Aβ₁₋₄₂ inhibition of LTP is prevented by manipulation of a signalling pathway involving caspase-3, Akt and GSK-3β

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Supplementary Figure 1. Pharmacological inhibition of caspases prevents the Aβ inhibition of LTP. (a) LTP was induced in Aβ-treated cultured hippocampal slices following pre-treatment with Z-VAD-FMK (n = 6). (b) Z-VAD-FMK alone had no effect on LTP (n = 6). (c) LTP was blocked following treatment with Aβ in acute slices (close symbol, n = 7). The inhibition of LTP is prevented by pre-treatment with Z-VAD-FMK (open symbol, n = 6). (d) Z-VAD-FMK alone had no effect on LTP (control: closed symbol, n = 8; Z-VAD-FMK: open symbol, n = 6; p > 0.05, control vs Z-VAD-FMK). Error bars indicate s.e.m.
Aβ_{1-42} inhibition of LTP is prevented by manipulation of a signalling pathway involving caspase-3, Akt and GSK-3β

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Supplementary Figure 2. Scrambled peptide was unable to prevent the inhibition of LTP by Aβ. Pooled data showing that Z-FA-FMK had no effect on LTP (closed symbol), while Z-FA-FMK was unable to prevent the inhibition of LTP by Aβ (open symbol).
Aβ₁₋₄₂ inhibition of LTP is regulated by a signalling pathway involving caspase-3, Akt and GSK-3β

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Supplementary Methods:

**Amyloid-β preparation:** The amyloid-β 1-42 peptide (Aβ; Ascent Scientific, Bristol, UK) was initially dissolved at a concentration of 1 mg / ml in 100 % HFIP (1,1,1,3,3,3-hexafluoro-2-propanol [Sigma-Aldrich]). This solution was then incubated at room temperature for 1 h with occasional vortexing at a moderate speed. Next, the solution was sonicated for 10 min in a water bath sonicator. The HFIP / peptide solution was then dried under a gentle stream of nitrogen gas. 100 % DMSO was then used to resuspend the peptide, which was then incubated at room temperature for 12 min with occasional vortexing. The final solution was aliquoted into smaller volumes and stored at −80 °C. For a working solution, 500 – 1000 µl (depending on the final concentration to be used) D-PBS (Invitrogen, UK) was added to the peptide stock solution and incubated for 2 h at room temperature to allow for peptide aggregation.

**Electrophysiology:** For whole-cell recordings, cultured slices were used. As required, slices were transferred to the recording chamber, where they were submerged in slice culture artificial CSF (aCSF), containing (in mM): 119 NaCl, 2.5 KCl, 26 NaHCO₃, 1 NaH₂PO₄, 4 CaCl₂, 4 MgCl₂, 11 D-glucose, 0.1 picrotoxin and 0.002 2-chloroadenosine (bubbled with 95 % O₂ / 5% CO₂). Stimulating electrodes were placed in the subiculum and CA2 (Schaffer collateral pathway). Whole-cell recording pipette (4 – 6 MΩ) solutions (280 mOsm [pH 7.2]) comprised (in mM) 130 CsMeSO₄, 8 NaCl, 4 Mg-ATP, 0.3 Na-GTP, 0.5 EGTA, 10 HEPES, 6 QX-314. Neurons were voltage clamped at −70 mV. Single- and dual-patch recordings were carried out using a MultiClamp 700B amplifier (Axon Instruments, Foster City, CA). Excitatory postsynaptic currents (EPSC) amplitude, series resistance, input resistance, and DC were monitored and analyzed online and offline using the WinLTP software (http://www.ltp-program.com). Only cells with series resistance < 25 MΩ with a
change in series resistance < 10 % from the start were included in this study. The amplitude of the EPSCs was measured and expressed relative to the normalized preconditioning baseline. For field recording, acute hippocampal slices were prepared from 4 - 5 - week old male Wistar rats. Experiments were carried out in accordance with the UK Animals Scientific Procedures Act of 1986. Animals were sacrificed by dislocation of the neck and then decapitated. The brain was rapidly removed and placed in ice-cold artificial CSF (aCSF) containing (in mM): 124 NaCl, 3 KCl, 26 NaHCO3, 1.25 NaH2PO4, 2 CaCl2, 1 MgSO4, 10 D-glucose, and 0.1 picrotoxin (bubbled with 95 % O2 / 5 % CO2). Transverse hippocampal slices (400 µm thick) were prepared using a McIllwain tissue chopper (Mickle Laboratory Engineering Co. Ltd., Gomshall, UK). Hippocampal slices were stored in aCSF (20 – 25 °C) for 1 – 2 h before transferring to the recording chamber, in which they were submerged in aCSF (30 °C) flowing at 2 ml / min. Extracellular field potentials were recorded in the CA1 region using glass electrodes containing NaCl (3 M). A stimulating electrode in CA2 was used to evoke field EPSPs (constant voltage, 100 µs duration, repeated at 30 sec intervals). Long-term potentiation was evoked by two trains of tetanus stimuli (each 100 Hz, 1 sec; repeated after a 30 sec interval). The slope of the evoked fEPSP was measured and expressed relative to the normalized preconditioning baseline. Experiments in which changes in the fiber volley occurred were discarded. The CT-99021 was obtained from Axon Medchem (The Netherlands). Z-VAD-FMK and Z-FA-FMK was obtained from BD Biosciences (UK).

