Patient-derived tau and amyloid ~~-A~~β facilitate long-term depression~~LTD~~ *in vivo*: role of tumor necrosis factor alpha~~TNFα~~ and the integrated stress response

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Abstract

Alzheimer's disease is characterized by progressive cognitive decline in older individuals accompanied by the deposition of two pathognomonic proteins amyloid β (Aβ) and tau. It is well documented that synaptotoxic soluble Aβ aggregates facilitate synaptic long-term depression (LTD), a major form of synaptic weakening which correlates with cognitive status in Alzheimer's disease. Whether synaptotoxic tau, which also associates strongly with progressive cognitive decline in patients with Alzheimer's disease and other tauopathies, also causes LTD facilitation remains to be clarified.

Young male adult and middle-aged rats were employed. Synaptotoxic tau and Aβ were obtained from different sources including i) aqueous brain extracts from patients with Alzheimer's disease and Pick's disease tauopathy, ii) the secretomes of induced pluripotent stem cell (iPSC)-derived neurons from individuals with trisomy of chromosome 21 (Ts21), and iii) synthetic Aβ. *In vivo* electrophysiology was performed in urethane anesthetized animals. Evoked field excitatory postsynaptic potentials were recorded from the stratum radiatum in the CA1 area of the hippocampus with electrical stimulation to the Schaffer collateral-commissural pathway. To study the enhancement of LTD, relatively weak low-frequency electrical stimulation was used to trigger peri-threshold LTD. Synaptotoxic forms of tau or Aβ were administered intracerebroventricularly. The ability of agents that inhibit the

cytokine tumor necrosis factor α (TNFα) or the integrated stress response to prevent the effects of Aβ or tau on LTD were assessed after local or systemic injection, respectively.

We found that diffusible tau from Alzheimer's disease or Pick's disease patients' brain aqueous extracts or the secretomes of Ts21 iPSC-derived neurons, like Alzheimer's disease brain-derived Aβ and synthetic oligomeric Aβ, potently enhanced synaptic LTD in live rats. We further demonstrated that LTD facilitation by both tau and Aβ was age-dependent, being more potent in middle-aged compared with young animals. Finally, at the cellular level, we provide pharmacological evidence that the TNFα and the integrated stress response are downstream mediators of LTD facilitation by both synaptotoxic tau and Aβ.

Overall, these findings reveal the promotion of an age-dependent synaptic weakening by both synaptotoxic tau and Aβ. Pharmacologically targeting shared mechanisms of tau and Aβ synaptotoxicity, such as TNFα/ the integrated stress response, provides an attractive strategy to treat early Alzheimer's disease.

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Introduction

Many debilitating symptoms of Alzheimer's disease (AD) are attributed to synaptic failure at both functional and structural levels [1-4]. Studies of mechanisms underlying synaptopathy have evaluated the synaptotoxic potential of diffusible forms amyloid β (Aβ) and, to a lesser extent, tau. Enduring changes in synaptic efficacy at glutamatergic synapses in vulnerable circuits, such as the entorhinal-hippocampal network, are very sensitive to disruption by Aβ. Exogenous application of diffusible forms of Aβ potently inhibits long-term potentiation (LTP) [5-7] and enhances long-term depression (LTD) [8-11] of synaptic transmission, supporting an Aβ-mediated synaptic depression model of early AD pathogenesis [12-15]. In this model, the inhibition of LTP and promotion of LTD by Aβ, including those in aqueous AD brain extracts [10,16], trigger synaptic depression mechanisms that ultimately cause the gradual loss of synapses in early AD.

Tau is also strongly implicated in the synaptic defects in AD with positive correlations between the extent of tau pathology, cognitive impairment and glutamatergic synapse loss observed both post-mortem [17-21] and recently *in vivo* [22,23]. Interestingly, endogenous physiological tau has been reported to be required for certain forms of LTD [24-26] (but see [27,28]), and Aβ-mediated inhibition of LTP [29] (but see [30]), although how the synaptic depression model might be extended to include pathological tau remains to be clarified [31-33]. Although tau is primarily an intracellular protein, we previously reported LTP impairing action by exogenous application of putative synaptotoxic tau from different sources including aqueous extracts of AD brain [34,35] and Pick's disease brain [36-38] (mainly intracellular forms of tau), and secretomes of induced pluripotent stem cell (iPSC)-derived neurons from people with trisomy of chromosome 21 (Ts21)[39] (mainly extracellular forms of tau). Given the diverse forms of tau which have been shown to be synaptotoxic, we wondered if any of these species might affect LTD.

The onset of the prodromal stage of AD starts many decades earlier than clinical diagnosis of dementia, which exponentially rises in people who are in their 80s [40]. We therefore hypothesized that any shared synaptic depression-promoting action of Aβ and tau would be accentuated in middle-aged animals [41]. Growing evidence indicates significant crosstalk between pro-inflammatory processes, in particular the master pro-inflammatory cytokine tumor necrosis factor α (TNFα) [42] and the integrated stress response (ISR) in AD [43,44]. Given the potential importance of such pro-inflammatory processes and the ISR in synaptic disruption in AD [45-47], we investigated if targeting TNFα or the ISR modulated any LTD-promoting actions of Aβ or tau.

Materials and methods

Animals and surgery

Young adult (2-3-month-old) and middle-aged (9-16-month-old) male Wistar and Lister Hooded rats were provided by the Comparative Medicine Unit of Trinity College Dublin. The animals were housed under a 12:12 h light-dark cycle (lights on at 8 AM) at room temperature (19-22^{o}C) with access to food and water *ad libitum.* Prior to the acute experiments, animals were anaesthetized with urethane (1.5-1.6 g/kg, i.p.). Lignocaine (10 mg, 1% adrenaline, s.c.) was injected over the area of the skull where electrodes and screws were to be implanted. The body temperature of the rats was maintained at 37-38^{o}C with a feedback-controlled heating blanket. All efforts were made to minimize the number of animals used and their suffering. Totally 199 rats were used in this study. Animal care and experimental protocols followed the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines 2.0 [48] and were approved by the Health Products Regulatory Authority, Ireland and the Animal Research Ethics Committee of Trinity College Dublin.

Cannula implantation

Cannula implantation followed the protocols described in our previous studies [10,39]. A stainless-steel cannula (22 gauge, 0.7-mm outer diameter) was located above the right lateral ventricle (1 mm lateral to the midline and 4 mm below the surface of the dura). For intracerebroventricular (i.c.v.) injection, an internal cannula (28 gauge, 0.36-mm outer diameter) was utilized, administering solutions of drugs or Aβ and tau were at a rate of 1.5 μL/min. The placement of the cannula was verified postmortem by observing the spread of ink dye following i.c.v. injection.

In vivo electrophysiology

Electrodes were made and implanted as described previously [10,39,49]. In brief, monopolar recording electrodes and twisted bipolar stimulating electrodes were crafted from Teflon-coated tungsten wires. Electrode sites were determined using stereotaxic coordinates relative to bregma: the recording electrode was positioned 3.4 mm posterior to bregma and 2.5 mm lateral to midline, and stimulating electrode was located 4.2 mm posterior to bregma and 3.8 mm lateral to midline. Field excitatory postsynaptic potentials (EPSPs) were recorded from the stratum radiatum in the CA1 region of the right hippocampus, in response to stimulation of the ipsilateral Schaffer collateral-commissural pathway. Test EPSPs were elicited by a single square wave pulse (0.2 ms duration) at 0.033 Hz frequency, with an intensity that evoked a 50% maximum EPSP response. A peri-threshold low-frequency electrical stimulation (LFS) protocol, used to study the Aβ-mediated facilitation of LTD [10], consisted of 300 pulses (0.2 ms duration) at 1 Hz, with an intensity that evoked 95% maximum amplitude. Throughout the experiments, none of the conditioning stimulation protocols induced any detectible abnormal changes in background EEG, which was continuously monitored from the hippocampus.

Compounds and antibodies

Etanercept (Enbrel), a soluble dimeric form of the TNF receptor that very selectively binds and neutralizes TNF [50], was purchased from the Pharmacy Department, St. James's Hospital, Dublin, Ireland. Etanercept was pre-injected (i.c.v.) immediately before the injection of Aβ or tau. Tau5 (Cat. No. 80601; binding epitope at amino acids 210-241 of full-length tau) and isotype control 6E10 (Cat. No. 803015; mouse IgG1, anti-Aβ N-terminus) antibodies were purchased from BioLegend. Trans-N,N′-(cyclohexane-1,4-diyl)bis(2-(4-chlorophenoxy) acetamide (ISRIB, Sigma, SML0843**),** a very potent and highly selective drug-like small

molecule ISR inhibitor that acts by reversing the effects of eukaryotic initiation factor 2α (eIF2α) phosphorylation [51], was dissolved in dimethyl sulfoxide (DMSO) with gentle warming in a 40°C water bath and vortexed until the solution became clear. Then the solution was diluted in polyethylene glycol 400 (PEG400) and again gently warmed in a 40°C water bath and vortexed. The solution was prepared freshly and diluted in warm saline (37°C) before injection. A 1:1 mixture of DMSO and PEG400 in saline was used as vehicle control. The dose and timing choices for injection of the different agents were based on previous reports. For co-injection of antibodies, the brain extracts were co-incubated for 5 min prior to i.c.v. injection using doses based on prior findings [34,35]. The dose of etanercept (50 μg in 5 μL, i.c.v.) used here was previously reported to restore disrupted LTP in freely-moving transgenic rats overexpressing AD-associated β-amyloid precursor protein at a pre-plaque stage of amyloidosis [52]. Similarly, the ISRIB dosing was based on our previous study of its pharmacokinetics in live rats [53] and the report that one day after a single ISRIB treatment systemically (2.5 mg/kg, i.p.), age-related changes in CA1 pyramidal neuron function and structure improved [54].

