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ORIGINAL ARTICLE

A Leptin Fragment Mirrors the Cognitive Enhancing and Neuroprotective Actions of Leptin

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Abstract

A key pathology of Alzheimer's disease (AD) is amyloid β ($A\beta$) accumulation that triggers synaptic impairments and neuronal death. Metabolic disruption is common in AD and recent evidence implicates impaired leptin function in AD. Thus the leptin system may be a novel therapeutic target in AD. Indeed, leptin has cognitive enhancing properties and it prevents the aberrant effects of $A\beta$ on hippocampal synaptic function and neuronal viability. However, as leptin is a large peptide, development of smaller leptin-mimetics may be the best therapeutic approach. Thus, we have examined the cognitive enhancing and neuroprotective properties of known bioactive leptin fragments. Here we show that the leptin (116–130) fragment, but not leptin (22–56), mirrored the ability of leptin to promote AMPA receptor trafficking to synapses and facilitate activity-dependent hippocampal synaptic plasticity. Administration of leptin (116–130) also mirrored the cognitive enhancing effects of leptin as it enhanced performance in episodic-like memory tests. Moreover, leptin (116–130) prevented hippocampal synaptic disruption and neuronal cell death in models of amyloid toxicity. These findings establish further the importance of the leptin system as a therapeutic target in AD.

Key words: Alzheimer's disease, amyloid beta, AMPA receptor trafficking, episodic memory, hippocampus, synaptic plasticity



Introduction

Alzheimer's disease (AD) is a complex, progressive brain disorder that results in profound cognitive deficits, particularly in memory. Accumulation of toxic amyloid plaques and neurofibrillary tangles comprising hyperphosphorylated tau are key pathological features of AD. Proteolytic processing of APP and generation of toxic amyloid beta ($A\beta$) is linked to aberrant synaptic function and neuronal degeneration. Several studies indicate that soluble $A\beta$ oligomers greatly influence activity-dependent hippocampal synaptic plasticity resulting in deficits in learning and memory. Indeed, $A\beta$ inhibits the induction of hippocampal long-term potentiation (LTP), but facilitates long-term

depression (LTD) (Walsh et al. 2002; Shankar et al. 2008). Furthermore, $A\beta$ significantly alters AMPA receptor trafficking to and away from synapses which contributes to hippocampal synaptic dysfunction (Hsieh et al. 2006; Liu et al. 2010).

Clinical evidence indicates that diet and lifestyle are major risk factors for developing AD and disruption to metabolic systems is linked to AD (Stranahan and Mattson 2012). The metabolic hormone leptin regulates energy homeostasis, but also markedly influences hippocampal synaptic function. Indeed, leptin-insensitive rodents exhibit impaired hippocampal LTP and spatial memory (Li et al. 2002). Moreover, direct administration of leptin into the hippocampus facilitates learning

(Wayner et al. 2004), whereas hippocampal dendritic morphology, glutamate receptor trafficking and synaptic plasticity are significantly altered after leptin treatment (Shanley et al. 2001; O'Malley et al. 2007; Moulton et al. 2010; Luo et al. 2015). Clinical studies indicate that aberrant leptin function is associated with an increased risk of AD, as AD patients exhibit significantly lower leptin levels than normal (Power et al. 2001). Individuals with lower circulating leptin also have a greater risk of developing AD (Lieb et al. 2009). Additionally, leptin levels are markedly reduced in rodent models with familial AD mutations (APP^{Swe}; PS1M146V; Fewlass et al. 2004). In cellular models of AD, leptin treatment prevents the aberrant effects of A β , as leptin reverses the ability of A β to inhibit LTP and facilitate LTD in hippocampal slices (Doherty et al. 2013) and it prevents A β -driven internalization of the AMPA receptor subunit, GluA1 in hippocampal neurons (Doherty et al. 2013). Chronic intracerebroventricular injection of leptin also reverses A β -induced impairments in LTP in vivo (Tong et al. 2015). In cell survival assays, leptin protects against A β -induced toxicity as neuronal viability is increased after leptin treatment. Moreover the cortical expression of 2 AD-linked biomarkers, endophilin 1 and phosphorylated tau, are enhanced in leptin-insensitive Zucker *fa/fa* rats (Doherty et al. 2013). Thus there is growing evidence that leptin-based therapies may be beneficial in AD.

However, using leptin therapeutically may not be the best approach due to its widespread central actions. One possibility is to develop small molecules that mimic leptin action. Indeed, studies have found that specific fragments of the leptin peptide are bioactive and mirror the antiobesity effects of leptin (Grasso et al. 1997; Rozhavskaia-Arena et al. 2000). Indeed, application of a C-terminal fragment of leptin (amino acids 116–130) or a shorter fragment (116–121) to leptin-deficient *ob/ob* mice reduced food intake and body weight (Grasso et al. 1997; Rozhavskaia-Arena et al. 2000; Grasso et al. 2001). However, another leptin fragment (22–56) is also bioactive as significant reductions in food intake have been observed after leptin (22–56) administration (Samson et al. 1996). Thus, it is feasible that different parts of the leptin molecule are active in the CNS. However, the cognitive enhancing and neuroprotective properties of the bioactive leptin fragments are unknown. Here we show that leptin (116–130), but not leptin (22–56), mirrored the effects of leptin on hippocampal synaptic plasticity and also prevented hippocampal synaptic disruption and cell death induced by A β . Moreover, leptin (116–130) has similar cognitive enhancing effects to leptin as it enhanced episodic-like memory. These findings establish further the importance of the leptin system as a therapeutic target in AD.

50 Methods

Primary Neuronal Culture

Hippocampal cultures were prepared from neonatal Sprague Dawley rats as before (O'Malley et al. 2007). Briefly, neonatal Sprague Dawley rats (1–3 days old) were killed by cervical dislocation in accordance with Schedule 1 of the United Kingdom Government Animals (Scientific Procedures) Act, 1986 and the hippocampi removed. After washing in HEPES buffered saline (HBS) comprising (mM): NaCl 135; KCl 5; CaCl₂ 1; MgCl₂ 1; HEPES 10; D-glucose 25 at pH 7.4; cells were treated with protease Type X and Type XIV (0.5 mg/ml; Sigma) for 25 min at room temperature. Dissociated cells were plated onto sterile dishes (Falcon 3001) treated with poly-L-lysine (20 μ g/ml; 1–2 h) and maintained in MEM with serum replacement-2 (Sigma) in a

humidified atmosphere of 5% CO₂ and 95% O₂ at 37°C for up to 3 weeks. 65

Human Neural Cell Line SH-SY5Y

The human neuroblastoma cell line, SH-SY5Y (ECACC, UK) was maintained in Dulbecco's Modified Eagle Medium supplemented with glucose (4500 μ g/l) and 10% (v/v) cosmic calf serum (Fisher Scientific, UK) at 37°C in a humidified atmosphere of 5% CO₂, 95% air and allowed to reach 70–80% confluence before seeding. Cells (passage 10–18) were plated at 10 000 cells per well in 96 well tissue culture plates (Nunc, VWR, UK) and at a density of 2×10^5 cells in 35 mm dishes for protein extraction. To induce differentiation, cells were cultured in DMEM supplemented with glucose (4500 μ g/l), 1% (v/v) cosmic calf serum and 10 μ M retinoic acid for 5 days. Thereafter they were incubated in DMEM supplemented with glucose (4500 μ g/l), serum replacement 2 (2%; Sigma, UK) and 18 μ M 5-fluorodeoxyuridine to inhibit proliferation of undifferentiated cells. The 50% of the medium was changed every 2–3 days and pharmacological treatment was carried out 7 days after switching to this medium. Reagents used were (from Sigma UK unless stated) 0.1–10 nM human leptin and leptin (116–130); 0.1–10 nM leptin (22–56; Bachem; Switzerland); 5 μ M copper chloride; 10 mM A β _{1–42}; 5 μ M WP1066 or 50 nM wortmannin. All treatments were added to the culture at the same time and survival assays were carried out after 96 h treatment. Protein samples for ELISA were extracted 3 h after exposure to the relevant reagents. 70 75 80 85 90

Cell Survival Assays

The concentration of lactate dehydrogenase (LDH) in the culture medium was used to monitor the level of cell death, as previously (Oldreive and Doherty 2010). A Crystal violet assay was used to assess total cell number. Cells were fixed in neutral buffered formalin and washed 3 times in PBS prior to staining with 0.01% crystal violet acetate for 5 min. Plates were washed 5–10 times in dH₂O and cells solubilised in 100 μ l dimethyl sulfoxide (DMSO) before reading the absorbance on a Biohit BP100 plate reader. For both assays, data is expressed as percentage relative to control, untreated wells to normalize for differences in plating density between individual experiments. 95 Q4 100 105

ELISA for Cell Signalling Pathways

Protein from cultures was extracted into 500 μ l Tris-buffered saline containing protease inhibitor cocktail, and a Bradford assay used to determine protein concentration. Samples were diluted to give equal loading onto ELISA plates. Commercially available ELISA kits were used in accordance with the manufacturer's instructions to determine the ratio of pan-STAT3 to phospho-STAT3 (Sigma, UK) and pan-Akt to phospho-Akt (Sigma, UK). Each protein sample was run in duplicate. 110 115

Surface Labelling of AMPA Receptors

To monitor GluA1 surface expression, immunocytochemistry was performed on hippocampal cultures (7–14 DIV) as before (Moulton et al. 2010). Neurons were treated with agents for 20 min at room temperature (20–22°C) before incubation with an antibody against an N-terminal region of GluA1 (sheep anti-GluR1; in house antibody against synthetic peptide (RTSDSRDHTRVDWKR) corresponding to 253–267 residues of GluR1; 1:100; Moulton et al. 2010) at 4°C. Neurons were then fixed with 4% paraformaldehyde (5 min) before adding an appropriate fluorescently labelled secondary 120 125

antibody (1:250; 30 min) to enable visualization of GluA1 surface staining. No labeling was observed after incubation with secondary antibody alone. For synaptic colocalization studies, surface GluA1 staining was compared with synaptophysin (antisynaptophysin antibody) immunolabelling and percentage of surface GluA1 at synapses was assessed as the number of GluA1-positive sites that colocalize with synaptophysin-positive sites.