**Organotypic Brain Slice Culture:** Hippocampal slice cultures were prepared from 7- to 8-day-old Wistar rats. After decapitation, the brain was immediately placed in ice-cold cutting solution, comprised of: (in mM) 238 Sucrose, 2.5 KCl, 26 NaHCO3, 1 NaH2PO4, 5 MgCl2, 11 D-glucose, and 1 CaCl2. Hippocampal slices (350 µm) were made using a McIllwain tissue chopper. Following washing, slices were plated on top of semipermeable membrane inserts (Millipore Corporation, Bedford, MA, USA) within a 6-well plate which contained culture medium, comprised of 78.8 % minimum essential medium, 20 % heat-inactivated horse serum, 25 mM HEPES, 10 mM D-glucose, 26 mM NaHCO3, 2 mM CaCl2, 2 mM MgSO4, 70 µM ascorbic acid, 1 µg / ml insulin, pH adjusted to 7.3 and 320 – 330 mOsm. The slices were then cultured in an incubator (35 °C, 5 % CO2) for 7 – 10 days in vitro (DIV). The medium
was changed every 2 days. Neurons were transected using a biolistic gene gun (Helios Gene-gun system, Bio Rad, U.S.A.) at DIV 3 - 4. 100 µg DNA was used to make the gene gun transfection bullets, at a ratio of 9 : 1 of the construct to test : pEGFP-C1. Electrophysiological recordings were performed at 3 - 4 days after transfection.

**Caspase-3 knockout mice:** Hippocampal slices (400 µm) were prepared from 28- to 36-day-old homozygous caspase-3 mutant (−/−) and littermate control (+/+ ) mice in C57Bl6 background (obtained from R.A. Flavell, Yale University) (Kuida et al., 1996). After 15 min of stable baseline, LTP was induced by theta burst stimulation (TBS), comprised of 10 bursts, at an interburst frequency of 5 Hz and an intraburst frequency of 100 Hz; each burst consisted of 5 pulses.

**Western blotting and antibodies.** For detection of active caspase-3 and phospho GSK-3β in cell lysates, hippocampi were lysed and protein samples were resolved on sodium dodecyl sulfate (SDS)-polyacrylamide gels and transferred to PVDF membranes (Millipore, Bedford, MA, USA). Primary antibodies against caspase-3, cleaved caspase-3 and GSK-3α / β-phospho S9 were purchased from Cell Signalling Technology (MA, USA). Immunoreactive bands were then probed with horseradish peroxidase-conjugated secondary antibody for 1 h and developed using the ECL detection system (Thermo Fisher Scientific, Rockford, IL, USA). Optical densities of immunoreactive bands were quantified using NIH ImageJ software (downloaded from http://rsb.info.nih.gov/ij/).

**Statistical Analyses:** Data were analyzed from one slice per rat (i.e., n = number of slices = number of rats). Data pooled across slices are expressed as the mean ± SEM. For electrophysiology experiments, the effects of the stimulation protocol were measured ~ 20 min after LTP induction. Data are expressed relative to a normalised baseline (100 % = no change). Significance (p < 0.05) from baseline was tested using two-tailed t-tests.