Aβ- and tau-containing aqueous extracts of human brain

Human brain tissue was used in accordance with the guidelines of Trinity College Dublin Faculty of Health Science Ethics Committee (under approval 16014) and the Harvard University Partner's Institutional Review Board (Protocol: Walsh BWH 2011). The detailed methods of preparation AD brain aqueous extracts were described previously [34,35]. Whereas the vast majority of studied extracts inhibited LTP in an Aβ-dependent manner, recently we found that approximately 1 in 15 AD brain extracts retained their deleterious effects on LTP after depletion of Aβ. These extracts inhibited LTP in a tau-dependent manner, an effect abrogated by selective depletion of tau [34]. In this study, we used frozen brain tissue obtained

from four cases, three of whom died with end-stage AD and one with Pick's disease (PiD1) (**Table 1**). Two AD brain extracts, referred to as "AD1" and "AD2" used in our previous report [34], inhibit LTP in a tau-dependent manner. The third AD brain extract, referred to as "AD7" used in a separate report [35], inhibits LTP in an Aβ-dependent manner (**Table 1**). Tissue from "AD1" was obtained from the Massachusetts ADRC Neuropathology Core, Massachusetts General Hospital, and tissue from "AD2" and "AD7" was acquired from Tissue Solutions, UK. "AD1" was a 75-year-old woman, "AD2" was a 79-year-old male, "AD7" was a 95-year-old woman, and "PiD1" was a 79-year-old male. All cases met post-mortem and clinical diagnostic criteria for the relevant disorder.

Synaptotoxic tau-containing secretomes from Ts21 iPSC-derived neurons

The Ts21 iPSC line was generated from a fibroblast biopsy of an individual with Down syndrome and used in accordance with the UK Code of Practice for the Use of Human Stem Cell Lines. The detailed methods of iPSC and cortical neuron culture generation, secretome collection and processing were described previously [39,55]. Briefly, secretomes were collected at 48-h intervals between days 70 and 80. Secretomes were clarified by 3-step centrifugation (200 g for 10 min, 10,000 g for 30 min, and 100,000 g for 2 h), intended to remove dead cells, debris and exosomes, and dialyzed against artificial cerebrospinal fluid (aCSF), to remove small molecules, before freezing at -80°C. Secretomes were characterized by analytical size exclusion chromatography (SEC) and western blot analysis. Detailed information was presented in our previous report (Supplemental Experimental Procedures) [39].

Patient-derived sample immunodepletion of Aβ or tau

The detailed methods of Aβ or tau immunodepletion (ID) of brain aqueous extracts and secretomes from Ts21 iPSC-derived neurons were described previously [34,35].

For Aβ immunodepletion, one portion of AD (AD1, AD2, and AD7) brain extract was immunodepleted by 3 rounds of 12-h incubations with the anti-Aβ polyclonal antibody, AW7 and Protein A Sepharose beads at 4°C. A second portion of AD brain extract was treated identically but incubated with pre-immune rabbit serum (PIS) plus Protein A Sepharose beads to yield "mock ID" samples. Aβ immunodepletion of Ts21 secretomes was performed by 2 rounds of 12-h incubation with AW7 and Protein A Sepharose beads at 4°C. Pre-immune serum was used as an identical control. Samples were cleared of beads and aliquots stored at -80°C.

For tau immunodepletion, one portion of AD1 brain extract was immunodepleted by 2 rounds of 16-h incubations with the anti-tau mAb Tau5 (10 μg) and Protein G Agarose (10 μL) beads at 4°C. A second portion of AD1 extract was treated identically with the control mAb 46-4 and protein G agarose beads at 4°C. Extract of PiD1 (0.5 ml aliquots) was immunodepleted of tau by 3 rounds (8, 16, and 8 h) of incubation with Tau5 (10 μg) and Protein G Agarose (10 μL) beads at 4°C. Mock ID was performed identically with isotype control mAb 46-4 (10 μg) and Protein G Agarose (10 μL) beads at 4°C. Tau immunodepletion of Ts21 secretomes was performed by 2 rounds of 12-h incubation with Tau5 (10 μg) and Protein G Agarose beads at 4°C. The isotype control mAb 46-4 (10 μg) and Protein G Agarose beads were used as a control in tau immunodepletion. The Tau5 and 46-4 treated samples were cleared of beads and then incubated with Protein G Agarose alone to remove residual IgG. The aliquots were stored at -80°C before using (**Table 1**). The volumes of brain extract or secretome to be injected were based on our previously published findings [34,35,39], and determined in dose-ranging pilot experiments. Further tau characterization and quantification was presented in our previous reports using these brain extracts[35] and secretomes[39].

Preparation of oligomeric Aβ

$A\beta_{1-42}$ (human sequence) was synthesized and purified using reverse-phase HPLC by Dr. James I. Elliott at the ERI Amyloid laboratory, Oxford, CT, USA. A single batch of oligomeric Aβ was prepared with the same protocol as described previously [10]. Briefly, the peptide was dissolved in ice-cold 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) to a concentration of 1 mM, sonicated for 10 min and left to stand at room temperature for 1 h. The HFIP was evaporated using a stream of dry air/N_2 to produce a clear, homogenous peptide film. This film was dissolved in anhydrous DMSO to produce a 5 mM solution and then diluted to 100 μM in phenol red-free Ham's F12 media and vortexed for 15 s. After 18 h incubation at 4°C, the sample was centrifuged at 16,000g for 5 min to remove large preformed aggregates, and the concentration of oligomeric Aβ in the supernatant was determined using NanoDrop with the extinction coefficient ε_{275} = 1,361 $M^{-1}cm^{-1}$ [56]. Oligomeric Aβ in the sample was characterized by analytical SEC (TSKgel superSW2000). Aliquots of Aβ solution were immediately frozen on dry ice and stored at -80°C.

Data analysis

The magnitude of LTD is expressed as the percentage of pre-LFS baseline EPSP amplitude (± s.e.m.). The EPSP data are grouped into 5 min epochs (average of 10 sweeps) for graphing purpose. The last 10 min prior to LFS was used to calculate the "Pre" -induction EPSP amplitude. Unless otherwise stated the magnitude of LTD was measured over the last 10 min at the end of recording "3h" after LFS. Control experiments were interleaved randomly throughout, and no data were excluded. To compare between two groups, repeated measures two-way ANOVA with Bonferroni *post hoc* test was used (Figure 1D, 2F, 3B, 3D, 3F, 4B, 6D, 7B, and 7D). To compare between groups of three or more, one-way ANOVA with Bonferroni multiple comparisons was used (Figure 1B, 2H, 5B, 5D, 5F, and 6B). A two-

tailed paired Student's *t*-test (paired *t*) was used to compare between "Pre" and "3h" within groups (Figure 1B, 1D, 2F, 2H, 3B, 3D, 3F, 4B, 5B, 5D, 5F, 6B, 6D, 7B, and 7D). Repeated measures (RM) one-way ANOVA was used to compare three or more treatments in one group (Figure 2B and 2D). Some experiments on the effect of Ts21-secretomes on LTD were performed blindly. A $P < 0.05$ was considered as statistically significant. All data were evaluated and graphed using Prism 9.0 (GraphPad Inc, San Diego, CA, USA).

Results

AD brain-derived synaptotoxic tau facilitates LTD in an age-dependent manner

Because certain forms of tau are potent synaptotoxins [17,19-23], we wondered if tau present in AD brain aqueous extracts would, similar to Aβ, facilitate LTD. Previously, we reported that a small number (~1 in 15) of AD brain aqueous extracts disrupt LTP in a tau-dependent and Aβ-independent manner [34]. Before testing the facilitation of LTD we first confirmed that a relatively weak LFS conditioning protocol [10] was just below the threshold for inducing stable hippocampal LTD *in vivo*. Thus, application of 300 high intensity pulses at 1 Hz (LFS-300) failed to induce stable LTD at CA3-to-CA1 synapses in young adult (2-3 months of age) anaesthetized rats (**Figure 1A, B**). Next, we tested the effect of one of these synaptotoxic tau-containing extracts ("AD2") on LTD in young adult rats (**Table 1**). We found that the weak LFS protocol induced robust and stable LTD after i.c.v. injection (10 μL) of the extract that had been mock-immunodepleted (with pre-immune serum) (**Figure 1A, B**). Moreover, the same volume of this "AD2" extract previously immunodepleted with anti-Aβ polyclonal antibody, AW7, still facilitated a robust and stable LTD (**Figure 1A, B**). In contrast, co-injection of a mAb targeting the mid-region of tau, Tau5, prevented the LTD facilitation

caused by "AD2". Importantly, co-injection of the isotype-matched anti-Aβ antibody 6E10, did not (**Figure 1C, D**). To further assess the role of tau in mediating the facilitation of LTD by extracts of AD brain which inhibit LTP in a tau-dependent manner, we tested another, similar, extract ("AD1") [34] (**Table 1**). After i.c.v. injection of 10 μL of AD1 that had been immunodepleted of Aβ, baseline synaptic transmission remained stable for 2.5 h and the application of LFS-300 induced significant LTD (**Figure 2A, B**), consistent with the proposal that tau in certain AD brain extracts can facilitate LTD.

Because ageing-related processes are strongly implicated in promoting cognitive decline in AD and other tauopathies, we wondered if the facilitation of LTD by synaptotoxic tau-containing AD brain extracts was age-dependent. Having seen the facilitation of LTD by acute i.c.v. injection of 10 μL "AD1" sample, we reduced the volume of the injection to 6 μL which failed to enable LTD by LFS-300 in young animals (**Figure 2C, D**). Similar to young rats, the application of LFS-300 failed to induce stable LTD in vehicle-injected middle-aged (9-16 months of age) anaesthetized animals (**Figure 2E, F**). Remarkably, in middle-aged rats the lower volume (6 μL) of the "AD1" extract, immunodepleted by anti-Aβ polyclonal antibody AW7, facilitated a robust and stable LTD (**Figure 2G, H**). Significantly, immunodepletion of tau with the Tau5 mAb abrogated the LTD facilitation, while mock-immunodepleted with a control mAb 46-4 (AD1/46-4) did not (**Figure 2G, H**), thus confirming that this facilitatory effect in middle-aged rats was indeed mediated by tau. Due to the limited amount of the sample, we did not test "AD2" in middle-aged rats.