An Axiovert 200M laser scanning confocal microscope was used for image acquisition and analysis. Dual labeling images were obtained in multitracking mode using a 15 s scan speed. Intensity of staining was determined offline using Lasershar software (Carl Zeiss). Analysis lines (50 μm) were drawn along randomly selected dendritic regions (50 nm), and mean fluorescence intensity was calculated. Data were obtained from at least 4 randomly selected cells for each condition, and all data were obtained from at least 3 different cultures from different animals. Within a given experiment, all conditions, including illumination intensity and photomultiplier gains, were kept constant. To quantify experimental data obtained from separate days, data were normalized relative to mean fluorescence intensity in control neurons.

Hippocampal Slice Preparation and Electrophysiology

Parasagittal hippocampal slices (300 μm) were prepared from either P13–21 or 12–24-week-old male Sprague Dawley rats as previously (Moult et al. 2010). Brains were rapidly removed and placed in ice-cold artificial CSF (aCSF; bubbled with 95% O_2 and 5% CO_2) containing the following (in mM): 124 NaCl, 3 KCl, 26 NaHCO_3 , 1.25 NaH_2PO_4 , 2 CaCl_2 , 1 MgSO_4 , and 10 D-glucose. Once prepared, parasagittal slices were allowed to recover at room temperature in oxygenated aCSF for 1 h before use. Slices were transferred to a submerged chamber maintained at room temperature and perfused with artificial cerebrospinal fluid at 2 ml min^{-1} . Standard extracellular recordings were used to monitor evoked field excitatory postsynaptic potentials (fEPSP) from stratum radiatum. The Schaffer collateral-commissural pathway was stimulated (constant voltage; 0.1 ms) at 0.033 Hz, using a stimulus intensity that evoked a peak amplitude $\sim 50\%$ of the maximum. Synaptic potentials were low pass filtered at 2 kHz and digitally sampled at 10 kHz. The fEPSP slope was measured and expressed relative to baseline. Synaptic records are the average of 4 consecutive responses and stimulus artefacts are blanked for clarity.

LTP was induced using a high frequency stimulation paradigm (10 trains; 8 stimuli; 100 Hz), whereas the subthreshold LTD paradigm used was low frequency stimulation (LFS; 1 Hz) for 5 min (300 shocks; 15). A primed burst stimulation paradigm (5 trains of 8 stimuli at 100 Hz; Rose and Dunwiddie 1986) was used to induce STP. Recordings were made using an Axopatch 200B amplifier and analyzed using LTP v2.4 software (Anderson and Collingridge 2007). In all experiments, the slope of fEPSPs was monitored continuously. For studies comparing the actions of leptin and the fragments on synaptic transmission, the mean slope (average of 5 min recording) of fEPSPs obtained during the 5 min period immediately prior to leptin and/or agent addition was compared with that after 25–30 min exposure to the agent. In synaptic plasticity studies, the degree of potentiation or depression was calculated 30–35 min after LFS or HFS and expressed as a percentage of baseline \pm SEM.

Episodic-Like Memory Testing

Mice were tested using the same protocol used to test object-place-context (OPC) recognition in rats (Wilson et al. 2013).

The size of the testing box was reduced to 30 cm \times 20 cm \times 20 cm and the household objects used in the task were approximately the size of the mice in at least 1 dimension. The box could be configured with 2 sets of contextual features (white vs. stripes) by using different wall and floor inserts. These created one environment that was plain white and one that had stripes and a mesh floor. Mice were handled extensively prior to training and then habituated to the box for 4 days before undergoing object recognition, object-place and object-context training. These data are not presented and served only to familiarize the mice to the testing procedures and the factors that are manipulated during the OPC task. On the OPC testing days, mice were given IP injections of either saline, leptin or leptin (116–130, both at 7.8 nM/ml) 30 min prior to testing. Testing consisted of 3 stages; 2 sample phases and a test phase (illustrated in Fig. 7A). Each phase was 3 min duration and exploration behaviour was scored using custom written software (Observe, University of St Andrews). Between all phases the box was cleaned and dried using F10 solution. All testing was recorded on a webcam connected to a laptop to allow offline scoring of behaviour. Twenty percent of trials were rescored by a researcher who was blind to the group identity of the mice and these scores were found to be within 10% of the original scoring. The dependent variable measured was a discrimination index that is calculated by subtracting the amount of time exploring the familiar object from the amount of time exploring the novel object and dividing by the total amount of exploration. This measure was taken from the first minute of the test trial as novelty discrimination has been shown to rapidly diminish after the first minute (Clarke et al. 2000).

Statistical Analyses

In immunocytochemical studies, data are expressed as mean \pm SEM and statistical analyses performed using one-way ANOVA with Tukey's post hoc test for comparisons between multiple groups. In electrophysiological studies, analyses were performed using repeated measures ANOVA for comparison between multiple groups. A one-way ANOVA with Tukey's post hoc test was used for comparison between multiple groups in cell viability assays. In ELISA assays, a t-test was used to determine differences between groups. $P < 0.05$ was considered significant. In behavioural studies a one-way ANOVA with Tukey's post hoc tests was used for comparison between the groups.

Materials

Recombinant human leptin (R&D Systems, Europe) was used in all experiments. Leptin was prepared as a 50 μM stock solution in normal aCSF and was diluted to the required concentration in aCSF or HBS. $\text{A}\beta_{1-42}$ peptide was synthesized and purified by Dr. Elliott at Yale University (New Haven, CT, USA) based on the human $\text{A}\beta$ sequence. The lyophilized powder was solubilized in DMSO and diluted to a working concentration. For cell viability and biochemical assays $\text{A}\beta$ was prepared as described previously as $\text{A}\beta$ has shown to elicit toxicity when in a β sheet conformation (Simmons et al. 1994). Briefly, lyophilized powder was solubilized in dH_2O to a concentration of 1 mM in phosphate-buffered saline and incubated for 24 h at 37°C prior to further dilution to working concentration in culture medium. Wortmannin, bisperoxovanadium (Calbiochem), WP1066 (Cayman Chemicals), and D-AP5 (Tocris Cookson) were all obtained commercially.



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Results

Leptin (116–130) Facilitates NMDA Receptor-Dependent Hippocampal Synaptic Plasticity

It is known that NMDA receptors contribute little to basal excitatory synaptic transmission but synaptic activation of NMDA receptors is crucial for LTP induction at hippocampal synapses (Bliss and Collingridge 1993). Previous studies indicate that leptin enhances the magnitude of activity-dependent LTP in acute hippocampal slices (Oomura et al. 2006) and following direct administration into the hippocampus in vivo (Wayner et al. 2004). We have also shown that leptin facilitates NMDA receptor-dependent synaptic plasticity as leptin promotes conversion of short term potentiation (STP) to hippocampal LTP (Shanley et al. 2001). In order to compare the effects of leptin and the leptin fragments on synaptic plasticity in juvenile hippocampal slices (P13–21), a primed burst stimulation paradigm (5 trains of 8 stimuli at 100 Hz; Rose and Dunwiddie 1986) was used to induce STP, which returned to baseline levels within 30–35 min ($n = 4$; Fig. 1A). In accordance with previous studies (Shanley et al. 2001), application of leptin (50 nM) prior to the

stimulation paradigm converted STP into a persistent increase in synaptic transmission ($155 \pm 6.1\%$ of baseline at 40 min; $n = 4$; $P < 0.05$; Fig. 1A). Similarly, treatment with 50 nM leptin (116–130) facilitated synaptic plasticity as synaptic transmission was enhanced to $143 \pm 6.4\%$ of baseline in leptin (116–130)-treated slices ($n = 4$; $P < 0.05$; Fig. 1B). In contrast, in slices exposed to 50 nM leptin (22–56), only STP was observed as synaptic transmission returned to baseline levels within 40 min ($n = 6$; Fig. 1C).

Leptin (116–130) Induces Synaptic Plasticity at Adult Hippocampal CA1 Synapses

We have shown that leptin regulation of excitatory synaptic transmission is age-dependent. Thus in contrast to its effects in juvenile tissue, leptin (25 nM) induces a novel form of LTP in adult hippocampus (Moult and Harvey 2011). In order to verify if the leptin fragments mirror leptin action in adult tissue, the effects of the leptin fragments were examined in hippocampal slices from adult (12–24 weeks) rats. In accordance with previous studies (Moult et al. 2010; Moult and Harvey 2011),

