The Neuroprotective Beta Amyloid Hexapeptide Core Reverses Deficits in Synaptic Plasticity in the 5x*FAD* APP/PS1 Mouse Model

Kelly H. Forest[#], Ruth Taketa, Komal Arora[¶], Cedomir Todorovic and Robert A. Nichols^{*,ID}

Department of Cell & Molecular Biology, John A. Burns School of Medicine,
University of Hawai'i at Manoa, Honolulu, HI, USA

Short title: Amyloid- β Core Peptide Reverses Synaptic Plasticity Deficits in a Familial Alzheimer's Disease Model

[#]Current address: Department of Biology, Dartmouth College, Hanover, NH USA

[¶]Current address: Department of Biology, College of Arts and Sciences, Georgia State University, Atlanta, GA USA

*Correspondence: robert.nichols@hawaii.edu

^{ID}ORCID: 0000-0003-0366-0162

Abstract

Alzheimer's disease (AD) is the most common cause of dementia in the aging population. Evidence implicates elevated soluble oligomeric A β as one of the primary triggers during the prodromic phase leading to AD, effected largely via hyperphosphorylation of the microtubule-associated protein tau. At low, physiological levels (pM-nM), however, oligomeric A β has been found to regulate synaptic plasticity as a neuromodulator. Through mutational analysis, we found a core hexapeptide sequence within the N-terminal domain of A β (N-A β core) accounting for its physiological activity, and subsequently found that the N-A β core peptide is neuroprotective. Here, we characterized the neuroprotective potential of the N-A β core against dysfunction of synaptic plasticity assessed in *ex vivo* hippocampal slices from 5 $\times$ FAD APP/PS1 mice, specifically hippocampal long-term potentiation (LTP) and long-term depression (LTD). The N-A β core was shown to reverse impairment in synaptic plasticity in hippocampal slices from 5 $\times$ FAD APP/PS1 model mice, both for LTP and LTD. The reversal by the N-A β core correlated with alleviation of downregulation of hippocampal AMPA-type glutamate receptors in preparations from 5 $\times$ FAD mice. The action of the N-A β core depended upon a critical di-histidine sequence and involved the PI3 kinase pathway via mTOR. Together, the present findings indicate that the non-toxic N-A β core hexapeptide is not only neuroprotective at the cellular level but is able to reverse synaptic dysfunction in AD-like models, specifically alterations in synaptic plasticity.

Introduction

Alzheimer's disease (AD) is clinically characterized by impairments in cognitive memory and function. Loss of critical pre- and post-synaptic markers have been reported for postmortem AD brain tissue [1,2], suggesting that AD-related cognitive impairments are based, in large part, on synaptic dysfunction and loss. Additionally, accumulating evidence shows a strong link between excess soluble oligomeric amyloid- β (A β) and synaptic dysfunction in AD [3-5]. Cognitive decline and synaptic plasticity deficits are reported to occur prior to the accumulation of A β plaques and tau neurofibrillary tangles in prodromic phases leading to AD [6], supporting the idea that synaptic dysfunction and mild cognitive impairment are early events driven by soluble oligomeric A β rising to abnormally high levels years prior to AD diagnosis.

Synaptic dysfunction and eventual degeneration lead to abnormal synaptic transmission and impaired long-term potentiation (LTP) and/or long-term depression (LTD), which are important in synaptic plasticity and learning and memory. Pathological levels (high nM to μ M) of A β have been shown to inhibit LTP-induction [3,7,8] and enhance LTD [9,10] in the hippocampus. On the other hand, low physiological levels (pM) of A β was found to enhance LTP and memory, indicating a hormetic effect of A β on synaptic plasticity [11-13].

Dysregulation of synaptic plasticity in AD pathogenesis involves altered regulation of NMDA-type and AMPA-type glutamate receptors. In addition to mediating A β -induced excitotoxicity, NMDA receptors can be depressed by A β at high concentrations [14], inducing LTD [15,16] as a consequence of subsequent downstream AMPA receptor internalization [15,16] and dendritic spine loss [16].