Synaptotoxic tau in Ts21 secretomes facilitates LTD in an age-dependent manner

Because synaptotoxic tau in AD brain aqueous extracts prepared by homogenization is mostly of intracellular origin, it is important to assess the effect of i.c.v. administration of

extracellular synaptotoxic tau on LTD. Previously, we reported that tau in the secretomes of iPSC-derived neurons from people with Ts21, the most common genetic cause of AD, inhibited hippocampal LTP *in vivo* [39]. We wondered if this extracellular source of synaptotoxic tau might also facilitate LTD in an age-dependent manner. Therefore, we tested the effect of Ts21-secretomes on LTD in both young and middle-aged rats. After i.c.v. injection of 10 μL, a volume that inhibits LTP *in vivo* [39], of a Ts21 secretome that had been immunodepleted of Aβ, the application of LFS-300 facilitated a very small LTD in young rats (**Figure 3A, B**). It is likely that the apparent low potency of these samples is due to the relatively low concentration of tau compared with brain extracts, although we did not increase the volume above 10 μL to test the efficacy of higher doses of tau in young adult rats. However, the same volume of this secretome facilitated the induction of a robust and stable LTD in middle-aged rats (**Figure 3C, D**). Immunodepletion of tau with the mAb Tau5 abrogated the LTD facilitation (**Figure 3E, F**). We conclude that synaptotoxic tau in AD brain and a human iPSC AD model facilitates LTD in an age-dependent manner.

Pick's disease brain-derived synaptotoxic tau enhances hippocampal LTD

To avoid the concomitant effects on LTD facilitation by both synaptotoxic tau and Aβ in AD brain extracts, we further tested brain aqueous extracts from a different tauopathy Pick's disease. Extracts of PiD1 brain had previously been reported to contain synaptotoxic tau that triggered neurite retraction in cultured human neurons [57] and inhibited hippocampal LTP *in vitro* [57] and *in vivo* [35]. We first injected 10 μL of mock immunodepleted PiD1 extract, a volume lacking effect on baseline synaptic transmission, into the cerebral ventricles of five young animals, application of LFS-300 induced robust and lasting LTD (**Figure 4A, B**). However, when we injected 10 μL of PiD1 extract that was depleted of tau with Tau5

antibody, the LTD enhancement was abrogated (**Figure 4A, B**). These findings, combined with the LTD-enhancing effects of AD brain aqueous extracts (AD1, AD2) and Ts21 secretomes, confirm our hypothesis that patient brain-derived synaptotoxic tau facilitates hippocampal synaptic LTD.

Age-dependence of the facilitation of LTD by Aβ

Previously, we reported that synthetic Aβ or synaptotoxic Aβ-containing AD brain extracts facilitated hippocampal LTD *in vivo* [10]. Because we found that LTD facilitation by AD-related tau was age-dependent and LTP inhibition by synthetic Aβ is increased in middle-aged rats [41], we wondered if synaptotoxic Aβ might also facilitate LTD in an age-dependent manner.

Since the most disease-relevant forms of Aβ are present in the brains of patients with AD, we first tested the effect of an aqueous extract of AD brain ("AD7") containing LTP-disrupting Aβ species [35] on LTD in young rats. Intracerebroventricular injection of 10 μL of Aβ-containing extract, a volume that inhibits LTP *in vivo* [35], enabled the weak LFS protocol to induce LTD whereas half this volume (5 μL) was without effect (**Figure 5A, B**). Nevertheless, this lower volume (5 μL) of Aβ-containing extract was sufficient to enhance LTD in middle-aged rats (**Figure 5C, D**). Importantly, Aβ-immunodepleted AD7 extract failed to facilitate LTD in young (**Figure 5A, B**) or middle-aged rats (**Figure 5C, D**) whereas co-injection of the anti-tau antibody Tau5 did not affect the facilitation of LTD by Aβ-containing AD7 extract (**Figure 5C, D**). These results indicate that AD7 facilitates LTD in an Aβ-dependent manner. Consistent with an age-dependent facilitation of LTD by Aβ-containing extract, synthetic Aβ also facilitated LTD in an age-dependent manner (**Figure 5E, F**). In young animals, i.c.v. injection of synthetic oligomeric Aβ enabled the induction of LTD by the weak LFS protocol when using a dose of 585 pmol (5 μL) (**Figure 5E, F**) that

inhibits LTP [58]. Half this Aβ dose (292.5 pmol, 2.5 μL) failed to enable LTD by weak LFS in young rats but this lower dose was sufficient to facilitate LTD in middle-aged rats (**Figure 5E, F**). Taken together, these data provide strong evidence that ageing enhances the synaptic plasticity disrupting action of Aβ.

A TNFα inhibitor, etanercept, ameliorates both tau and Aβ-facilitated LTD in middle-aged animals

Our findings that both AD-related synaptotoxic tau and Aβ facilitated LTD in an age-dependent manner implicate a shared synaptic depression-promoting action of Aβ and tau in early AD pathogenesis. Both Aβ and ageing trigger an increase in pro-inflammatory cytokines, including the master regulator of inflammation TNFα [46], and TNFα is known to mediate the inhibition of LTP by Aβ [59-61] and ageing [62]. Furthermore, tau triggers pro-inflammatory states which are associated with increased TNFα levels [63,64]. Because TNFα can promote certain forms of LTD, via activation of TNFR1, [59,65], we hypothesized that TNFα would be required for AD brain tau- and Aβ-facilitated LTD. We tested the role of this cytokine in middle-aged rats using etanercept which is a decoy receptor for TNFα [50]. In middle-aged rats, co-injection of etanercept (50 μg in 5 μL, i.c.v.) [52] significantly reduced the induction of LTD by LFS-300 in the presence of AD brain tau (**Figure 6A, B**). Similarly, in middle-aged rats, neither synthetic nor human/patient brain-derived Aβ facilitated LTD in the presence of etanercept (**Figure 6C, D**), leading us to conclude that TNFα could serve as a critical mediator of LTD-facilitating effects of tau and Aβ present in AD brain aqueous extracts. However, the failure to fully prevent tau-mediated LTD facilitation by etanercept indicates the possibility that other molecular mechanisms are also involved in AD-related tau-enhanced LTD.

Systemic administration of ISRIB prevents the facilitation of LTD by tau and Aβ in middle-aged animals

Pro-inflammatory stimuli can trigger cellular stress [43,47,66,67] which in turn activates numerous cellular pathways, including the integrated stress response (ISR) [47]. The ISR initiates a decrease in general protein synthesis [68], which is necessary for many forms of long-lasting synaptic plasticity and memory [47,68]. Indeed, reducing the ISR facilitates certain forms of LTP and memory in Aβ-overexpressing [69-71] and aged [54] mice. Having found that a brain-penetrant, small molecule inhibitor of the ISR, trans ISRIB [51] prevents Aβ-facilitated LTD and abrogates spatial learning and memory deficits in rats injected with synthetic Aβ [53], we systemically administered ISRIB (2.5 mg/kg, i.p.) 24 h prior to i.c.v. injection of AD brain tau in middle-aged rats. Consistent with the ISR playing an essential role, ISRIB completely abrogated the facilitation of LTD by AD brain tau (**Figure 7A, B**). Having found that AD brain tau-mediated LTD facilitation required the ISR, next we assessed its role in the facilitatory action of AD brain Aβ. Similarly, pre-treatment with ISRIB completely prevented the facilitation of LTD by AD brain Aβ (**Figure 7C, D**). We conclude that the facilitation of LTD by AD brain tau and Aβ share similar mechanisms.

Discussion

We report here that patient-derived tau, like Aβ, can facilitate the induction of synaptic LTD. Furthermore, the facilitation of LTD by tau and Aβ was age-dependent, such that doses of either protein that did not affect LTD in young rats, lowered the threshold for LTD induction in middle-aged animals. Consistent with a role for pro-inflammatory cytokines and protein synthesis disruption, both tau- and Aβ-facilitated LTD were prevented by agents inhibiting TNFα and the integrated stress response.

Our new data, that diffusible tau obtained from AD brain facilitated the induction of LTD by LFS, support an extended synaptic weakening hypothesis for synaptic failure in early AD. Whereas previous proposals had restricted themselves to a role for certain synaptotoxic forms of Aβ in driving synaptic weakening, the present work, taken in combination with research reporting LTP inhibitory actions of AD brain tau [34,39,72,73], helps establish tau as a key player as well. Very recently, we reported the interaction of Aβ and tau synaptotoxicity, with relatively low volumes of certain AD brain extracts inhibiting LTP in a manner that requires the presence of both Aβ and tau [35]. Whether or not this interaction affects LTD facilitation by Aß and tau in AD brain extracts remains to be determined. Indeed, a number of authors have suggested that the impairment of LTP and promotion of LTD mechanisms are likely two sides of the same coin enabling synaptic pruning, with long-lasting structural plasticity including synaptic loss [3,74-77].

Aqueous brain extracts contain multiple forms of tau derived from both intracellular and extracellular sources [78,79]. Although tau is primarily an intracellular protein where detergent insoluble fibrils are deposited as neurofibrillary tangles, potentially synaptotoxic forms of tau are released extracellularly [38,79-84]. For example, an extracellular, Tau5-binding form of tau secreted by Ts21 iPSC-derived neurons potently inhibits LTP [39] and facilitates LTD (this study). These secretomes contain an array of tau fragments with little or no detectable full-length protein [39]. Therapeutic targeting of different forms of extracellular tau with selective antibodies has become a major area of clinical development in the potential treatment of AD [85,86]. It is a matter of some controversy whether to target the N-terminus, mid-region or C-terminus of tau. Our finding that a mid-region-directed anti-tau antibody abrogate the LTD facilitation by patient-derived tau lends support to these strategies [87]. Future studies should further determine the relative efficacy of targeting different tau species on synaptic plasticity disruption by patient-derived tau.

Whereas tau in AD and PiD brain aqueous extracts, as well as Ts21 secretomes facilitate LTD, relatively soluble tau aggregates prepared by sonication of recombinant tau fibrils were found to inhibit, rather than facilitate, LTD [88]**.** Interestingly, tau oligomers in AD brain have been reported to have a relatively low molecular weight [36-38]. Very low amounts of full-length tau, but abundant mid-region fragments and lower levels of N-terminal and C-terminal fragments, are found in the Ts21-iPSC secretomes which inhibit hippocampal LTP [39] and facilitated LTD in the present study. Future studies will be needed to establish which isoforms and species (e.g. aggregation, truncation and phosphorylation status) of tau mediate the potent facilitation of LTD by tau found in Ts21-iPSC secretomes and AD brain extracts.

The finding that the facilitation of LTD by synthetic Aβ is enhanced in middle-aged rats complements a previous report that the threshold for the inhibition of LTP by synthetic Aβ is lowered in similarly aged animals [41]. The discovery that both AD brain Aβ- and tau-mediated LTD facilitation were also enhanced in an age-dependent manner lends further support to the potential importance of synaptic weakening processes in mediating early AD [12-15,89]. This finding is consistent with evidence in humans that changes in Aβ and tau fluid markers of AD commence in mid-life [90], usually decades before the onset and clinical diagnosis of dementia. Although the present findings leave open the question as to the mechanisms how the threshold for Aβ- and tau-induced synaptotoxicity is lowered in middle-aged rats, they help explain why ageing is a primary risk factor and support the development of interventions that target Aβ and tau in middle age prior to presentation with full-blown clinical dementia in older life.