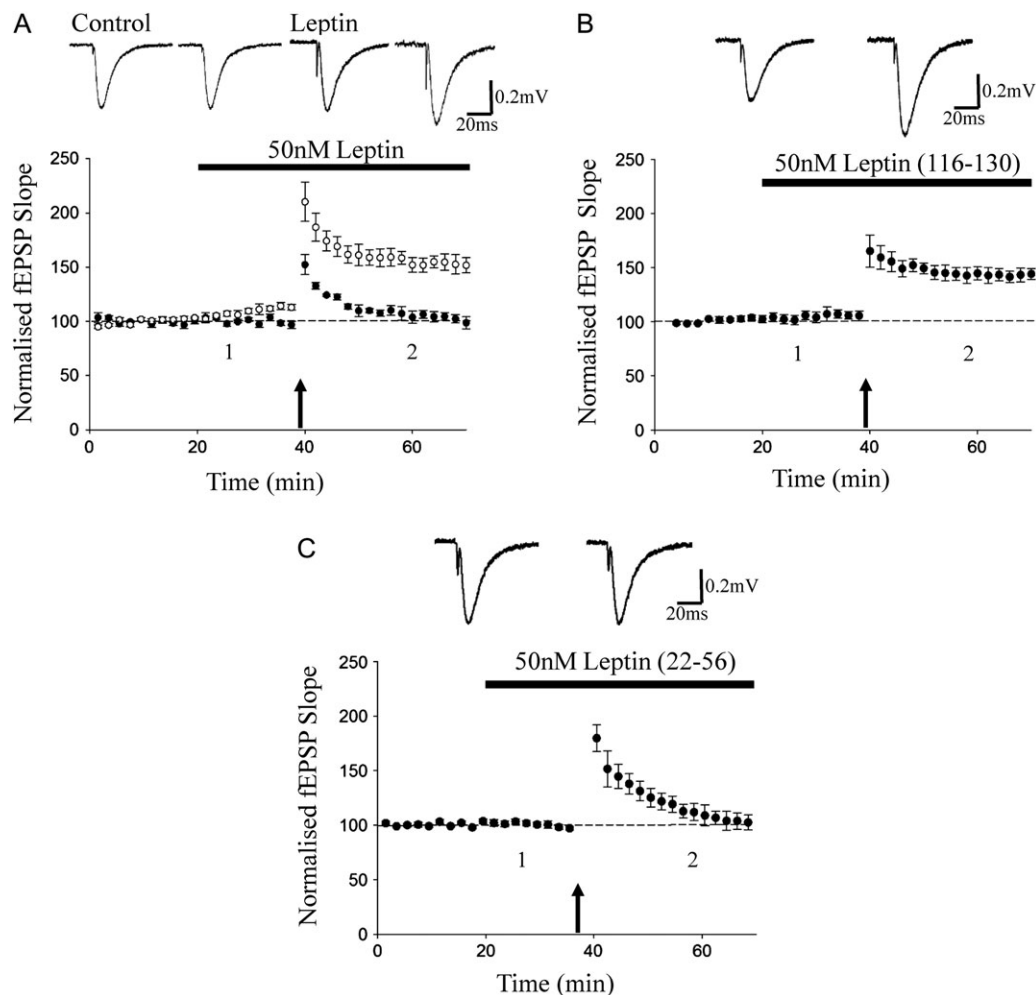


Figure 1. Leptin (116–130) promotes conversion of STP into a persistent increase in synaptic transmission in juvenile hippocampus. (A) Pooled data showing that primed burst stimulation (indicated by the arrow) delivered in the absence of leptin induced STP (filled circle) in juvenile hippocampal slices. In contrast, in leptin-treated slices (open circle) the same stimulation paradigm resulted in a persistent increase in synaptic transmission. In this and subsequent figures, each point is the average of 4 successive responses. Top, Representative synaptic records (average of 4 consecutive records) are shown for the times indicated. (B) During exposure to leptin (116–130; 50 nM), application of the primed burst stimulation paradigm resulted in a persistent increase in synaptic transmission (B), whereas only STP was evident in slices treated with leptin (22–56; C).

application of leptin (25 nM; 20 min) to adult slices rapidly enhanced synaptic transmission (to $188 \pm 13\%$ of baseline; $n = 4$; $P < 0.01$; data not shown) which was sustained for the duration of recordings. Synaptic transmission was also markedly increased (to $140 \pm 13\%$ of baseline; $n = 5$; $P < 0.05$; Fig. 2A) after treatment with 25 nM leptin (116–130), whereas application of 25 nM leptin (22–56) had no significant effect on synaptic efficacy ($99 \pm 2\%$ of baseline; $n = 4$; $P > 0.05$; Fig. 2B). These data indicate that leptin (116–130) mirrors the ability to leptin to enhance synaptic efficacy in adult hippocampus.



Leptin (116–130), But Not Leptin (22–56) Enhances the Surface Expression of GluA1

Trafficking of AMPA receptors to and away from synapses is crucial for various forms of activity-dependent synaptic plasticity (Collingridge et al. 2004). Our studies indicate that leptin regulates AMPA receptor trafficking as leptin promotes trafficking of GluA1 to hippocampal synapses (Moult et al. 2010). Moreover the ability of leptin to induce LTP in adult hippocampus requires the delivery of GluA1 to synapses by leptin (Moult et al. 2010). Thus, to assess if the leptin fragments also influence AMPA receptor trafficking processes, the surface expression of GluA1 was assayed in cultured hippocampal neurons (Moult et al. 2010). In agreement with previous studies, application of leptin (50 nM; 15 min) increased surface GluA1 expression to $184 \pm 7\%$; ($n = 36$; $P < 0.001$; Fig. 2C,D) compared with control hippocampal neurons. Similarly GluA1 surface expression was elevated (to $160 \pm 6\%$ of control; $n = 36$; $P < 0.001$) after exposure to 50 nM leptin (116–130; Fig. 2C,D), whereas GluA1 surface expression ($98 \pm 4\%$ of control; $n = 36$; $P > 0.05$) was not altered after treatment with 50 nM leptin (22–56). These data indicate that leptin (116–130), but not leptin (22–56) mirrors the plasticity enhancing effects of leptin by increasing GluA1 surface expression in hippocampal neurons.

As excitatory synaptic strength is governed by the density of AMPA receptors expressed at synapses, the effects on synaptic AMPA receptors was examined by comparing the colocalization between surface GluA1 and synaptophysin immunolabelling in hippocampal cultures (Moult et al. 2010). In agreement with previous studies (O'Malley et al. 2007; Moult et al. 2010), leptin (50 nM; 30 min) increased synaptophysin staining to $144 \pm 9\%$ of control ($n = 48$; $P < 0.001$) and it also enhanced the degree of colocalization between surface GluA1 and synaptophysin immunostaining from $43 \pm 5.4\%$ to $62 \pm 4.4\%$ ($n = 36$; $P < 0.05$; Fig. 2E). Similarly, in hippocampal neurons treated with leptin (116–130), synaptophysin staining was increased to $122 \pm 9\%$ ($n = 36$; $P < 0.05$) and a significant increase (to $64 \pm 4.9\%$; $n = 36$; $P < 0.01$) in GluA1-synaptophysin colocalization was observed (Fig. 2E). Conversely, exposure to leptin (22–56) had no significant effect on either synaptophysin staining ($105 \pm 9\%$; $n = 36$; $P > 0.05$) or the degree of colocalization ($49 \pm 5.4\%$; $n = 36$; $P > 0.05$; Fig. 2E). These data indicate that leptin (116–130), but not leptin (22–56) promotes the trafficking of GluA1 to synapses in hippocampal neurons.

Inhibition of the phosphatase, PTEN underlies leptin-driven trafficking of GluA1 to synapses (Moult et al. 2010). In order to determine if similar leptin dependent signalling cascades mediate the actions of leptin (116–130), the effects of pharmacological inhibition of PTEN with bisperoxovanadium (bpV; Schmid et al. 2004) were assessed in hippocampal cultures. Application of bpV (50 nM; 30 min) increased GluA1 surface expression to $148 \pm 8\%$ of control ($n = 36$; $P < 0.001$; Fig. 2F). In accordance with previous studies (Moult et al. 2010), leptin

resulted in a significant increase in surface GluA1 labelling ($140 \pm 7.2\%$; $n = 36$; $P < 0.001$; Fig. 2F). Similarly, GluA1 surface expression was enhanced (to $167 \pm 10\%$ of control; $n = 36$; $P < 0.001$) after exposure to leptin (116–130). Moreover, treatment with bpV occluded the effects of either leptin or leptin (116–130) such that GluA1 staining was $131 \pm 6.6\%$ and $148 \pm 8.1\%$ of control ($n = 36$; $P < 0.001$; for both; Fig. 2F) in the presence of bpV and either leptin or leptin (116–130), respectively. These data indicate that the ability of leptin (116–130) to regulate GluA1 trafficking involves inhibition of PTEN.

Leptin (116–130), But Not Leptin (22–56) Reverses $A\beta_{1-42}$ Inhibition of Hippocampal Synaptic Plasticity

Several studies indicate that soluble $A\beta$ oligomers impair activity-dependent synaptic plasticity, as exposure to $A\beta$ inhibits hippocampal LTP (Shankar et al. 2008; Li et al. 2009) and enhances LTD (Shankar et al. 2008). Moreover, our recent studies indicate that leptin reverses the detrimental effects of $A\beta_{1-42}$ on both LTP and LTD (Doherty et al. 2013). Thus we assessed if either of the fragments mirrored the protective actions of leptin on hippocampal synaptic plasticity. Initially we determined that application of leptin prevented the acute effects of $A\beta_{1-42}$ on LTP. In control slices, synaptic plasticity was induced using high frequency stimulation (HFS; 100 Hz 10 trains of 8 stimuli) which increased synaptic transmission to $127 \pm 3.4\%$ of baseline ($n = 8$; $P < 0.01$). Similarly in slices treated with the inactive peptide $A\beta_{42-1}$ ($1 \mu\text{M}$; 40 min), an enhancement of synaptic transmission ($132 \pm 8.8\%$ of baseline) was induced ($n = 5$; $P < 0.05$; Fig. 3A). However, in accordance with previous studies (Doherty et al. 2013), exposure to $A\beta_{1-42}$ not only blocked hippocampal synaptic plasticity ($96 \pm 7.5\%$ of baseline; $n = 5$; $P > 0.05$; Fig. 3A) but this effect was reversed by leptin (25 nM; $135 \pm 6.3\%$ of baseline; $n = 4$; $P < 0.05$; Fig. 3B). Similarly, treatment with 25 nM leptin (116–130) before $A\beta_{1-42}$ resulted in a robust increase in synaptic transmission (to $136 \pm 5.5\%$ of baseline; $n = 6$; $P < 0.05$; Fig. 3C). In contrast, HFS failed to increase synaptic efficacy ($107 \pm 3.3\%$ of baseline; $n = 5$; $P > 0.05$) in slices exposed to $A\beta_{1-42}$ and leptin (22–56; Fig. 4D), although some STP was observed. Thus these data indicate that like leptin, leptin (116–130) prevents the detrimental effects of $A\beta_{1-42}$ on hippocampal synaptic plasticity.

