $F_{[1,4]} = 345.2$; $p < 0.001$; Figure 6e,f). Leptin (25 nM) was applied to slices 20 min prior to application of tau (10 nM), which was applied 20 min before HFS. Application of either leptin alone (101.5 ± 1.0% of baseline; $n = 5$ slices; $F_{[3,12]} = 32.3$; $p = 1.00$) or leptin plus tau (104.4 ± 1.1% of baseline; $n = 5$ slices; $F_{[3,12]} = 32.3$; $p = 0.84$) had no effect on basal synaptic transmission (Figure 6e,f). However, leptin attenuated the tau-driven block of LTP, as HFS resulted in a persistent increase in synaptic transmission (136.7 ± 2.1% of baseline; $n = 5$ slices; $F_{[3,12]} = 32.3$; $p < 0.001$; Figure 6e,f) in leptin-treated slices. The magnitude of LTP in the combined presence of leptin and tau was not significantly different to that observed in control slices ($n = 5$ slices; $F_{[3,16]} = 193.3$; $p = 0.99$; Figure 6e,f). Together these data indicate that leptin prevents tau block of hippocampal LTP at juvenile SC-CA1 synapses.

4 | DISCUSSION

Increasing evidence implicates the hormone, leptin, in a wide range of CNS functions out-with the hypothalamus, where it regulates energy balance (Spiegelman & Flier, 2001). Leptin receptors are highly expressed in the hippocampus and numerous studies indicate that leptin has pro-cognitive properties, via its ability to influence hippocampal excitatory synaptic efficacy, AMPAR trafficking and dendritic morphology (Moult et al., 2010; O'Malley et al., 2007; Shanley et al., 2001). However, impairments in the leptin system are associated with AD pathogenesis, such that non-physiological circulating levels of leptin are linked to a higher risk of AD (Bigalke et al., 2011; Bonda et al., 2013; Lieb et al., 2009). This has fuelled interest in the leptin system as a potential novel therapeutic target for AD. In support of this, we have shown that leptin protects against the synapto-toxic effects of Aβ, by preventing Aβ-driven inhibition of hippocampal synaptic plasticity and internalisation of AMPARs (Doherty et al., 2013; Malekizadeh et al., 2017). Here, we extend these findings to provide the first compelling evidence that leptin prevents the aberrant effects of tau on hippocampal synaptic function. Specifically, we show that leptin prevents $A\beta_{1\text{-}42}$-driven targeting of tau and p-tau from hippocampal axons to synapses, via a process involving PI3K driven inhibition of GSK3β. Aβ-induced synaptic targeting of tau leads to