Diffusible forms of AD Aβ have been shown to induce hyperexcitability in individual neurons and neural circuits via disrupting excitatory/inhibitory balance [91]. These mechanisms may underlie the facilitation of hippocampal LTD by Aβ [15,32]. In the case of tau, epileptogenic hyperexcitability has been found in animal models including Tau4RTg2652

mice exhibiting ~12-fold overexpression of human tau relative to endogenous mouse tau[92], transgenic mice with neuronal expression of A152T-variant human tau (hTau-A152T)[93], and FTDP-17 (frontotemporal dementia with parkinsonism linked to chromosome 17) mice over-expressing a human tau isoform with 2 N-terminal inserts, 4-microtubule-binding-repeat elements and with the three FTDP-17-linked mutations G272V, P301L, and R406W under the control of the neuron-specific Thy-1 promoter (human Tau^{VLW}) [94]. Whether, like Aβ, tau also causes hyperexcitability via excitatory/inhibitory imbalance, and if so, which variant of tau, remains elusive [87].

It is remarkable that apart from sharing the ability to facilitate LTD in a similar age-dependent manner, both Aβ and tau require TNFα and the ISR to disrupt this form of plasticity. Our working hypothesis was based on previous reports that Aβ increases TNFα in the brain [58,95-97] and thereby drives the ISR excessively [66]. Furthermore, etanercept and related TNFα-neutralizing strategies prevent Aβ-mediated activation of the ISR [66] and inhibition of LTP [60,98], and suppression of the ISR also prevents Aβ-mediated LTP inhibition [69-71]. Indeed, both TNFα activation of TNFR1 [59,65] and other activators of the ISR [53,99-102] can promote certain forms of LTD. Less is known about how tau affects TNFα [103] but tau triggers pro-inflammatory states which are associated with increased TNFα levels [63,64]. The ISR is also engaged in certain models of tauopathy [104-106] but not others [107,108]. The ISR is a complex intracellular signaling pathway that helps the cell, tissue, and organism to adapt to variable intrinsic and extrinsic stress. Given that synaptotoxic Aβ and tau species induce ISR hyperactivation, suppression of the ISR by ISRIB could mediate its beneficial effects against the synaptic plasticity disruptive actions of both Aβ and tau. Although the safety of ISRIB in humans has yet to be evaluated, systemic administration of ISRIB restores translation downstream of kinase phosphorylation of the eukaryotic initiation factor 2 α-subunit (eIF2α), without obvious behavioural side effects at the dose used in the present experiments [53]. ISRIB

blocks metabotropic glutamate receptor (mGluR)- dependent LTD induced by the mGluR agonist DHPG [99] and Aβ-mediated mGluR-LTD [53], while it does not observably affect control LTD induced by LFS-900 in live rats [109]. Direct activation of the ISR by synaptotoxic Aβ and tau species may also drive TNFα production, possibly in a positive feedback loop. Normal concentrations of TNFα can directly affect neurons expressing TNFα receptors, impacting basal synaptic transmission and synaptic plasticity by affecting NMDARs or AMPARs [110]. At higher concentration, such as that found in AD brain, TNFα also signals to astrocytes expressing TNFR1. The activation of TNFR1 in astrocytes in the hippocampus triggers the release of glutamate [111-114]. The astrocyte-released glutamate enhances presynaptic glutamate release via activating presynaptic GluN2B-containing NMDAR [114]. The activation of NMDARs, both synaptic and extrasynaptic, is critical for the induction of many forms of LTP and LTD [115]. In Aβ AD models, extrasynaptic glutamate receptors, in particular GluN2B and mGluR5, are responsible for LTP impairment [60,116] and LTD enhancement [9-11,53]. TNFα has been found to mediate LTP impairment in different animal models of AD [59,98,117]. Having found Aβ-mediated facilitation of mGluR5-LTD [10,11,53] and the involvement of TNFR1 in the induction of mGluR5-LTD [118], we report here that anti-TNFα agent prevents LTD facilitation mediated by both Aβ and tau.

Inflammaging, a chronic low-grade inflammation in the body caused by accumulated oxidative stress and cellular senescence events [119], leads to age-associated pro-inflammatory changes in the brain [120], including increased TNFα. Such changes may contribute to the lowered threshold for LTD facilitation by both Aβ and tau in middle-aged rats in the present study. Whether inflammaging is involved in the age-dependent LTD facilitation, or whether ISR-inhibition and TNFα-neutralization provide beneficial effects to ameliorate inflammaging needs to be carefully investigated.

The finding that diffusible forms of tau and Aβ species in AD brain both promote LTD via shared downstream mechanisms lends support to further exploring the synaptic weakening hypothesis of AD and detailed underlying cellular processes. In addition to the interplay between pro-inflammatory processes and the ISR, other shared mechanisms for Aβ and tau, such as cellular prion protein (PrP^C) and low-density lipoprotein receptor-related protein 1 (LRP1), have been reported. Indeed, we recently discovered that PrP^C plays a pivotal role in the synaptotoxicity mediated by soluble Aβ, tau, and α-synuclein [57]. Furthermore, PrP^C is implicated in Aβ-facilitated LTD [10] and in the impairment of LTP by both Aβ and tau [34,39]. LRP1 is known to regulate the uptake of both Aβ [121] and tau [122]. The potential involvement of these and other putative shared mechanisms in synaptic weakening by Aβ and tau warrants future investigation.

Data availability

All data supporting this study are available from the corresponding author upon reasonable request.

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Competing interests

The authors declare that they have no competing interests.

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Figure legends

Figure 1 Synaptotoxic tau in AD brain aqueous extracts facilitates hippocampal LTD in young rats. (**A**) The application of weak LFS (bar, LFS-300, 300 pulses at 1 Hz) did not induce stable LTD in young adult (2-3 months-old) anaesthetized rats. Acute i.c.v. injection of an AD brain aqueous extract containing LTP disrupting tau [34], mock immunodepleted with pre-immune serum (AD2/PIS, 10 µL, i.c.v., hash), enabled the induction of a robust and stable LTD by the weak LFS-300 protocol. After immunodepleting Aβ with a polyclonal antibody, this extract (AD2/AW7, 10 µL, i.c.v., hash) still facilitated LTD by LFS-300. (**B**) At 3 h post LFS, the EPSP measured 96.5 ± 3.0% in the vehicle control group ($n = 5$, $P > 0.05$ compared with Pre; paired t), 66.2 ± 8.3% in the AD2 mock immunodepleted group (AD2/PIS, $n = 5$, $P < 0.05$ compared with Pre and $P < 0.05$ compared with the vehicle group; paired t and one-way ANOVA-Bonferroni) and 70.0 ± 4.3% in the Aβ-ID group (AD2/AW7, $n = 5$, $P < 0.05$ compared with Pre and $P < 0.05$ compared with the vehicle group; paired t and one-way ANOVA-Bonferroni). (**C**) Co-injection of this tau-containing AD brain aqueous extract with the anti-tau antibody Tau5 prevented the facilitation of LTD by LFS-300. In contrast, co-injection of the same extract with the anti-Aβ antibody 6E10 enabled the induction of LTD by LFS-300. Open triangle, Tau5 or 6E10 antibodies; hash, "AD2" brain extract. (**D**) At 3 h post LFS, the EPSP measured 92.2 ± 5.5% in the Tau5 co-injected group (AD2/AW7+Tau5, $n = 6$, $P > 0.05$ compared with Pre; paired t) and 59.1 ± 4.0% in the 6E10 co-injected group (AD2/AW7+6E10, $n = 5$, $P < 0.05$ compared with Pre, paired t; $P < 0.05$ compared with AD2/AW7+Tau5, two-way ANOVA-Bonferroni). Calibration bars for EPSP traces: vertical, 2 mV; horizontal, 10 ms. Each data-point represents the average of 10 EPSP sweeps (5 min) and the magnitude of LTD is expressed as the percentage of pre-LFS baseline EPSP amplitude in (**A**) and (**C**).

Figure 2 AD brain tau facilitates hippocampal LTD in an age-dependent manner. (**A**) In young rats, i.c.v. injection of another AD brain aqueous extract that contains LTP disrupting tau [34] (AD1/AW7, 10 μL, i.c.v., hash) did not affect baseline synaptic transmission for 2.5 h, and enabled the induction of a small but significant LTD by the weak LFS-300 protocol. This extract had been immunodepleted of Aβ with a polyclonal antibody. As summarized in (**B**), the EPSP measured 98.1 ± 2.9% 2.5 h after i.c.v. injection of AD1/AW7 ($n = 5$, $P > 0.05$ compared with Pre-injection, RM one-way ANOVA-Bonferroni) and 81.7 ± 6.3% 1 h post LFS-300 ($P < 0.05$ compared with Pre-injection and Pre-LFS, RM one-way ANOVA-Bonferroni). (**C**) However, a lower dose (6 μL) of the same extract of AD brain did not enable the induction of LTD even after application of LFS-300 twice in young rats. As summarized in (**D**), the EPSP measured 90.8 ± 2.3% at 1 h post LFS1 ($n = 4$, $P > 0.05$ compared with Pre, RM one-way ANOVA-Bonferroni) and 87.2 ± 4.1% at 1 h post LFS2 ($P > 0.05$ compared with Pre and Post-LFS1, RM one-way ANOVA-Bonferroni). (**E**) Similar to young animals, the application of LFS-300 did not induce stable LTD in middle-aged (9-16 months-old) anaesthetized rats. (**F**) At 3 h post LFS, the EPSP measured 93.3 ± 3.6% in young rats (Young Veh, $n = 9$, $P > 0.05$ compared with Pre; paired *t*), 94.5 ± 3.3% in middle-aged group (Mid-age Veh, $n = 7$, $P > 0.05$ compared with Pre, paired *t*; $P > 0.05$ compared with young animals, two-way ANOVA-Bonferroni). (**G**) In middle-aged rats, the lower dose (6 μL) of same AD brain extract that had been immunodepleted of Aβ (AD1/AW7) enabled the induction of robust and persistent LTD by LFS-300. In contrast, the same extract of AD brain that had been immunodepleted of tau, using the anti-tau antibody Tau5 (AD1/Tau5), failed to enable the induction of LTD by LFS-300. In contrast, samples mock-immunodepleted with the isotype control mAb 46-4 (AD1/46-4) facilitated LTD. (**H**) As summarized at 3 h, the EPSP measured 57.4 ± 6.1% in AD1/46-4 group ($n = 5$, $P < 0.05$ compared with Pre, paired *t*), 59.9 ± 6.2% in AD1/AW7 group ($n = 5$, $P < 0.05$ compared

with Pre, and $P > 0.05$ compared with the AD1/46-4 group; paired *t* and one-way ANOVA-Bonferroni), 88.3 ± 6.2% in AD1/Tau5 group ($n = 5$, $P > 0.05$ compared with Pre, $P < 0.05$ compared with the AD1/46-4 group and AD1/AW7 group; paired *t* and one-way ANOVA-Bonferroni). Calibration bars for EPSP traces: vertical, 2 mV; horizontal, 10 ms. Each data-point represents the average of 10 EPSP sweeps (5 min) and the magnitude of LTD is expressed as the percentage of pre-LFS baseline EPSP amplitude in (**A**), (**C**), (**E**), and (**G**).