Leptin (116–130) Reverses $A\beta_{1-42}$ -Induced LTD

It is known that oligomeric $A\beta$ promotes the induction of LTD (Shankar et al. 2008) and that exposure to a low concentration of leptin (10 nM) prevents facilitation of hippocampal LTD by $A\beta_{1-42}$ (Doherty et al. 2013). Thus we assessed if either of the leptin fragments mirror leptin action. Initially we verified that leptin prevented $A\beta_{1-42}$ -induced LTD. In agreement with previous studies (Doherty et al. 2013), application of the subthreshold LFS paradigm failed to induce LTD in vehicle-treated slices ($94 \pm 5.6\%$ of baseline; $n = 5$; $P > 0.05$), whereas robust LTD ($73 \pm 3.8\%$ of baseline; $n = 5$; $P < 0.001$) was induced in $A\beta_{1-42}$ -treated slices (Fig. 4A). Furthermore, leptin (10 nM) reduced the magnitude of $A\beta_{1-42}$ -induced LTD such that LFS depressed synaptic transmission to $101 \pm 5.3\%$ of baseline in leptin-treated slices ($n = 4$; $P > 0.05$; Fig. 4B). To establish if the leptin fragments mirrored leptin action the effects of leptin (116–130) or leptin (22–56) were also examined. Application of 10 nM leptin (116–130) or leptin (22–56) had no significant effect on basal synaptic transmission ($n = 6$ for leptin [116–130] and $n = 5$ for

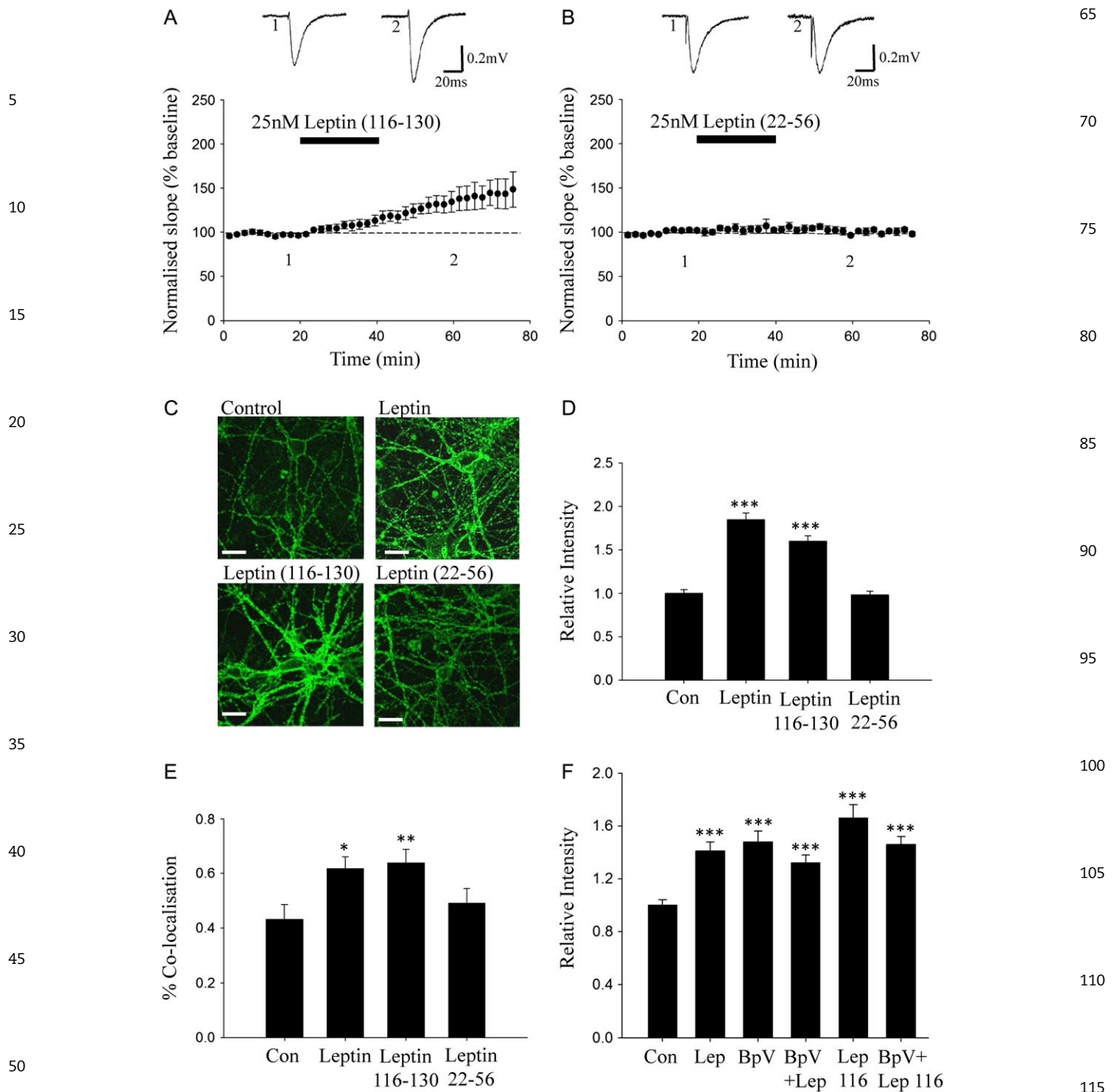


Figure 2. In adult, leptin (116-130) induces a persistent increase in synaptic efficacy and increases GluA1 trafficking to hippocampal synapses. (A) Pooled data showing that application of leptin (116-130) results in a persistent increase in excitatory synaptic transmission in adult hippocampal slices. (B) In contrast application of leptin (22-56) failed to alter excitatory synaptic strength. (C) Representative confocal images of surface GluA1 staining in control cultured hippocampal neurons and after exposure to leptin, leptin (116-130) or leptin (22-56). Leptin (116-130) mirrors the effects of leptin by increasing GluA1 surface labelling. Scale bars, 10 μ m. (D) Pooled data showing relative changes in surface GluA1 labelling in control conditions, and after exposure to leptin, leptin (116-130) or leptin (22-56) in hippocampal neurons. (E) Pooled data of the percent colocalization of surface GluA1 and synaptophysin immunolabelling in cultured hippocampal neurons. Leptin (116-130), but not leptin (22-56) increased GluA1 surface labelling associated with synapses. (F) Pooled data of relative changes in surface GluA1 labelling in hippocampal cultures in control conditions, after bpV, leptin or leptin (116-130) treatment, and in the presence of bpV and either leptin, or leptin (116-130). Inhibition of PTEN mimicked and occluded the effects of leptin (116-130).

leptin [22-56]). Application of 10 nM leptin (22-56) failed to alter the magnitude of $A\beta_{1-42}$ -induced LTD such that synaptic transmission was depressed to $73 \pm 4.0\%$ of baseline ($n = 5$; $P < 0.001$; Fig. 4D). In contrast, a significant reduction in the magnitude of

$A\beta_{1-42}$ -induced LTD ($94 \pm 5.2\%$ of baseline; $n = 5$; $P > 0.05$; Fig. 4C) was observed in hippocampal slices treated with leptin (116-130; 10 nM; 45 min) indicating that leptin (116-130) also inhibits facilitation of LTD by $A\beta_{1-42}$. Thus these data indicate