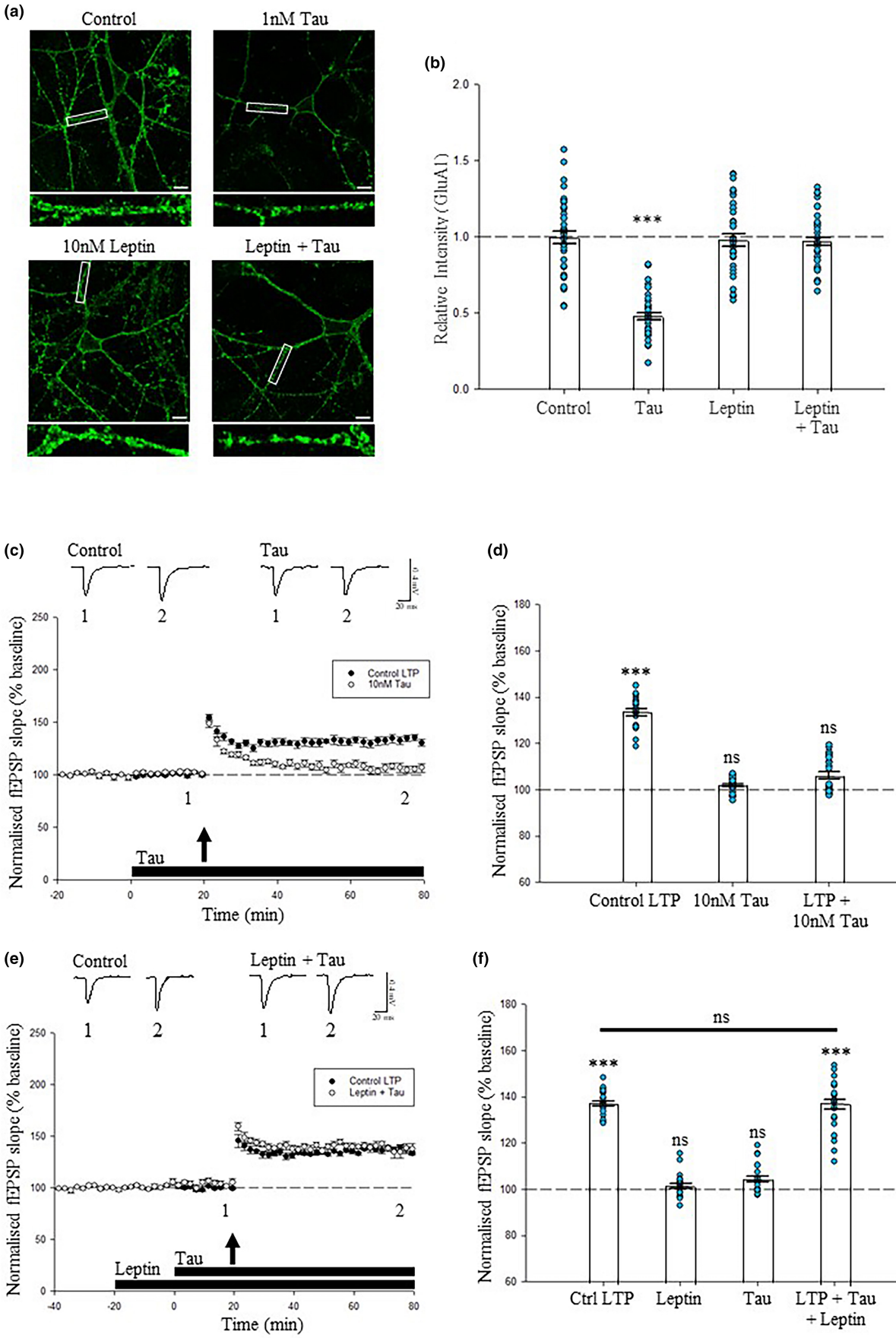

FIGURE 6 Leptin prevents internalisation of GluA1-containing AMPARs and block of LTP induced by oligomeric tau. (a) Representative images of surface GluA1 labelling in cultured hippocampal neurons (11-14DIV; scale bar = 10 μm). (b) Pooled data showing surface GluA1 levels in control neurons and after treatment with tau, leptin, and leptin plus tau. Leptin prevents the tau-induced decrease in surface GluA1 expression. (c) Pooled data showing the effects of tau (open circle) on long-term potentiation (LTP) induced by HFS (100 Hz, 1 s) compared to control LTP (filled circle) at SC-CA1 synapses in juvenile hippocampal slices. Each point is the average of four successive responses, and representative field excitatory postsynaptic potentials (fEPSPs) are shown above each plot and for the time indicted. (d) Histogram of pooled data illustrating the effects on synaptic transmission. Tau (10 nM) had no effect on basal synaptic transmission but it blocked induction of LTP. (e) Pooled data showing that treatment with leptin prevents oligomeric tau-induced block of LTP. (f) Histogram of pooled data illustrating the effects on synaptic transmission. Tau block of LTP was prevented by prior addition of leptin. All data are mean ± SEM % of baseline from 5 slices obtained from separate animals.

removal of GluA1-containing AMPARs from synapses. This process is also attenuated by leptin and likely involves PI3K activation and subsequent inhibition of GSK-3β. Leptin also prevented AMPAR removal from synapses in hippocampal neurons treated with oligomeric tau. In functional studies, treatment of hippocampal slices with leptin inhibited the ability of tau to block induction of hippocampal LTP at juvenile SC-CA1 synapses. These findings identify previously unknown protective roles for leptin in cellular models of tau-related synaptic dysfunction, and add to the growing body of evidence of leptin's protective effects in AD.

In hippocampal neurons, exposure to Aβ results in abnormal targeting of tau to synapses; a process thought to be an early aberrant synaptic event in tau-related pathology (Miller et al., 2014). In accordance with this, here we show that $A\beta_{1\text{-}42}$ significantly enhanced dendritic tau levels indicating that $A\beta_{1\text{-}42}$ promotes movement of endogenous tau from axons to dendrites. Additionally, $A\beta_{1\text{-}42}$ treatment increased synaptic expression of tau as a significant increase in % co-localisation between tau and PSD-95 was observed. Tau targeting to dendrites and synapses is likely to be an early pre-clinical event as the observed changes in tau localisation were not associated with any change in synaptic density, and thus are not likely to reflect loss of synapses.

Hyperphosphorylation of tau is a fundamental pathological event in AD. It is known that Aβ drives tau phosphorylation (Jin et al., 2011), thereby reducing its association with microtubules, which enables p-tau movement to synapses (Miller et al., 2014). Mislocalisation of tau to synapses is highly dependent on its phosphorylation status, as expression of phospho-null mutant tau prevents its targeting to synapses (Hoover et al., 2010; Miller et al., 2014; Teravskis et al., 2021). Here, we show that exposure to $A\beta_{1\text{-}42}$ decreased expression of p-tau in axonal regions and increased the dendritic and synaptic levels of p-tau. It is known that elevated levels of p-tau, particularly at Ser396, occurs in AD, and that phosphorylated Ser396 is linked to neurodegeneration (Abraha et al., 2000; Bramblett et al., 1993). In line with this, we show when tau Ser396 is phosphorylated, there is increased targeting of tau to synapses, suggesting that phosphorylation of this serine residue is necessary for tau movement. However, as this study utilised an antibody that only recognised p-Ser396, it is feasible that $A\beta_{1\text{-}42}$-driven phosphorylation of other AD-associated sites on tau (Gong & Iqbal, 2008; Mondragón-Rodríguez et al., 2020; Neddens et al., 2018) occurs in our model. Consequently, we cannot rule out the possibility that the observed synaptic levels of p-tau are under-represented in this study, or that phosphorylation of tau at other AD-associated phospho-epitopes also contribute to the targeting of tau to synapses.