Figure 3 Extracellular soluble tau in Ts21 secretomes facilitates hippocampal LTD in an age-dependent manner. (**A**) The application of LFS-300 did not induce obvious LTD in young rats after i.c.v. injection of either vehicle (PBS) or secretome of Ts21 iPSC-derived cortical neurons (Ts21/AW7, 10 μL, hash). The secretome had been immunodepleted of Aβ with the polyclonal antibody AW7. As summarized in (**B**), the EPSP measured 92.2 ± 5.5% in the vehicle control group ($n = 5$, $P > 0.05$ compared with Pre; paired *t*) and 93.4 ± 2.5% in the Ts21/AW7 group ($n = 7$, $P > 0.05$ compared with Pre, and $P > 0.05$ compared with the vehicle control group; paired *t* and two-way ANOVA-Bonferroni) three hours after LFS. (**C**) In contrast, the same volume of this secretome (Ts21/AW7) enabled the induction of robust and persistent LTD by LFS-300 in middle-aged rats. As summarized in (**D**), at 3 h post LFS, the EPSP measured 92.2 ± 4.3% in the vehicle control group ($n = 4$, $P > 0.05$ compared with Pre; paired *t*) and 53.6 ± 8.7% in the Ts21/AW7 group ($n = 5$, $P < 0.05$ compared with Pre, and $P < 0.05$ compared with the vehicle control group; paired *t* and two-way ANOVA-Bonferroni). (**E**) Immunodepletion of tau with the anti-tau antibody Tau5 (Ts21/Tau5) prevented the facilitation of LTD by Ts21 secretome in middle-aged rats whereas secretome that had been mock-immunodepleted with a control antibody 46-4 (Ts21/46-4) enabled the induction of robust and persistent LTD by LFS-300. (**F**) As summarized at 3 h, the EPSP measured 69.2 ± 6.9% in the Ts21/46-4 group ($n = 5$, $P < 0.05$ compared with Pre, paired *t*)

and 98.2 ± 6.87% in the Ts21/Tau5 group (n = 5, P > 0.05 compared with Pre, and P < 0.05 compared with the Ts21/46-4 group; paired t and two-way ANOVA-Bonferroni). Calibration bars for EPSP traces: vertical, 2 mV; horizontal, 10 ms. Each data-point represents the average of 10 EPSP sweeps (5 min) and the magnitude of LTD is expressed as the percentage of pre-LFS baseline EPSP amplitude in (**A**), (**C**), and (**E**).

Figure 4 Synaptotoxic tau in Pick's disease brain aqueous extracts facilitates hippocampal LTD in young rats. (**A**) In young animals, acute i.c.v. injection of a Pick's disease brain aqueous extract containing LTP disrupting tau [35], mock immunodepleted with the isotype control mAb 46-4 (PiD1/46-4, 10 µL, i.c.v., hash), enabled the induction of a robust and stable LTD by the weak LFS-300, whereas immunodepletion of tau with the anti-tau antibody Tau5 (PiD1/Tau5, 10 µL, i.c.v., hash) prevented the facilitation of LTD. Each data-point represents the average of 10 EPSP sweeps (5 min) and the magnitude of LTD is expressed as the percentage of pre-LFS baseline EPSP amplitude. (**B**) At 3 h post LFS, the EPSP measured 77.1 ± 2.6% in the PiD1/46-4 group (n = 5, P < 0.05 compared with Pre, paired t), and 103.5 ± 2.0% in the PiD1/Tau5 group (n = 5, P > 0.05 compared with Pre, paired t; P < 0.05 compared between two groups, two-way ANOVA-Bonferroni). Calibration bars for EPSP traces: vertical, 2 mV; horizontal, 10 ms.

Figure 5 AD brain synaptotoxic Aβ and synthetic Aβ facilitates hippocampal LTD in an age-dependent manner. (**A**) Acute i.c.v. injection of an AD brain aqueous extract containing LTP-disrupting Aβ [34] (AD7/PIS, 10 µL) enabled the induction of robust and stable LTD by the weak LFS-300 protocol in young animals while i.c.v. injection of Aβ-ID (AD7/AW7, 10 µL) extract failed to facilitate LTD. However, half this volume of the same brain extract

(AD7/PIS, 5 μL) did not enable the induction of LTD by LFS-300. (**B**) As summarized at 3 h, the EPSP measured 101.5 ± 6.3% in the AD7/AW7 (10 μL) group (n = 5, P > 0.05 compared with Pre; paired t), 55.4 ± 6.2% in the AD7/PIS (10 μL) group (n = 5, P < 0.05 compared with Pre and the AD7/AW7 group; paired t and one-way ANOVA-Bonferroni), 97.8 ± 8.0% in the AD7/PIS (5 μL) group (n = 5, P > 0.05 compared with Pre and the AD7/AW7 group; P < 0.05 compared with 10 μL treated animals, paired t and one-way ANOVA-Bonferroni). (**C**) In middle-aged rats, the lower volume (5 μL) of the same AD brain extract (AD7/PIS) enabled the induction of LTD by LFS-300 and immunodepletion of Aβ using a polyclonal anti-Aβ antibody (AD7/AW7) prevented the facilitation of LTD. In contrast, co-injection of the anti-tau antibody Tau5 (AD7/PIS+Tau5) failed to prevent the facilitation of LTD. (**D**) As summarized at 3 h, the EPSP measured 49.9 ± 10.0% in the AD7/PIS (5 μL) -injected middle-aged rats (n = 5, P < 0.05 compared with Pre; paired t), 89.3 ± 2.8% in the AD7/AW7 (5 μL) group (n = 5, P > 0.05 compared with Pre, and P < 0.05 compared with the AD7/PIS group; paired t and one-way ANOVA-Bonferroni), 59.5 ± 8.1% in the AD7/PIS+Tau5 group (n = 5, P < 0.05 compared with Pre, and P > 0.05 compared with the AD7/PIS group; paired t and one-way ANOVA-Bonferroni). (**E**) In young rats, whereas acute i.c.v. injection of 585 pmol (5 μL) synthetic Aβ facilitated the induction of a robust and persistent LTD by LFS-300, injection of half this dose (292.5 pmol in 2.5 μL) failed to facilitate LTD. In middle-aged rats, in contrast, the lower dose of Aβ enabled the induction of robust LTD by the same weak conditioning stimulation protocol. (**F**) As summarized at 3 h, the EPSP measured 60.0 ± 4.9% in higher dose (585 pmol in 5 μL) Aβ-injected young rats (n = 6, P < 0.05 compared with Pre; paired t), 93.0 ± 3.4% in lower dose (292.5 pmol in 2.5 μL) Aβ-injected young rats (n = 5, P > 0.05 compared with Pre and P < 0.05 compared with the young (5 μL) group; paired t and one-way ANOVA-Bonferroni) and 62.8 ± 11.2% in lower dose (292.5 pmol in 2.5 μL) Aβ-injected middle-aged rats (n = 5, P < 0.05 compared with Pre and P < 0.05

compared with lower dose Aβ-injected young rats; paired *t* and one-way ANOVA-Bonferroni). Calibration bars for EPSP traces: vertical, 2 mV; horizontal, 10 ms. Each data-point represents the average of 10 EPSP sweeps (5 min) and the magnitude of LTD is expressed as the percentage of pre-LFS baseline EPSP amplitude in (**A**), (**C**), and (**E**).

Figure 6 The TNFα inhibitor etanercept ameliorates the facilitation of LTD by synaptotoxic tau and Aβ in middle-aged rats. (**A**) Pre-injection of etanercept (Etn, 50 μg/5 μL, i.c.v.) reduced the facilitation of LTD by i.c.v. injection of synaptotoxic tau-containing AD brain extract AD1/46-4 (6 μL) in middle-aged rats. Triangle: Etanercept; hash: AD1/46-4. (**B**) As summarized at 3 h, the EPSP measured 82.2 ± 6.0% in Etn+AD1/46-4 group ($n = 5$, $P > 0.05$ compared with the vehicle control group (94.5 ± 3.3%, $n = 7$) and $P < 0.05$ compared with the AD1/46-4 group (57.4 ± 6.1%, $n = 5$), one-way ANOVA- Bonferroni). (**C**) The same dose of etanercept prevented LTD facilitation by both synthetic Aβ (292.5 pmol in 2.5 μL) and synaptotoxic Aβ-containing AD brain extract (AD7/PIS, 5 μL) in middle-aged rats. (**D**) As summarized at 3 h the EPSP measured 93.9 ± 3.1% in Etn+Aβ group, $n = 5$, $P > 0.05$ compared with Pre, paired *t*; 94.7 ± 3.9% in Etn+AD7/PIS group, $n = 5$, $P > 0.05$ compared with Pre, paired *t*; $P > 0.05$ compared between two groups, two-way ANOVA-Bonferroni). Calibration bars for EPSP traces: vertical, 2 mV; horizontal, 10 ms. Each data-point represents the average of 10 EPSP sweeps (5 min) and the magnitude of LTD is expressed as the percentage of pre-LFS baseline EPSP amplitude in (**A**) and (**C**).