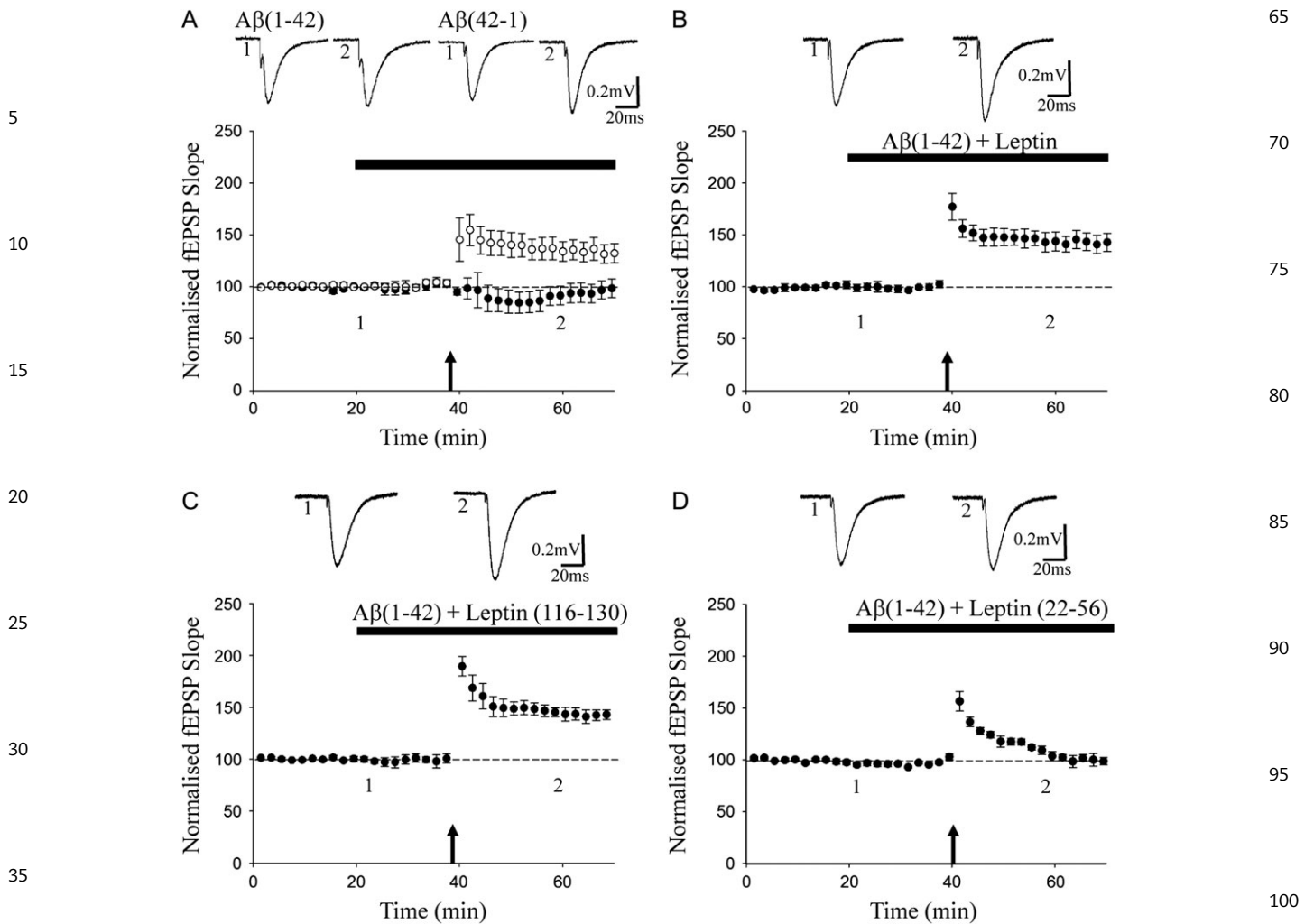


Figure 3. Leptin (116–130) inhibits the aberrant effects of amyloid- β ($A\beta$)₁₋₄₂ on hippocampal synaptic plasticity in juvenile slices. (A) Pooled data showing that HFS (indicated by the arrow) induces synaptic plasticity in $A\beta$ ₄₂₋₁-treated (open circles) slices, whereas $A\beta$ ₁₋₄₂ inhibits synaptic plasticity (filled circles). (B) Exposure to leptin prevented $A\beta$ ₁₋₄₂-inhibition of synaptic plasticity. Treatment with leptin (116–130; C) but not leptin (22–56; D) reversed $A\beta$ ₁₋₄₂-inhibition of synaptic plasticity.

that leptin (116–130), but not leptin (22–56) reverses $A\beta$ ₁₋₄₂-induced facilitation of LTD.



Leptin (116–130) Prevents $A\beta$ -Induced Internalization of GluA1

Previous studies indicate that $A\beta$ promotes internalization of the AMPA receptor subunit, GluA1 (Hsieh et al. 2006; Liu et al. 2010); an effect that is prevented by leptin (Doherty et al. 2013). To determine if the leptin fragments mirror this effect, the cell surface density of GluA1 was probed in cultured hippocampal neurons (Moult et al. 2010). In accordance with previous studies (Doherty et al. 2013), treatment with $A\beta$ ₁₋₄₂ (500 nM; 20 min) significantly attenuated (to $70 \pm 2\%$ of control) GluA1 surface expression compared with control ($A\beta$ ₄₂₋₁-treated) hippocampal neurons ($n = 48$; $P < 0.001$; Fig. 4E). Application of a low concentration of leptin (10 nM) induced a small increase in GluA1 surface expression ($113 \pm 7\%$ of control; $n = 36$; $P < 0.01$). However, in leptin-treated neurons, $A\beta$ ₁₋₄₂ failed to significantly alter GluA1 surface expression ($101 \pm 4\%$ of control; $n = 36$; $P > 0.05$). In contrast, treatment with a low concentration (10 nM) of leptin (22–56) had no significant effect on GluA1 trafficking *per se* ($n = 36$; $P > 0.05$). Moreover, leptin (22–56) failed to prevent the effects of

$A\beta$ on GluA1 trafficking as surface GluA1 was reduced to $51 \pm 2\%$ of control ($n = 36$; $P < 0.001$) in the presence of $A\beta$ and leptin (22–56; Fig. 4E). Conversely, after exposure to leptin (116–130), $A\beta$ ₁₋₄₂ failed to significantly reduce GluA1 surface expression ($97 \pm 4\%$ of control; $n = 36$; $P > 0.05$). These data indicate that leptin (116–130) mirrors leptin action by preventing $A\beta$ ₁₋₄₂-induced internalization of GluA1 in cultured hippocampal neurons.

Leptin (116–130) Prevents Copper and $A\beta$ -Induced Cell Death

We have demonstrated previously that leptin attenuates cortical neuronal death triggered by $A\beta$ ₁₋₄₂ or divalent copper ions (Doherty et al. 2013). To determine whether leptin (116–130) has neuroprotective actions, the effects of leptin (116–130) on the viability of differentiated human neural (SH-SY5Y) was examined after exposure to either $5 \mu\text{M}$ CuCl_2 or $10 \mu\text{M}$ $A\beta$ ₁₋₄₂. Cells were treated with the toxin alone or with a range of concentrations (10–0.1 nM) of leptin or leptin (116–130). Determination of membrane leakage by LDH assay revealed a significant reduction in LDH release after treatment with either leptin or leptin (116–130; both 0.1–10 nM). Thus for CuCl_2 -treated cells, 10 nM leptin reduced LDH release by $39.5 \pm 2.73\%$

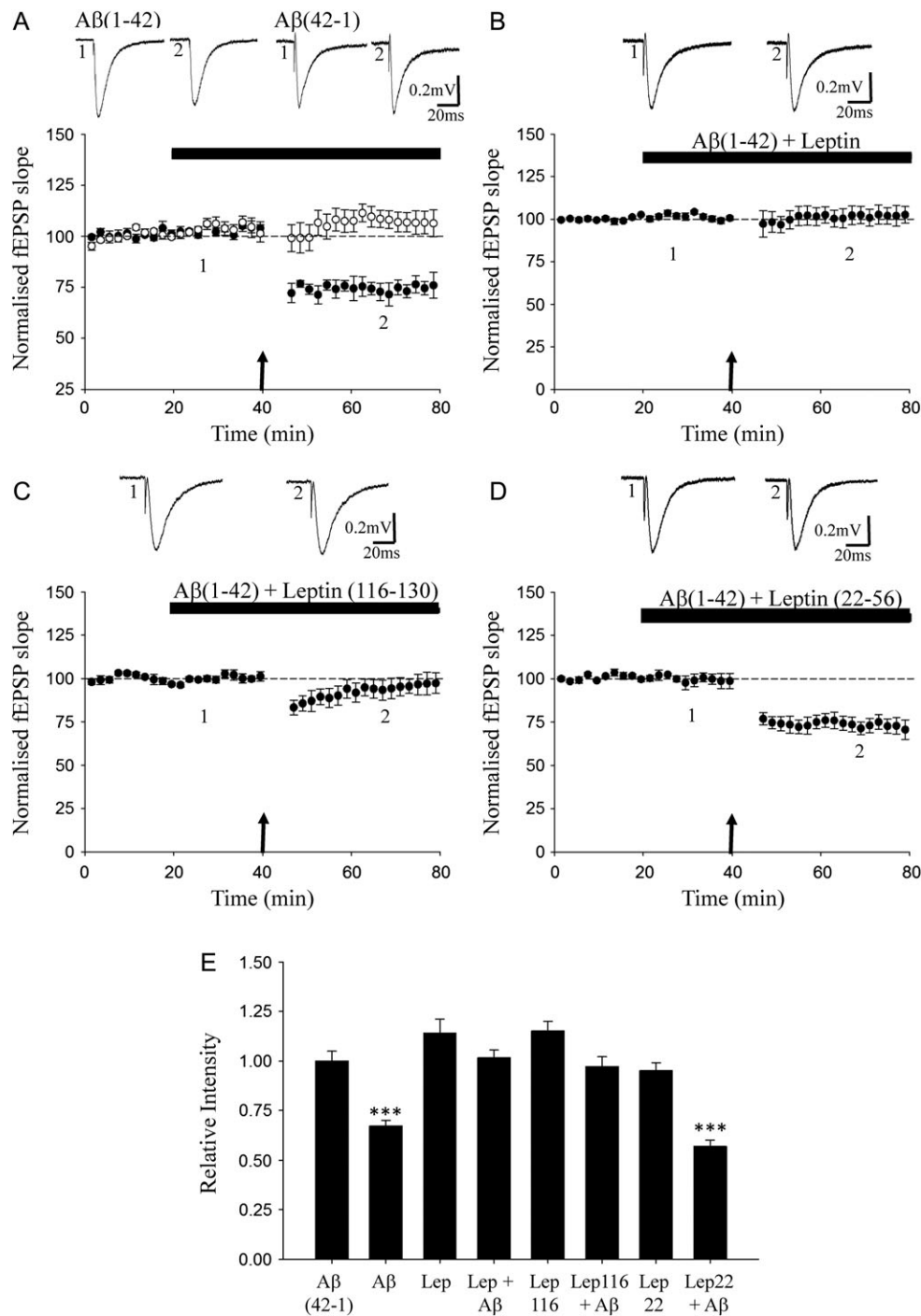


Figure 4. Leptin (116–130) prevents A β -induced AMPA receptor internalization and facilitates of hippocampal LTD. (A) Pooled data showing that the subthreshold low frequency stimulation (indicated by the arrow) failed to induce long-term depression (LTD) in A β_{42-1} -treated (open circles) slices, whereas robust LTD (filled circles) is induced in A β_{1-42} -treated juvenile slices. (B) Exposure to leptin prevented A β_{1-42} -induced LTD. Similarly treatment with leptin (116–130; C) but not leptin (22–56; D) prevented A β_{1-42} -induced LTD. E, Pooled data showing relative changes in GluA1 surface labelling in cultured hippocampal neurons in control (A β_{42-1}) conditions and after treatment with leptin, A β_{1-42} , leptin (116–130), leptin (22–56) and in the presence of A β_{1-42} plus either leptin, leptin (116–130) or leptin (22–56), respectively. Leptin and leptin (116–130) prevent A β -driven internalization of GluA1.

compared with CuCl₂ alone ($n = 5$; $P < 0.001$; Fig. 5A); an effect that was mirrored by 10 nM leptin (116–130; $45.6 \pm 2.92\%$ [$n = 5$; $P < 0.001$; Fig. 5A]). In A β_{1-42} treated cells, leptin also significantly reduced LDH release by $31.8 \pm 13.2\%$ (0.1 nM) and $47.9 \pm 7.45\%$ (10 nM), respectively ($n = 5$; $P < 0.001$; Fig. 5B). Similarly, application of 0.1 nM or 10 nM leptin (116–13) decreased LDH release by

$26.7 \pm 17.3\%$ or $46.6 \pm 9\%$, respectively ($n = 5$; $P < 0.05$; Fig. 5B). Thus like leptin, treatment with leptin (116–130) reduces neuronal death in response to AD-linked toxins in vitro.