Here, we show that leptin prevents aberrant targeting of tau and p-tau to synapses, following chronic treatment with $A\beta_{1\text{-}42}$. Previous evidence indicates that leptin protects against tau-related toxicity, by reducing the levels of p-tau in animal models of AD (Doherty et al., 2013; Greco et al., 2010). Given that mis-localisation of tau to hippocampal synapses is dependent on tau phosphorylation (Hoover et al., 2010; Miller et al., 2014) and that leptin can reduce phosphorylation of tau (Greco et al., 2010), it is likely that leptin inhibits tau/p-tau movement to synapses by limiting tau phosphorylation. Previous studies have demonstrated that leptin restricts tau phosphorylation by inhibiting GSK-3β (Greco et al., 2009). In line with this, we show that inhibition of GSK-3β is also required for the protective effects of leptin in this study. Indeed, addition of a broad-spectrum GSK-3, or selective inhibitor of GSK-3β mimicked and occluded the ability of leptin to inhibit targeting of tau to synapses, thereby suggesting overlapping signalling mechanisms. Consequently, the likeliest scenario is that leptin prevents the aberrant targeting of tau to synapses by inhibiting GSK-3β and thereby reducing tau phosphorylation.

PI3K activation is also essential for the protective effects of leptin, as pharmacological inhibition of PI3K, with two structurally distinct inhibitors, blocked the protective effects of leptin. As GSK-3β is a key downstream target following PI3K activation, it is likely that leptin receptor activation promotes stimulation of PI3K signalling leading to inhibition of GSK-3β which in turn limits tau phosphorylation and movement to synapses.

In agreement with previous studies, (Fá et al., 2016; Hu et al., 2018; Ondrejcak et al., 2018; Puzzo et al., 2017), we show that exogenous application of tau inhibits the induction of hippocampal LTP at SC-CA1 synapses. Additionally, we show that direct application of tau to hippocampal neurons significantly decreases the surface expression of GluA1-containing AMPA receptors on dendrites. The exact mechanism by which tau internalises GluA1-containing AMPARs in this model is unknown. However, it is feasible that extracellular tau is taken up into neurons and influences GluA1 trafficking when located intracellularly, as has been shown for tau-mediated inhibition of LTP (Fá et al., 2016; Puzzo et al., 2017). As recent studies indicate that tau also interacts with NSF, a vesicular ATPase that is critically involved in AMPAR trafficking (Prikas et al., 2022), it is possible that this mechanism plays a role in this study.

Here, we provide evidence that leptin prevents both the block of hippocampal LTP and removal of surface GluA1 induced by exogenous tau. Although the mechanism underlying the neuroprotective effect of leptin remains to be determined, as pharmacological inhibition of GSK-3β prevents Aβ-induced block of LTP and AMPA receptor internalisation (Jo et al., 2011; Rui et al., 2010), it is possible that similar mechanisms underlie the aberrant effects of tau on synaptic function. As PI3K signalling is a key leptin driven signalling pathway that underlies leptin regulation of hippocampal synaptic plasticity and AMPA receptor trafficking (Luo et al., 2015; Moult et al., 2010; Moult & Harvey, 2011), and inhibition of GSK-3β lies downstream of PI3K activation, it is feasible that this signalling mechanism contributes to leptin's ability to counteract the aberrant effects of tau on synaptic plasticity, although this remains to be determined.

In conclusion, it is well established that leptin has potent neuroprotective properties against the aberrant effects of Aβ (Doherty et al., 2013, 2022; Magalhães et al., 2015; Malekizadeh et al., 2017; McGregor & Harvey, 2018). Recent studies have highlighted the pivotal role of tau in mediating Aβ-induced toxicity and have also identified the crucial role tau plays in AD-related synaptic dysfunction. Consequently, the ability of leptin to prevent aberrant targeting of tau and p-tau to hippocampal synapses extends our understanding of leptin's neuroprotective actions at synapses and has important implications for leptin's role in neurodegenerative disorders like AD.

AUTHOR CONTRIBUTIONS

J.H. supervised and designed the experiments and wrote the manuscript. K.H., E.M., and K.M. performed all the experiments. K.H. analysed all the data and helped to write the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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