Figure 7 ISRIB prevents the facilitation of LTD by AD brain tau and Aβ in middle-aged rats. (A) Systemic pre-treatment of ISRIB (24h pre, 2.5 mg/kg, i.p.) completely abolished the facilitation of LTD by AD1/46-4 (6 μL) in middle-aged rats. **(B)** Summary at 3 h (95.1 ±

7.1% in the ISRIB treated group, $n = 4$, $P > 0.05$ compared with Pre and $P < 0.05$ compared with the Veh+AD1/46-4 group; paired *t* and two-way ANOVA-Bonferroni). **(C)** Acute i.c.v. injection of the lower volume of diffusible Aβ-containing AD brain extract (AD7/PIS, 5 μL) enabled LTD by LFS-300. Systemic pre-treatment of ISRIB (24h pre, 2.5mg/kg, i.p.) abolished LTD facilitation by AD7/PIS in middle-aged rats. **(D)** Summary at 3 h (99.9 ± 5.6% in the ISRIB treated group, $n = 5$, $P > 0.05$ compared with Pre and $P < 0.05$ compared with the Veh+AD7/PIS group; paired *t* and two-way ANOVA-Bonferroni). hash: AD1/46-4- or AD7/PIS. Calibration bars for EPSP traces: vertical, 2 mV; horizontal, 10 ms. Each data-point represents the average of 10 EPSP sweeps (5 min) and the magnitude of LTD is expressed as the percentage of pre-LFS baseline EPSP amplitude in (**A**) and (**C**).

Table 1. Previously reported effectiveness of Aβ ID with AW7 and tau ID with Tau5 on LTP inhibition by the brain extracts and Ts21 secretomes used in the present studies

Figure in this study	*Patient's Code*	*Immunodepletion information*	*Percent reduction of Aβ or tau*	*LTP inhibition*	*Reference*
Fig. 1	AD2	mock ID (PIS)		Yes	*Ondrejcak et al., 2018* [34]
		Aβ ID (AW7)	nd	Yes	*Ondrejcak et al., 2018* [34]
Fig. 2,6,7	AD1	Aβ ID (AW7)	~ 82%	Yes	*Ondrejcak et al., 2018* [34]
		mock ID (46-4)		Yes	*Ondrejcak et al., 2018* [34]
		tau ID (Tau5)	~ 82%	No	*Ondrejcak et al., 2018* [34]
Fig. 3	PiD1	mock ID (46-4)		Yes	*Ondrejcak et al., 2023* [35]
		tau ID (Tau5)	~ 48%	No	*Ondrejcak et al., 2023* [35]
Fig. 4	Ts21	Aβ ID (AW7)	<LLoQ	Yes	*Hu et al., 2018* [39]
		mock ID (46-4)		Yes	*Hu et al., 2018* [39]
		tau ID (Tau5)	~ 79%	No	*Hu et al., 2018* [39]
Fig. 5,6,7	AD7	mock ID (PIS)		Yes	*Ondrejcak et al., 2023* [35]
		Aβ ID (AW7)	~ 98%	No	*Ondrejcak et al., 2023* [35]

nd: not determined; LLoQ: the lower limit of quantitation; PIS: pre-immune serum

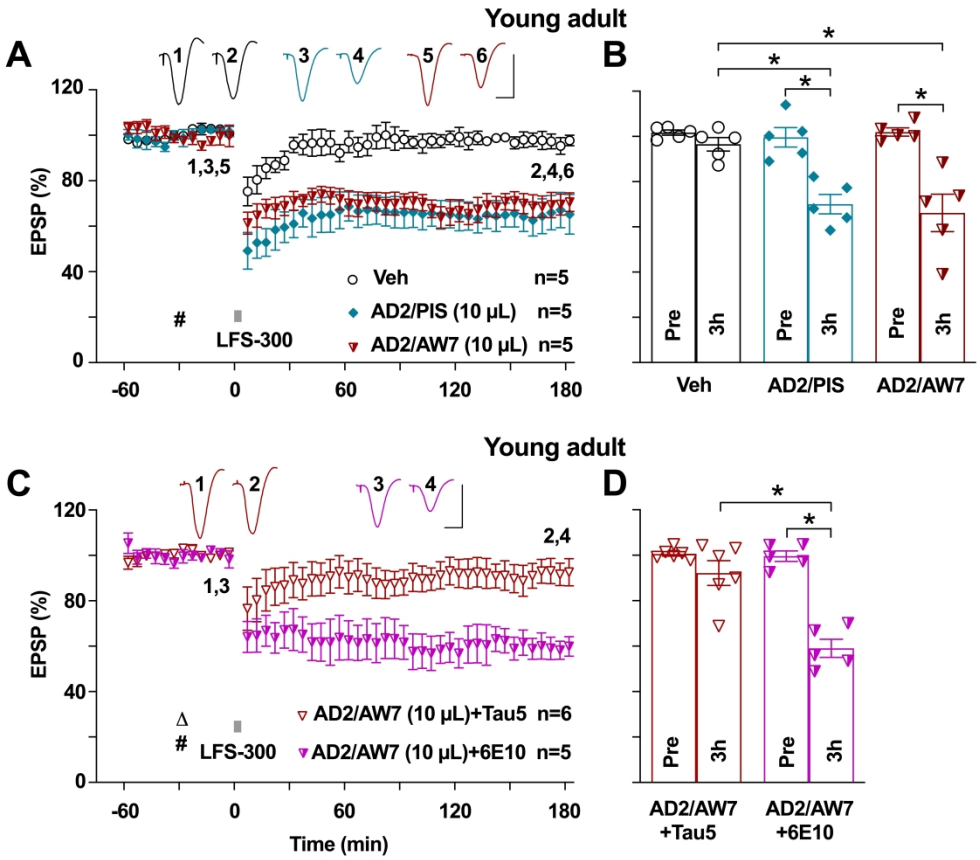

Figure 1 Synaptotoxic tau in AD brain aqueous extracts facilitates hippocampal LTD in young rats.

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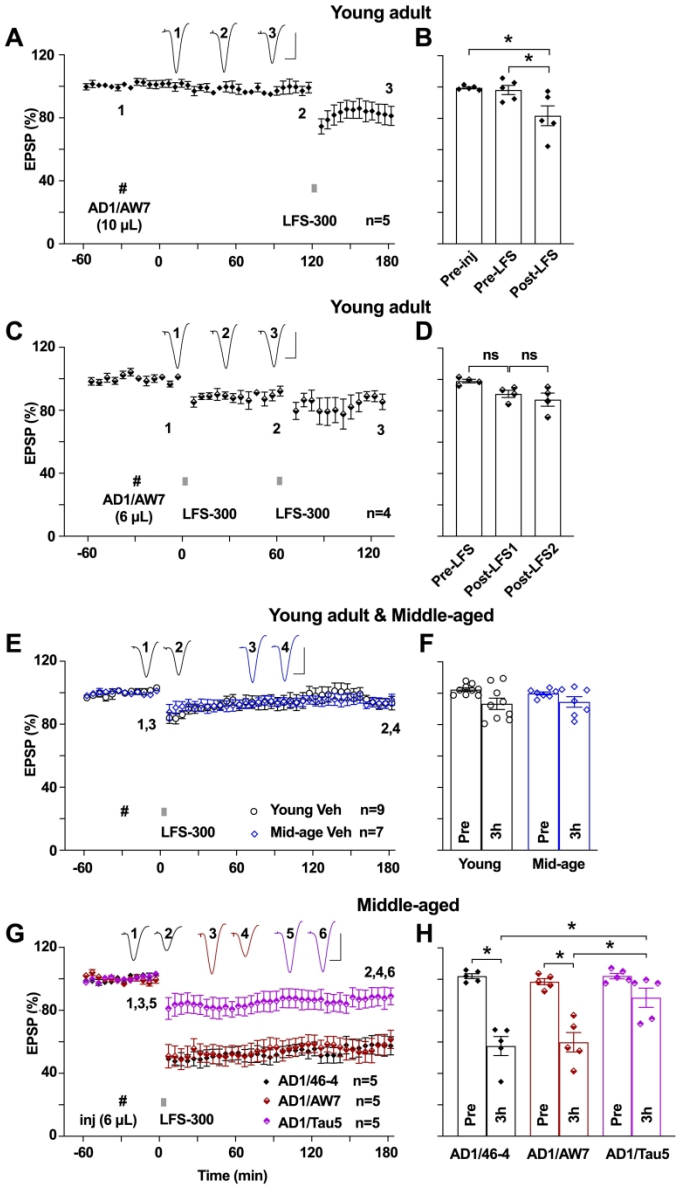

Figure 2 AD brain tau facilitates hippocampal LTD in an age-dependent manner.

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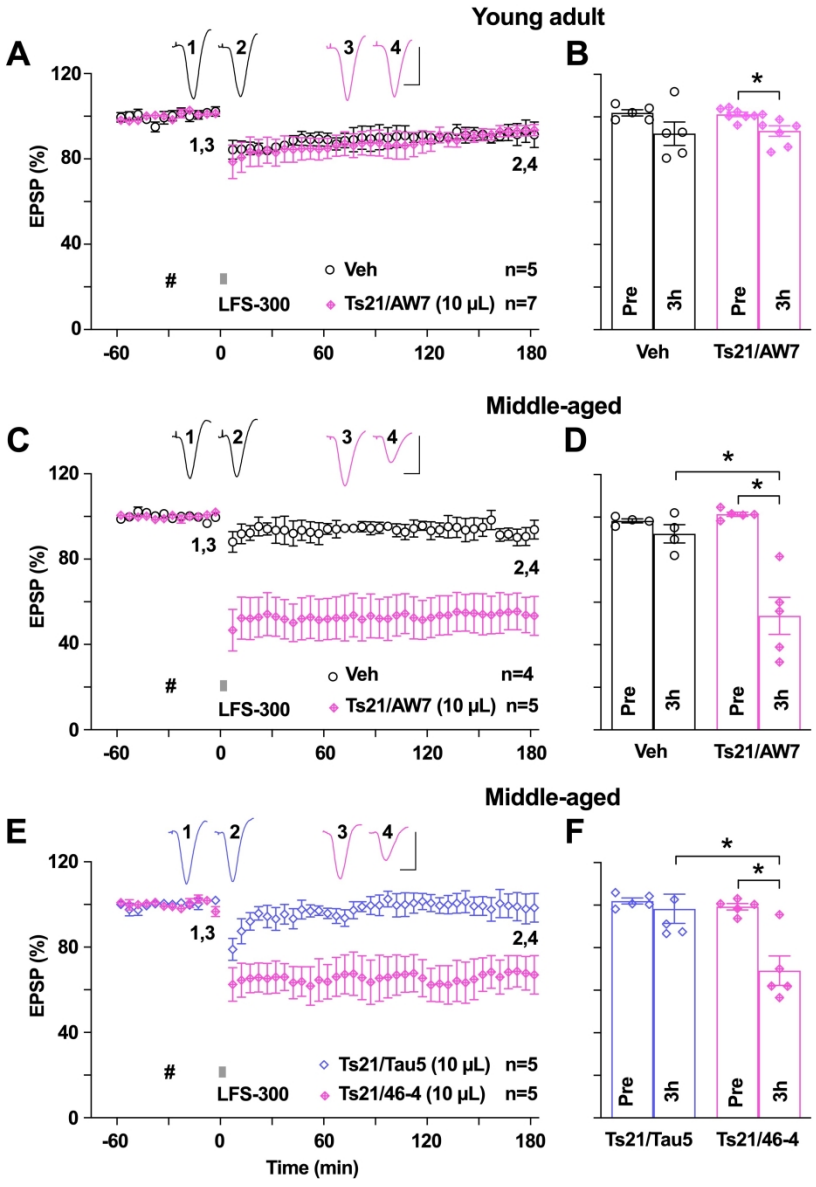

Figure 3 Extracellular soluble tau in Ts21 secretomes facilitates hippocampal LTD in an age-dependent manner.