In parallel studies a crystal violet assay was used to verify these findings by assessing cell number. In CuCl₂-treated cells, there was a concentration-dependent increase in the survival

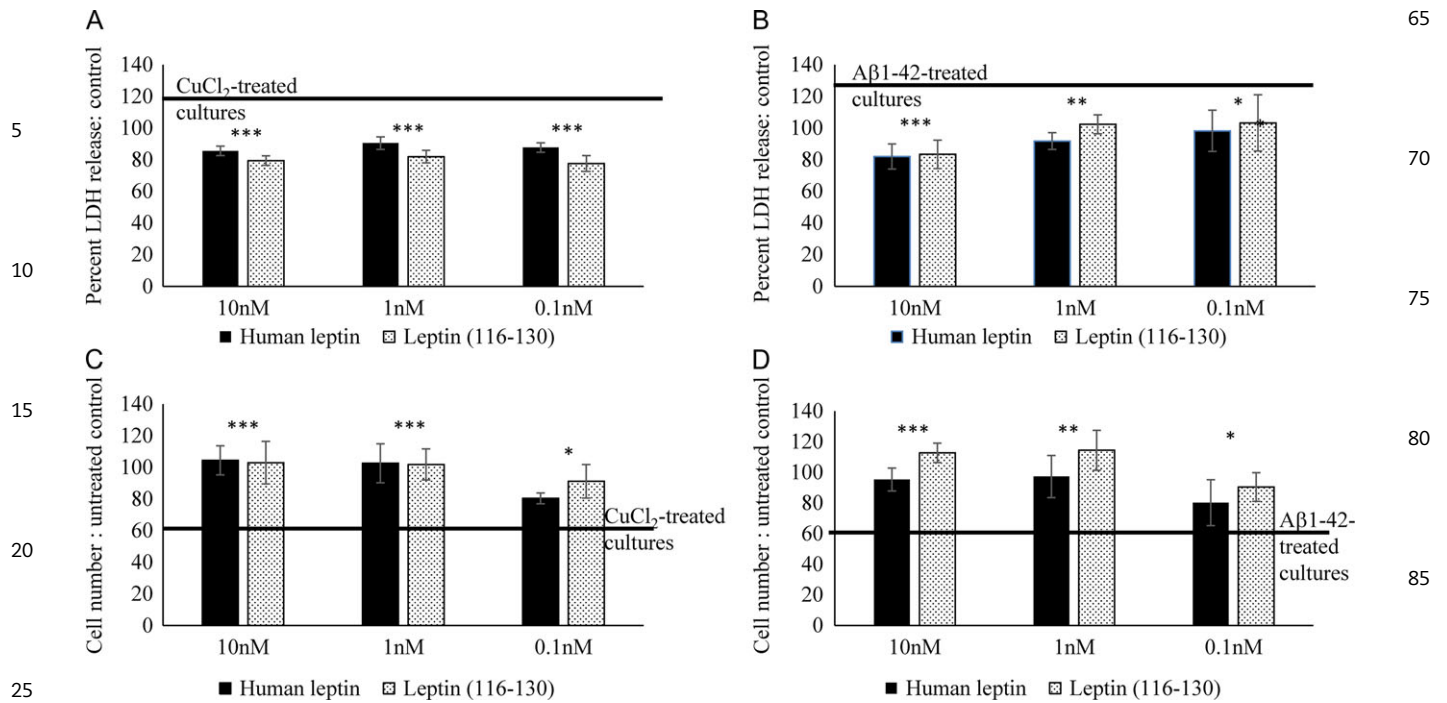


Figure 5. Leptin (116–130) prevents copper and A β -induced cell death in human SH-SY5Y cells. (A) Pooled data revealing that leptin and leptin (116–130) prevent LDH release induced by administration of 5 μ M CuCl₂. (B) Similar pooled data was obtained for cultures treated with 10 μ M A β ₁₋₄₂. (C) Pooled data showing that in 5 μ M CuCl₂ treated cultures enhanced numbers of cells are detected with a crystal violet assay when cultures are cotreated with either leptin or leptin (116–130) with a similar trend observed when cultures were induced to die with 10 μ M A β ₁₋₄₂ (D).

of cells treated with either leptin or leptin (116–130). Thus, treatment with leptin (0.1 nM) resulted in a $16.7 \pm 3.4\%$ increase in cell number and this increased to $43.4 \pm 9.2\%$ in the presence of 10 nM leptin ($n = 5$; $P < 0.01$). Similarly, exposure to 0.1 or 10 nM leptin (116–130) increased cell number by $27.8 \pm 10.6\%$ and $39.9 \pm 13.5\%$, respectively ($n = 5$; Fig. 5C). Treatment with leptin (116–130) also mirrored leptin action by increasing cell viability in A β ₁₋₄₂-treated cultures as cell number increased by $19.2 \pm 15\%$ and $44.3 \pm 7.5\%$ after treatment with 0.1 nM or 10 nM leptin, respectively ($n = 5$ for each; $P < 0.01$). Exposure to leptin (116–130) also resulted in significant increases in cell number (0.1 nM: $29.4 \pm 9.4\%$ increase; 10 nM: $51.8 \pm 6.3\%$ increase; $n = 5$ for both; $P < 0.001$; Fig. 5D).

As leptin (22–56) has biological activity in other systems, the specificity of the leptin (116–130) fragment in promoting cell survival was examined by determining whether leptin (22–56) inhibited neuronal death induced by A β ₁₋₄₂. In contrast to leptin (116–130), treatment with leptin (22–56) had no effect on the viability of cells exposed to A β ₁₋₄₂ ($41.2 \pm 5.8\%$ survival following A β ₁₋₄₂ treatment and $48.6 \pm 9.3\%$ in A β ₁₋₄₂ with 10 nM leptin (22–56) treated cells; $n = 5$; $P > 0.5$; data not shown). These data reveal a potent neuroprotective effect of the leptin fragment (116–130) that is comparable to the survival actions of leptin. Moreover, this **antiapoptotic** response is specific to leptin (116–130) as leptin (22–56) failed to influence neuronal viability.

The Neuroprotective Effects of Leptin (116–130) Involve Activation of STAT3 and PI3-Kinase-Dependent Signalling Pathways

Our previous studies indicate a crucial role for STAT3 and PI3-kinase/Akt signalling in the neuroprotective actions of leptin (Doherty et al. 2013). To determine whether leptin (116–130) acts

via similar signalling cascades we examined the effects of pharmacological inhibitors of STAT3 (WP1066) or PI3-kinase (wortmannin). In A β ₁₋₄₂-treated SH-SY5Y cells, application of either inhibitor significantly reduced the ability of leptin (116–130) to alleviate neuronal death. When neurons were treated with the STAT3 inhibitor, an $18.3 \pm 3.2\%$ increase in LDH release in leptin (116–130) and A β ₁₋₄₂-treated cultures was observed, which is similar to the $26.7 \pm 4.4\%$ increase observed with A β ₁₋₄₂ alone ($n = 5$; $P > 0.5$; Fig. 6A). Thus STAT3 inhibition blocks the neuroprotective actions of leptin (116–130), suggesting a role for STAT3 in this process. Furthermore, following inhibition of PI3-kinase with wortmannin an $26.9 \pm 9.8\%$ increase in LDH release was observed in leptin (116–130) and A β ₁₋₄₂-treated cells which is not significantly different from cells treated with A β ₁₋₄₂ alone ($n = 5$; $P > 0.5$; Fig. 6B). Thus these data also indicate a role for PI3-kinase in mediating the neuroprotective actions of leptin (116–130).

To verify that leptin (116–130) directly activates these signalling pathways, SH-SY5Y cells were exposed to 1 nM leptin (116–130; 3 h) or left untreated prior to protein extraction for ELISA. The ratio of phosphorylated STAT3 to pan STAT3 increased markedly following leptin (116–130) administration ($n = 3$; $P < 0.01$; Fig. 6C). Similarly an increase in the ratio of phosphorylated Akt to pan Akt was observed following exposure to leptin (116–130; $n = 3$; $P < 0.01$; Fig. 6D). These data indicate that leptin (116–130) reduces cell death by a mechanism involving activation of STAT3 and PI3-kinase. Furthermore, exposure to leptin (116–130) resulted in a significant increase in the active components of these signalling cascades.