We have shown that at low concentration (pM-nM) the N-terminal A β fragment comprising amino acids 1-15/16 of the A β sequence, an endogenous peptide cleaved from A β via α -secretase [17], is more effective as a neuromodulator than full-length A β 1-42, stimulating receptor-linked increases in neuronal Ca²⁺, enhancing synaptic plasticity and enhancing contextual fear memory [13]. The A β 1-16 peptide sequence corresponds to the C-terminal 16 amino acid sequence in soluble amyloid precursor protein- α (sAPP- α), referred to as the CT α 16, which has also been shown to enhance synaptic plasticity [18]. An essential core sequence comprising amino acids 10-15 of A β (N-A β core) was identified as the active region of the N-terminal A β fragment and was further shown to protect against A β -induced neuronal toxicity [19]. Here, we aimed to better understand the neuroprotective mechanism of the N-A β core on synaptic plasticity. We investigated whether the N-A β core could rescue LTP and LTD dysfunction resulting from prolonged, elevated levels of A β in an APP/PS1 transgenic mouse model harboring mutations found in familial Alzheimer's disease (FAD), while assessing the impact on AMPA-type glutamate receptor expression in reference to the neuroprotective action of the N-A β core in A β -synaptotoxicity.

Materials and methods

Transgenic mice and cannulation surgery

All animal handling, surgery, use and euthanasia were performed under an approved IACUC protocol (11-1219-6 / 16-2282-2), compliant with NIH and Society for Neuroscience guidelines for use of vertebrate animals in neuroscience research. The human APP/PSEN1 mouse line, 5×FAD (Tg6799), on the B6.SJL background (B6SJL-Tg(APP^{SwFILOn},PSEN1*^{M146L}*^{L286V}) 6799Vas/Mmjax; obtained from JAX stock #006554, MMRRC034840 hemizygous) was used as a well characterized model for Aβ-based pathology and neurodegeneration [20], along with age-matched control (B6.SJL background) mice (MMRRC034840 Non-carrier). Mice at 7- to 8-months of age of both sexes (weight range: 28-35g), obtained from established in-house colonies and housed in ventilated enrichment cages in the John A. Burns School of Medicine AAALAC-accredited Vivarium with *ad libitum* access to food and water, were used at roughly equal numbers, this age range selected for displaying pronounced LTP deficits in the transgenic line. Inclusion/exclusion criteria were based on animal health.

For bilateral cannulation and injection, the following protocol was employed (as per ref 19). Cannulation into the dorsal aspects of both hippocampi of the 5×FAD mice at 7- to 8-months of age of both sexes was performed under full anesthesia (general: 1.2% Avertin; local at site: lidocaine) using stereotaxis (coordinates: -1.5mm anteroposterior; ±1mm lateral; -2mm depth). After the brief surgical procedure and recovery (full righting reflex), mice were subsequently housed in sound-isolated, ventilated hotels prior to peptide injection one week later. On the day of microinjection (morning), sterile saline or 500 nM N-Aβcore peptide was administered bilaterally through the cannulae in the 5×FAD

mice via microinjectors over 30s (0.5µL/side) and the mice were returned to their cages in the mouse hotel. Hippocampi were collected from euthanized mice 24 h after the bilateral microinjection of the saline or peptide, and lysates extracted from the hippocampi were prepared for immunoblot analysis (30 µg each). Euthanasia was performed under an approved IACUC protocol (11-1219-6 / 16-2282-2). This study was not preregistered and followed ARRIVE guidelines.

Preparation of Aβ peptides

Solutions of Aβ₁₋₄₂ (American Peptide; Anaspec) were prepared from aqueous stock solutions, followed by bath sonication. This preparation of full-length Aβ was previously shown to exist predominantly in the oligomeric state [see 13]. The N-Aβcore peptide (synthesized and purified to Peptide 2.0) was prepared from aqueous stock solutions.

Extracellular field potential recordings in hippocampal slices

Hippocampal slices were prepared from 7- to 8- month-old 5×FAD (Tg6799) or B6.SJL (control mice) (as per ref 13). Cervical dislocation and decapitation were performed under an approved IACUC protocol (11-1219-6 / 16-2282-2), compliant with NIH and Society for Neuroscience guidelines for use of vertebrate animals in neuroscience research. Brains were removed into ice-cold artificial cerebral spinal fluid (aCSF) consisting of 130mM NaCl, 3.5 mM KCl, 10mM glucose, 1.25mM NaH₂PO₄, 2.0mM CaCl₂, 1.5mM MgSO₄, and 24mM NaHCO₃, bubbled in 95% O₂/5% CO₂. Transverse brain slices of 400µm were obtained using a Leica vibrating microtome (Leica, VT1200S) and quickly transferred to fresh ice-cold aCSF for hippocampi isolation. Extracted hippocampi slices were incubated in bubbled aCSF in a holding chamber for 30 mins at room temperature

(23°C) after which the holding chamber was transferred to a 32°C water bath for 1 h. The chamber was then removed from the water bath and placed at room temperature for another 1 h prior to recording. The slices were subsequently transferred to a recording chamber and perfused at 3mL/min with aCSF (bubbled with 95% O₂/5% CO₂) at 32°C. The Schaffer collateral fibers were stimulated at a frequency 0.1 Hz using a bipolar stimulating electrode and CA1 field excitatory postsynaptic potentials (fEPSPs) were recorded with a glass electrode filled with 3M NaCl (resistance 1-1.5 MΩ). Basal synaptic transmission was assessed by comparing stimulus strength against fEPSP slope to generate input/output (I/O) curves. A minimum of 20 min baseline stimulation was then performed, recording every minute. The baseline and stimulus current were adjusted during this period so that fEPSP stabilized at 30-40% of maximum.