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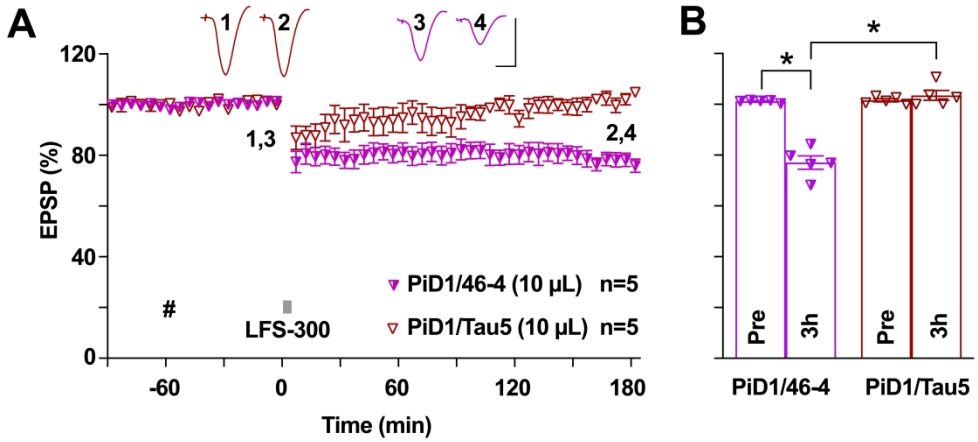

Figure 4 Synaptotoxic tau in Pick's disease brain aqueous extracts facilitates hippocampal LTD in young rats.

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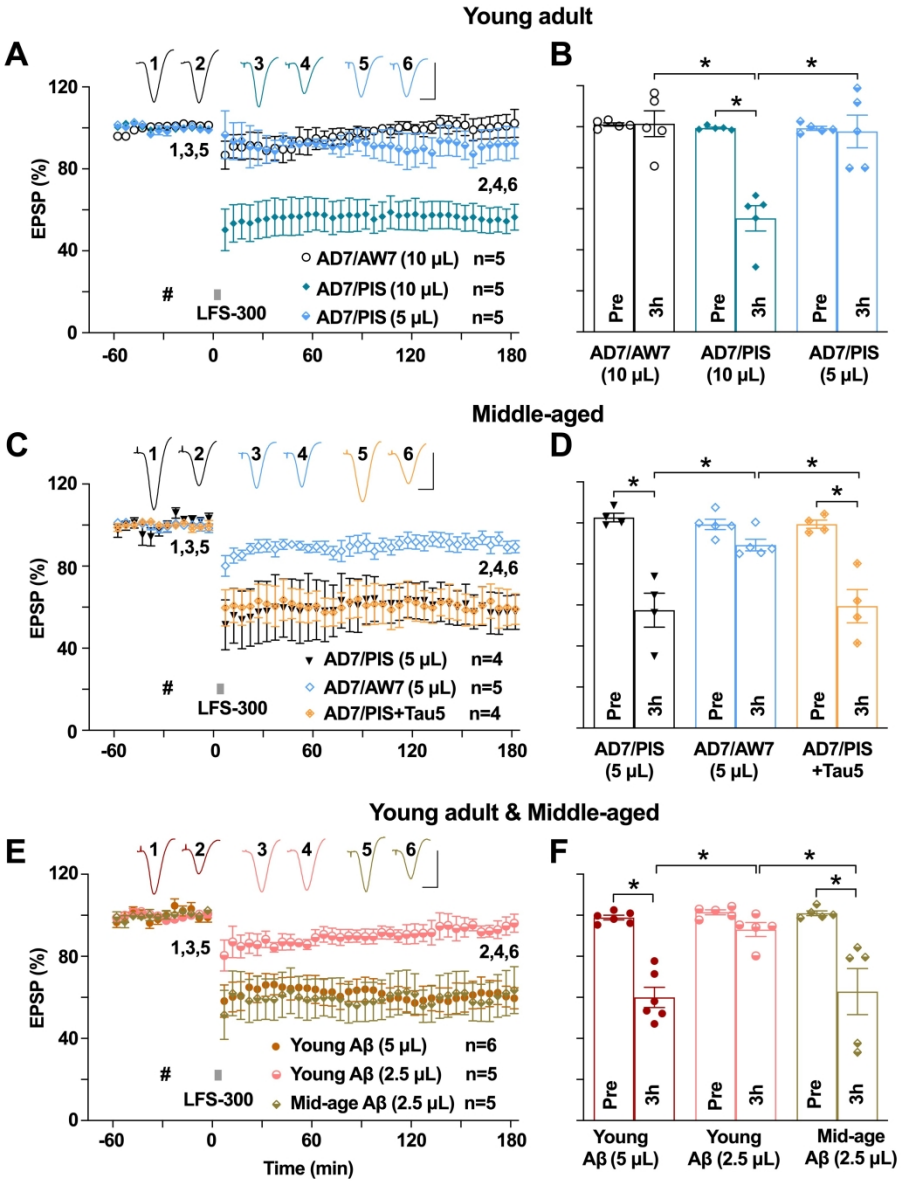


Figure 5 AD brain synaptotoxic Aβ and synthetic Aβ facilitates hippocampal LTD in an age-dependent manner.

137x179mm (600 x 600 DPI)

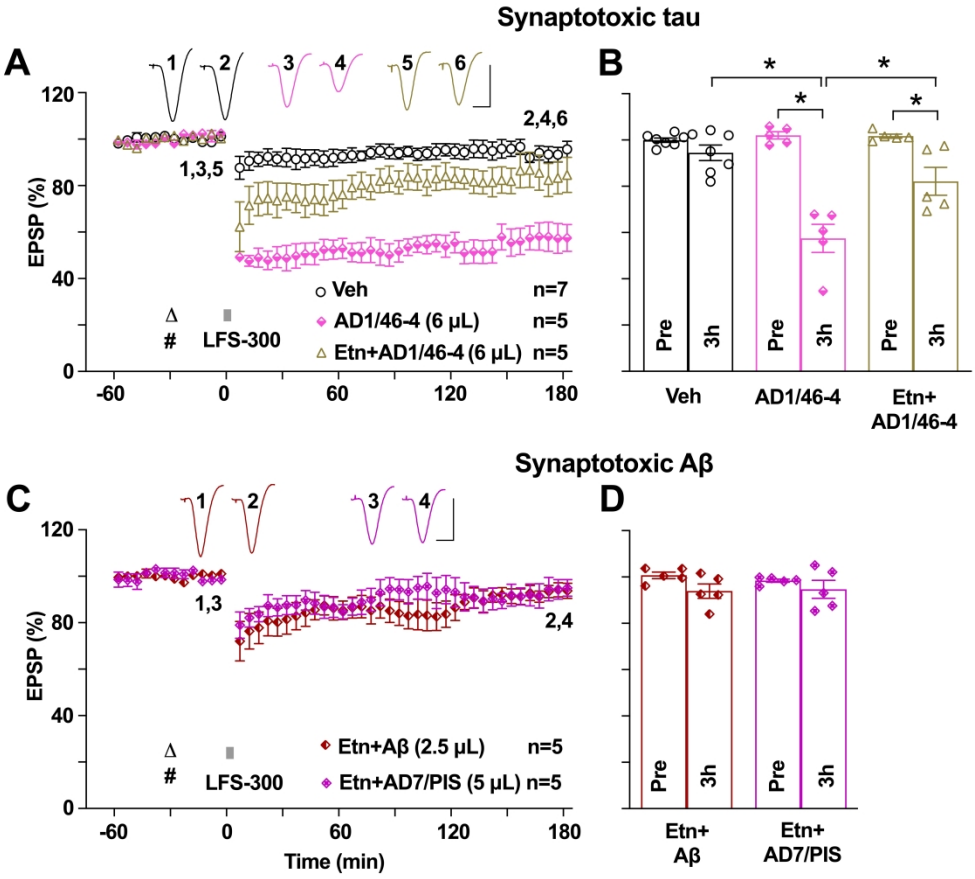


Figure 6 The TNFα inhibitor etanercept ameliorates the facilitation of LTD by synaptotoxic tau and Aβ in middle-aged rats.