Leptin (116–130) Enhances Episodic-Like Memory

The current data demonstrate that leptin (116–130) enhances hippocampal synaptic plasticity mechanisms and has

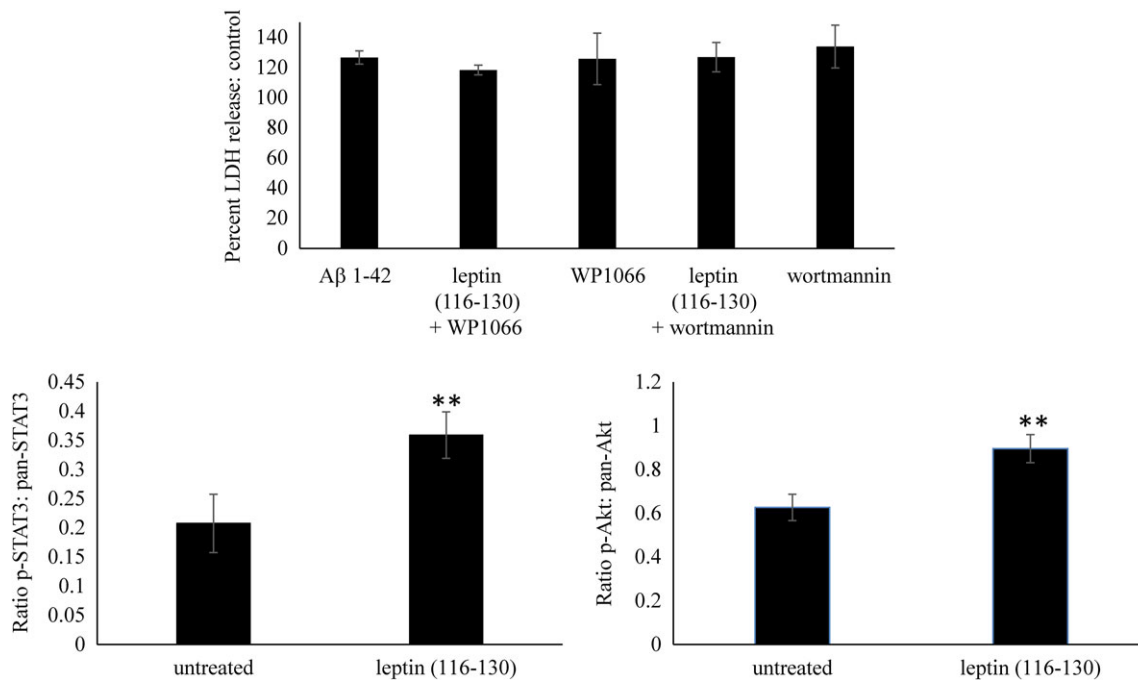


Figure 6. The neuroprotective effects of leptin (116–130) involve activation of STAT3 and PI3-kinase-dependent signalling pathways. Pooled data revealed that treatment of SH-SY5Y cells with the STAT-3 inhibitor WP1066 (5 μ M) prevented leptin (116–130)-mediated neuroprotection from the effects of 10 μ M A β ₁₋₄₂ (Fig. 7B). Pooled data from LDH assays demonstrating that the PI3-kinase inhibitor wortmannin (50 nM) attenuated leptin (116–130)-mediated neuroprotection against 10 μ M A β ₁₋₄₂ (Fig. 7C). Pooled data from ELISA assays showing that leptin (116–130) stimulates phosphorylation of STAT3 (Fig. 7D) and Akt (Fig. 7E). Pooled data from ELISA assays showing that leptin (116–130) stimulates phosphorylation of Akt.

neuroprotective effects. To further assess its therapeutic potential we next asked if this fragment has similar cognitive enhancing properties to the whole leptin molecule. Previous studies indicate that leptin enhances hippocampal-dependent memory (Oomura et al. 2006; Farr et al. 2006), whereas resistance to leptin results in impaired spatial memory (Li et al. 2002). We used the object–place–context (OPC) recognition task which models human episodic memory, the first cognitive process to be compromised in the early stages of AD (Swainson et al. 2001). Performance on this task has been shown to be impaired in murine models of AD (Davis et al. 2013) and is compromised in animals with lesions of hippocampus (Langston and Wood 2010) and lateral entorhinal cortex (Wilson et al. 2013). The task is based on the object recognition paradigm and models the integrated aspect of human episodic memory by exposing rodents to novel combinations of objects, the spatial locations in which they are experienced and the contextual features of the environment (Fig. 7A; Eacott and Norman 2004). A total of 42 C57/BL6 mice were habituated to a testing environment and then trained on object recognition, object–place recognition and object–context recognition. Following training mice were tested on 4 days of the episodic-like OPC task. On these days mice were assigned to 1 of 3 groups (control, leptin, or fragment) and on each day mice were given 100 μ l IP injections of saline, 7.8 nM/ml leptin, or 7.8 nM/ml leptin (116–130) 30 min prior to testing. One-way ANOVA on the discrimination indices during the first minute of the test phase revealed a significant effect of group (Fig. 7B; $F_{(2,41)} = 4.318$, $P = 0.02$). Post hoc comparisons (Tukey's test) revealed that both the leptin and leptin (116–130) treated mice showed enhanced performance on the task relative to the control group ($P < 0.05$) and did not differ from each other. One sample t-tests confirmed that all groups performed significantly better than chance level performance

($P < 0.01$). Finally analysis of the overall exploration time in both sample and test phases of the task revealed no change in total levels of exploration between groups demonstrating that neither leptin nor leptin (116–130) produced a nonspecific change in exploration behaviour (Fig. 7C; $P > 0.05$). The increased discrimination index was driven by an increase in exploration of the novel object combined with a decrease in the exploration of the familiar object (Fig. 7D). Together these data indicate that leptin (116–130) mirrors leptin's action by enhancing performance in episodic-like memory tasks.

Discussion

It is well established that the hormone leptin circulates in the plasma and enters the brain via transport across the blood brain barrier. In the hypothalamus, leptin plays a major role in regulating food intake and body weight (Spiegelman and Flier 2001). However, the central actions of the hormone leptin are not restricted to the hypothalamus and the regulation of energy homeostasis. Indeed, a number of extrahypothalamic brain regions, including the hippocampus display high levels of leptin receptor expression (Irving and Harvey 2014). Leptin mRNA and protein are also highly expressed in the hippocampal formation (Morash et al. 1999) and emerging evidence suggests brain-specific production of leptin (Eikelis et al. 2006). Thus, it is likely that a combination of locally released leptin as well as peripherally derived leptin reach hippocampal synapses and can influence synaptic function. Indeed, numerous studies indicate that leptin has potential cognitive enhancing properties as it readily facilitates the cellular events underlying hippocampal learning and memory. Thus, leptin has rapid effects on activity-dependent synaptic plasticity, glutamate receptor trafficking and dendritic morphology (Irving and Harvey 2014).

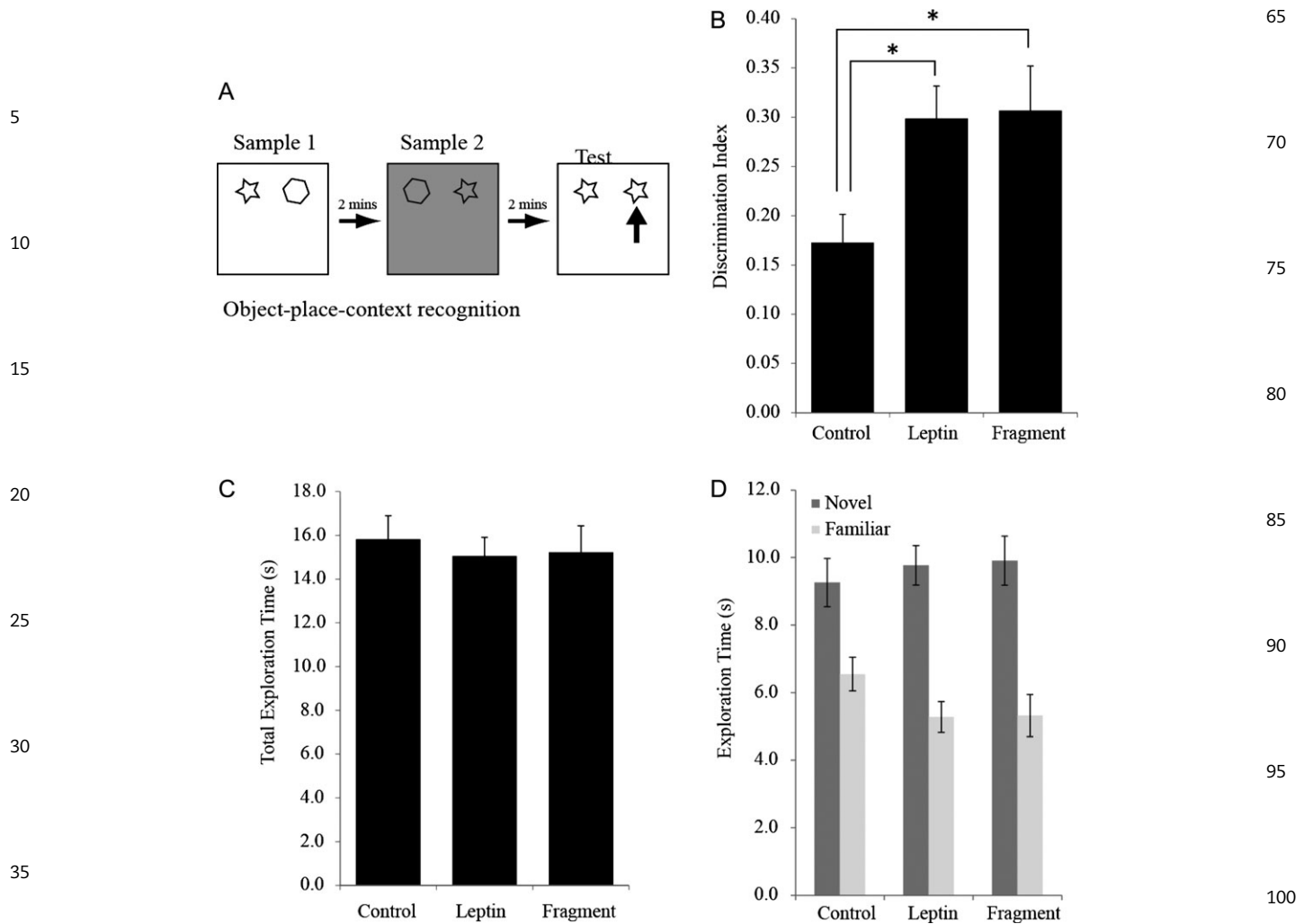


Figure 7. Leptin (116–130) enhances episodic-like memory. (A) Object-place-context task used to assess episodic-like memory. There are 2 sample phases in which mice are exposed to different copies of 2 different objects (star and hexagon) and allowed to explore for 3 min. In the test phase they see 2 new copies of 1 of the objects. The arrow points to the object that has not been previously seen in that place within that context. (B) Mean ± SEM discrimination index for the 3 groups. *P < 0.05. (C) Total exploration time in the test phase is not different between groups. (D) Exploration of the novel and familiar objects in the test phase.