LTP was induced by a 3-theta-burst stimulation (TBS) protocol, where each burst consisted of 4 pulses at 100 Hz with a 200-ms interburst interval. LTD was induced using a low frequency stimulation (LFS) protocol, consisting of a 1Hz single pulse stimulus (900 pulses for 15 min). TBS and LFS were administered after a 20-min baseline recording period for aCSF alone or a 35-min baseline recording period in aCSF (15 min) followed by inclusion of N-Aβcore in the absence or presence of tested reagents (20 min). For the latter, TBS and LFS were administered in the presence of N-Aβcore in the absence or presence of tested reagents.

Immunoblot analysis

Hippocampi injected with N-Aβcore. Hippocampi removed from euthanized 8-month-old 5×FAD mice previously bilaterally injected with saline or N-Aβcore were

homogenized with 200-250 μ L of Pierce I.P Lysis Buffer (ThermoFisher Scientific, # 87788, lot# MJ162614) with 1x Halt Protease and Phosphatase Inhibitor Cocktail (ThermoFisher Scientific, # 78441, lot# SF248390). The homogenates were centrifuged at 18,000g for 20 min at 4°C and the supernatant was collected. The total amount of protein was quantified by a Pierce™ BCA Protein Assay Kit (ThermoFisher Scientific, # 23225).

For each condition, gel sample buffer (4x; ThermoFisher Scientific, # B0007, lot # 1920132) and reducing agent (10x; ThermoFisher Scientific, # B0009, lot # 1901009) were added to diluted SDS-solubilized protein samples for a final protein concentration of 2 μ g/ μ L. The samples were boiled at 95°C for 10 min, immediately cooled on ice and then centrifuged. Equal amounts of protein were subjected to electrophoretic separation on a 4-20% Tris-Glycine polyacrylamide gel (ThermoFisher Scientific, #XP04200), transferred to Nitrocellulose membrane (LI-COR, # 92631092) via the iBlot2 semidry system (ThermoFisher). Recovered blots were incubated in primary antibody (see below) overnight at 4°C. The transfer blots were washed 3x (10 min each wash) in 0.1% Tween-20 in Tris-buffered saline (TBS) and incubated in the appropriate IR-dye-conjugated secondary antibody (LI-COR Biosciences) for 1h. An Odyssey IR imaging system (LI-COR Biosciences, Lincoln, NE) was used for signal detection. Analysis was performed via Image Studio v5.2.5 software (LI-COR Biosciences).

The following primary antibodies were used for detection and normalization, respectively: anti-pAMPA1 rabbit monoclonal antibody (pS831-GluA1; Cell Signaling Technology; RRID:AB_2799873) and anti-AMPA1 rabbit monoclonal antibody (GluA1;

Cell Signaling Technology; RRID:AB_2732897), both at 1:1000 and anti-beta actin mouse monoclonal antibody (Sigma-Aldrich; RRID:AB_476697) at 1:10,000 dilution.

Cultured hippocampal neurons

Hippocampal neuron cultures were prepared as described [21] from neonatal mouse pups (0-2d old; one litter (6-10 mice)/preparation) from female breeders housed in the John A. Burns School of Medicine AAALAC-accredited Vivarium of either gender (equivalent numbers) obtained from established colonies of wild-type B6.SJL (background) mice. All animal procedures (handling; euthanasia) followed an IACUC-approved protocol (16-2282-2), compliant with NIH and Society for Neuroscience guidelines for use of vertebrate animals in neuroscience research. Following rapid decapitation, brains were removed from the mice into ice-cold Neurobasal A medium (NB) containing B-27 supplement, 5% fetal bovine serum (FBS) and Gentamicin. Hippocampi were then dissected under a stereomicroscope (Olympus SZ61). The isolated hippocampi were treated with papain (Worthington, LS003126, Lot # 35N16202) in Hanks buffer with 10mM cysteine at 37°C for 15mins. The partially digested tissue was washed by centrifugation in NB plus FBS. The cells were dissociated using sequential trituration (polished Pasteur pipettes of decreasing diameter) and collected by low-speed centrifugation. The dissociated cells were pre-plated in standard tissue culture dishes to remove adherent non-neuronal cells (glia; fibroblasts) for 10-15 mins. The neuron-enriched preparation was diluted to 1×10^5 cells/mL and then plated into poly-D-lysine-coated 24-well dishes in NB plus serum and Gentamicin. The cultures were washed with Neurobasal A medium containing B27 and