134x120mm (1200 x 1200 DPI)

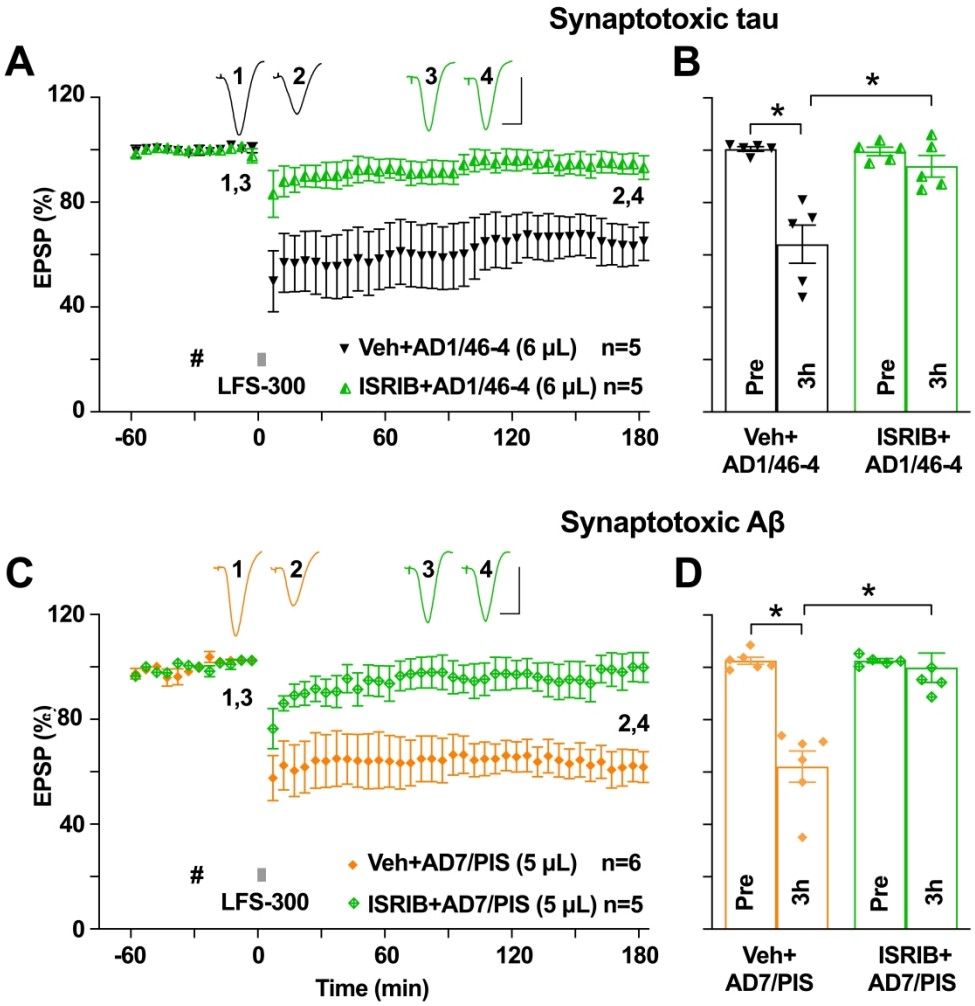
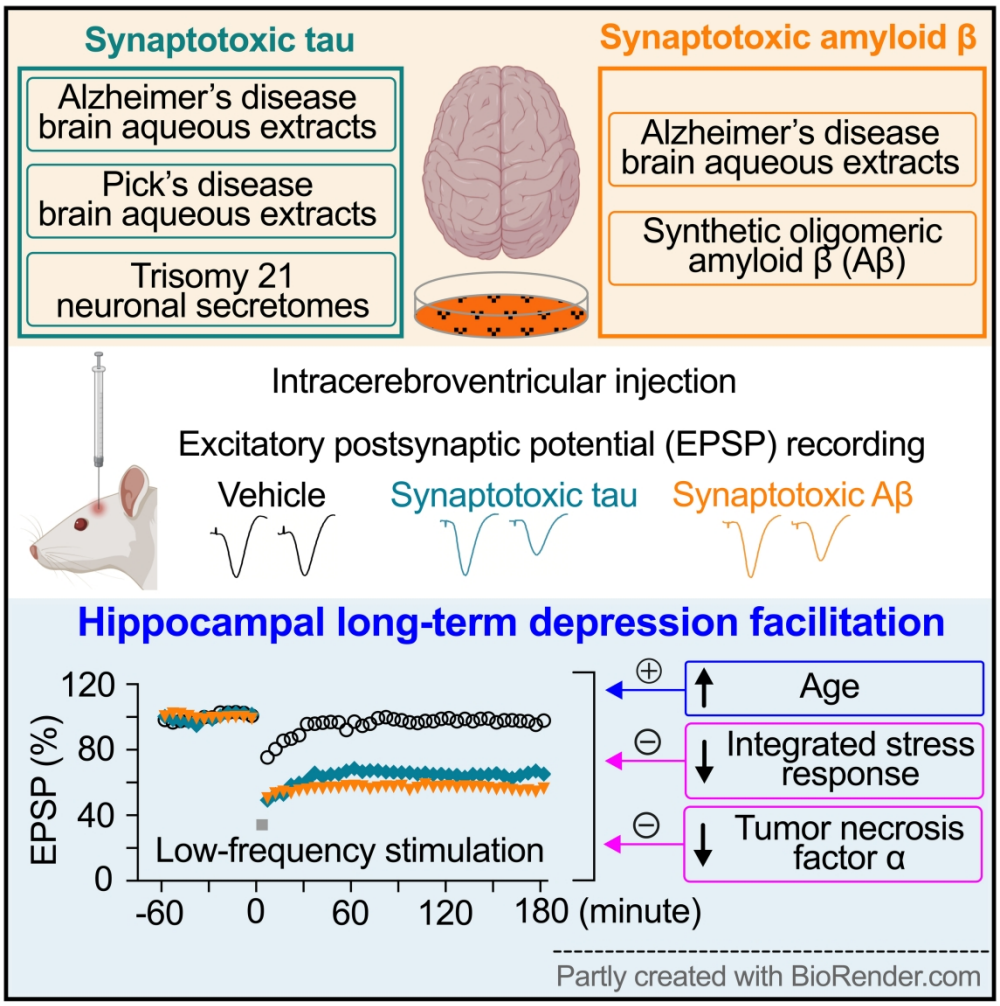


Figure 7 ISRIB prevents the facilitation of LTD by AD brain tau and Aβ in middle-aged rats.

118x122mm (1200 x 1200 DPI)



GRAPHICAL ABSTRACT

122x122mm (600 x 600 DPI)

Hu et al. report that Alzheimer's disease-related proteins tau and amyloid-β both facilitate synaptic long-term depression, a process by which synapses become less efficient in transmitting neuronal signals. Thus, targeting common pathways of both proteins could be a promising approach for Alzheimer’s disease treatment.

The ARRIVE guidelines 2.0: author checklist

The ARRIVE Essential 10

These items are the basic minimum to include in a manuscript. Without this information, readers and reviewers cannot assess the reliability of the findings.

Item		Recommendation	Section/line number, or reason for not reporting
Study design	1	For each experiment, provide brief details of study design including: a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated. b. The experimental unit (e.g. a single animal, litter, or cage of animals).	Data analysis section (page 10) Animals and surgery section (page 4)
Sample size	2	a. Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used. b. Explain how the sample size was decided. Provide details of any *a priori* sample size calculation, if done.	Figure Legends section (page 38-44); Animals and surgery section (page 4) Animals and surgery section (page 4)
Inclusion and exclusion criteria	3	a. Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established *a priori.* If no criteria were set, state this explicitly. b. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so. c. For each analysis, report the exact value of *n* in each experimental group.	Data analysis section (page 10) Data analysis section (page 10) Data analysis section (page 10)
Randomisation	4	a. State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence. b. Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly.	Data analysis section (page 10) Animals and surgery section (page 4)
Blinding	5	Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).	Data analysis section (page 10)
Outcome measures	6	a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes). b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.	Results section (page 11-16) Results section (page 11-16)
Statistical methods	7	a. Provide details of the statistical methods used for each analysis, including software used. b. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met.	Data analysis section (page 10) Data analysis section (page 10)
Experimental animals	8	a. Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight. b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.	Animals and surgery section (page 4) Animals and surgery section (page 4)
Experimental procedures	9	For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: a. What was done, how it was done and what was used. b. When and how often. c. Where (including detail of any acclimatisation periods). d. Why (provide rationale for procedures).	Materials and methods (page 4-10) Materials and methods (page 4-10) Materials and methods (page 4-10) Materials and methods (page 4-10)
Results	10	For each experiment conducted, including independent replications, report: a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range). b. If applicable, the effect size with a confidence interval.	Figure Legends section (page 38-44) Figure Legends section (page 38-44)

The Recommended Set

These items complement the Essential 10 and add important context to the study. Reporting the items in both sets represents best practice.

Item		Recommendation	Section/line number, or reason for not reporting
Abstract	11	Provide an accurate summary of the research objectives, animal species, strain and sex, key methods, principal findings, and study conclusions.	Abstract section (page 1-2)
Background	12	a. Include sufficient scientific background to understand the rationale and context for the study, and explain the experimental approach. b. Explain how the animal species and model used address the scientific objectives and, where appropriate, the relevance to human biology.	Introduction section (page 3-4) Introduction section (page 3-4)
Objectives	13	Clearly describe the research question, research objectives and, where appropriate, specific hypotheses being tested.	Results section (page 11-16)
Ethical statement	14	Provide the name of the ethical review committee or equivalent that has approved the use of animals in this study, and any relevant licence or protocol numbers (if applicable). If ethical approval was not sought or granted, provide a justification.	Animals and surgery section (page 4)
Housing and husbandry	15	Provide details of housing and husbandry conditions, including any environmental enrichment.	Animals and surgery section (page 4)
Animal care and monitoring	16	a. Describe any interventions or steps taken in the experimental protocols to reduce pain, suffering and distress. b. Report any expected or unexpected adverse events. c. Describe the humane endpoints established for the study, the signs that were monitored and the frequency of monitoring. If the study did not have humane endpoints, state this.	Animals and surgery section (page 4) Animals and surgery section (page 4) Animals and surgery section (page 4)
Interpretation/ scientific implications	17	a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature. b. Comment on the study limitations including potential sources of bias, limitations of the animal model, and imprecision associated with the results.	Results section (page 11-16) Discussion section (page 16-21)
Generalisability/ translation	18	Comment on whether, and how, the findings of this study are likely to generalise to other species or experimental conditions, including any relevance to human biology (where appropriate).	Discussion section (page 16-21)
Protocol registration	19	Provide a statement indicating whether a protocol (including the research question, key design features, and analysis plan) was prepared before the study, and if and where this protocol was registered.	Materials and methods section (page 4-10)
Data access	20	Provide a statement describing if and where study data are available.	Data availability (page 21)
Declaration of interests	21	a. Declare any potential conflicts of interest, including financial and non-financial. If none exist, this should be stated. b. List all funding sources (including grant identifier) and the role of the funder(s) in the design, analysis and reporting of the study.	Competing interests (page 21) Funding section (page 21)