In addition, several studies have identified neuroprotective effects of leptin as the viability of central and peripheral neurons is markedly influenced by this hormone (Weng et al. 2007; Doherty et al. 2008; Guo et al. 2008). Recent clinical evidence has established a link between circulating leptin levels and the incidence of AD (Power et al. 2001; Lieb et al. 2009) that has fuelled the possibility of using the leptin system as a novel therapeutic target in AD. Indeed, treatment of various AD models with leptin prevents the detrimental effects of A β that occur at both early and late stages of the disease (Fewlass et al. 2004; Farr et al. 2006; Doherty et al. 2013). However, as leptin is a very large peptide, developing small leptin-like molecules may be a better therapeutic approach. Several fragments of leptin peptide are biologically active and mirror the antiobesity effects of leptin (Grasso et al. 1997; Rozhavskaya-Arena et al. 2000; Grasso et al. 2001). However, the cognitive enhancing and neuroprotective effects of the leptin fragments are not known. Here we provide the first compelling evidence that leptin (116–130), but not leptin (22–56), has a potent effect on hippocampal synaptic function as it promotes trafficking of AMPA receptors to synapses and facilitates hippocampal synaptic plasticity. Moreover in cellular models that mimic amyloid toxicity, leptin

(116–130), but not leptin (22–56) prevents the aberrant effects of A β on hippocampal synaptic function and neuronal viability. These findings indicate that one particular leptin fragment, namely (116–130), mirrors the beneficial actions of leptin in preventing the detrimental effects of A β at the early and late stages of AD. Finally we have shown that the leptin fragment that enhances hippocampal synaptic plasticity and has neuroprotective effects, namely leptin (116–130), is also a cognitive enhancer as it improves performance on tests of episodic memory.

Here we show that, in accordance with previous studies (Shanley et al. 2001; Wayner et al. 2004), NMDA receptor-dependent synaptic plasticity is enhanced by leptin as treatment with leptin promoted conversion of STP into a persistent increase in synaptic transmission in juvenile hippocampal slices. Similarly exposure to the leptin fragment (116–130) readily facilitated synaptic plasticity as an increase in synaptic strength was evident in leptin (116–130), but not leptin (22–56)-treated slices. We have shown that the efficacy of excitatory synaptic transmission is also regulated by leptin in adult hippocampus (Moult et al. 2010). In agreement with this, application of either leptin or leptin (116–130) to adult hippocampal slices

resulted in the induction of a persistent increase in synaptic transmission. In contrast, however leptin (22–56) failed to alter excitatory synaptic strength in adult hippocampus.

AMPA receptor trafficking is pivotal for activity-dependent synaptic plasticity (Collingridge et al. 2004) and leptin regulates trafficking of GluA1 to synapses (Moult et al. 2010). In this study, treatment with either leptin or leptin (116–130) increased GluA1 surface expression in cultured hippocampal neurons, whereas leptin (22–56) was without effect. In colocalization studies, the density of GluA1 subunits associated with synapses was increased after application of leptin or leptin (116–130), suggesting that leptin (116–130) parallels the actions of leptin by boosting the synaptic insertion of AMPA receptors. We have shown that leptin-driven trafficking of GluA1 involves inhibition of PTEN (Moult et al. 2010). Similarly in this study, the ability of leptin (116–130) to influence GluA1 trafficking involves inhibition of PTEN, as application of the PTEN inhibitor bpV blocked the increase in GluA1 surface expression induced by leptin (116–130) in hippocampal neurons. These data indicate that like leptin, treatment with leptin (116–130) promotes GluA1 trafficking to hippocampal synapses via inhibition of PTEN. Thus, overall these data indicate that the leptin fragment (116–130) mirrors the actions of leptin as it markedly influences the cellular events underlying learning and memory by regulating AMPA receptor trafficking.

It is known that $A\beta$ inhibits the induction of hippocampal LTP (Shankar et al. 2008), and this detrimental effect of $A\beta_{1-42}$ is reversed by leptin (Doherty et al. 2013). Similarly, leptin (116–130) reversed the acute effects of $A\beta_{1-42}$ in this study as synaptic plasticity was readily induced in hippocampal slices exposed to leptin (116–130) and $A\beta_{1-42}$. Contrastingly, application of leptin (22–56) failed to prevent the detrimental effects of $A\beta_{1-42}$ as no increase in synaptic strength was induced after exposure to $A\beta_{1-42}$ and leptin (22–56). However, in slices exposed to leptin (22–56) post-tetanic potentiation (PTP) and some STP was observed after HFS, suggesting that this fragment may influence the transient enhancement of synaptic strength induced by HFS. As PTP and STP are thought to involve presynaptic expression mechanisms (Zucker and Regehr 2002; Lauri et al. 2007), it is feasible that leptin (22–56) can act presynaptically to influence glutamate release mechanisms.

Several studies indicate that $A\beta_{1-42}$ also facilitates the induction of hippocampal LTD (Shankar et al. 2008; Li et al. 2009), and this effect is also reversed by leptin (Doherty et al. 2013). In accordance with these findings, treatment with leptin reduced the magnitude of LTD in $A\beta_{1-42}$ -treated slices. Similarly, leptin (116–130), but not leptin (22–56) attenuated the effects of $A\beta_{1-42}$ as the magnitude of LTD was significantly decreased in the presence of leptin (116–130). Moreover, application of either leptin or leptin (116–130) inhibited $A\beta_{1-42}$ -driven AMPA receptor removal from hippocampal synapses, whereas treatment with leptin (22–56) was without effect. Thus overall these data demonstrate that leptin (116–130) mirrors the actions of leptin in counteracting the detrimental acute effects of $A\beta_{1-42}$ on hippocampal synaptic function.

Evidence is growing that leptin has neuroprotective actions in various models of neurodegenerative disease. In Parkinson's disease models, treatment with leptin protects dopaminergic neurons from various toxic insults (Weng et al. 2007; Doherty et al. 2008), whereas in AD models of amyloid toxicity, leptin increases neuronal viability via activation of STAT3 and PI3-kinase signalling (Doherty et al. 2008; Guo et al. 2008; Doherty et al. 2013). In this study, leptin and leptin (116–130) enhanced the survival of human neural (SH-SY5Y) cells treated with

either $A\beta_{1-42}$ or Cu^{2+} . Conversely no change in cell viability was evident after treatment with leptin (22–56), thereby providing further evidence that leptin (116–130) but not leptin (22–56) mirrors the protective actions of leptin.

In these studies, we reveal that signalling via PI3-kinase and STAT3 is essential for leptin (116–130)-mediated neuroprotection as selective inhibition of these pathways eliminated the protective effects of leptin (116–130). Moreover, direct activation of key components of PI3-kinase and STAT3 signalling pathways was observed following administration of leptin (116–130). As stimulation of both PI3-kinase (Doherty et al. 2013; Doherty et al. 2008) and STAT3 signalling cascades (Doherty et al. 2013; Guo et al. 2008) mediate the neuroprotective actions of leptin, these data indicate that leptin (116–130) is activating the same signalling pathways as the full length leptin peptide to induce neuronal survival. This provides further evidence that leptin (116–130) is mirroring the neuronal effects of leptin.

These studies demonstrate that the 116–130 fragment of the leptin molecule enhances hippocampal synaptic plasticity and has neuroprotective effects. As such this fragment is a very interesting therapeutic target to treat memory dysfunction and protect against neurodegeneration in the early stages of AD. To test the functional implications of the effects of leptin 116–130 we examined the effects of acute doses of this fragment on a test of episodic-like memory. This test is particularly appropriate as it models the type of memory that is first compromised in AD. Performance on the task has been shown to be impaired in rodents with damage to the lateral entorhinal cortex (Wilson et al. 2013), the first region to be damaged in AD, and the hippocampus (Langston and Woods, 2010). It has also been shown that the triple transgenic murine model of AD show impaired performance on this task at 6 months of age (Davis et al. 2013). The current data demonstrate powerful cognitive enhancing effects of both leptin and leptin (116–130) as both groups performed significantly better than controls on the OPC task. This is the first time that leptin has been shown to enhance the specific type of memory that degrades in AD and the fact that this cognitive enhancement is also produced by leptin (116–130) suggests that this fragment is a viable tool to treat memory dysfunction caused by damage to the hippocampal-entorhinal network. Recent studies indicate that administration of leptin also protects against $A\beta$ -induced impairments in spatial memory tasks (Tong et al. 2015). Thus, it is feasible that administration of leptin (116–130) will also mirror the effects of leptin and protect against the chronic effects of $A\beta$ on hippocampal-dependent learning and memory.

The current experiments demonstrated enhancement of memory for object–place–context associations. Enhancement of this hippocampal-dependent task is consistent with our findings showing enhancement of hippocampal synaptic plasticity but it remains a possibility that leptin 116–130 may also enhance simpler forms of recognition memory such as object recognition or object–place recognition. These simpler forms of recognition memory are dependent on other areas of the medial temporal lobe network and so future work could examine whether the cognitive enhancement is specific to the hippocampus or also affects the surrounding cortical inputs. One other consideration is the anxiolytic properties of leptin that have been reported in both normal (Liu et al. 2010) and chronically stressed rats (Lu et al. 2006). Reduced anxiety could potentially affect performance on the spontaneous recognition tasks as less anxious animals may explore more freely. This was not found to be the case in the current study as the levels of exploration in both sample and test phases of the OPC experiment

were not different between groups. This is not surprising as animals had extensive handling and pretraining before the OPC test and so levels of anxiety would have been very low in all animals.

In conclusion, these data indicate that the leptin (116–130) fragment mirrors the cognitive enhancing effects of leptin as it promotes trafficking of the AMPA receptor subunit GluA1 to synapses, facilitates hippocampal synaptic plasticity and improves performance in an episodic-like memory task. In addition, leptin (116–130) counteracts the detrimental effects of A β _{1–42} on hippocampal synaptic function and neuronal viability in various cellular models of amyloid toxicity. Our findings not only reinforce the consensus that the leptin system is an important therapeutic target in AD, but also establish that leptin (116–130) may be useful in the development of leptin-mimetic agents for therapeutic use.

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Notes

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