Gentamicin to remove the serum and then cultured in this media for 7-10 days prior to treatment with A β , N-A β core or the combination for an additional 7 days.

qPCR

RNA was extracted from treated cultured hippocampal neurons using the PureLink® RNA Mini Kit (Ambion, Life Technologies, #12183025) as per the manufacturer's protocol. Genomic DNA contamination was eliminated the RNA preparation by digesting with RNase-free DNase (Qiagen, #79254). The iScript™ cDNA Synthesis Kit (Bio-Rad) was used to synthesize cDNA. The expression levels of various genes were then determined using SYBR green via qPCR (Bio-Rad iCycler iQ™ Multicolor Real-Time PCR Detection System) using the primers listed in the accompanying table (Table 1). Cycling conditions were as follows: 95 °C for 15 min, followed by 40 cycles of 94 °C for 15 s and 60 °C for 60 s and the fold-changes in the variously treated samples compared to untreated (vehicle control) samples were calculated after normalizing to GAPDH gene expression.

Table 1

Gene (Accession number)	Sequence (5'-3')
GluA1 (NM_001113325)	
Forward	AAGAGAAACAAGAGAAACCT
Reverse	GATGTACGGCATATTCCTT
GluA2 (NM_001083806)	
Forward	TTTGTGGATGCTCTACTT
Reverse	ATCTGTATGCTGTTAGAAGA
Akt1 (NM_009652)	
Forward	AGAAGAGACGATGGACTT
Reverse	GCTTCAGGTACTCAAACCT
Akt3 (NM_011785)	
Forward	GATGTGGACTTACCTTATC
Reverse	TTCCTCTGGTGTATCTAC
mTOR (NM_020009)	
Forward	GGCACACATTTGAAGAAG
Reverse	GTGATCTCCTCCATCTCT
PI3KCa (NM_008839)	
Forward	TGATTCTGACTCCATAAGG
Reverse	GTCACATCATTCCATTGG
PI3KCb (NM_029094)	
Forward	GATGTTATGGAAGCAAGT
Reverse	CATATACAGCAGTTTGATTC

Data and statistical analysis

Treatment and units were randomized as to order for all assays and experiments. Biological replicates were based on independent samples (n). All experiments were repeated at least three times unless otherwise noted. After testing for normality, multiple comparisons of the data were made using one-way ANOVA with Bonferroni or Tukey's post hoc tests, as indicated. Paired comparison was made using Student's t -tests. P -values <0.05 were considered the minimum for significance (as rejection of the null hypothesis). Unless otherwise noted, data were analyzed and graphed with GraphPad Prism 5 (GraphPad v5.0b; RRID:SCR_002798) using the appropriate statistical tests.

Results

The N-A β core reversed LTP deficits induced by pathological levels of full-length A β

We have previously shown that the N-terminal A β fragment (A $\beta_{1-15/16}$) enhances synaptic plasticity and contextual fear memory while protecting against A β -linked synaptic impairment [13]. Considering the evidence that the N-A β core accounts for the neuromodulatory activity of the N-terminal A β fragment, we assessed whether the N-A β core is capable of reversing A β -linked synaptic dysfunction in an *ex vivo* model. We utilized hippocampal slices from a mutant APP/PS1 transgenic AD-like endophenotype mouse model (5 $\times$ FAD) and their wild-type counterparts (B6.SJL) to examine synaptic transmission. Basal synaptic transmission at the Schaffer collateral-CA1 synapses represented by input-output curves shows that the fEPSP slopes versus stimulus strength for both the 5 $\times$ FAD and B6.SJL mice were comparable (Fig 1A), ruling out any issues with regard to impact of the A β fragment peptides on baseline synaptic strength. Interestingly, treatment with the N-A β core during baseline recordings induced increases in baseline synaptic transmissions for both 5 $\times$ FAD and B6.SJL but was only significant in the B6.SJL slices (Fig 1B; average increase relative to untreated controls: 110% $\pm$ 2% s.d.).

To assess sustained changes in synaptic plasticity, we used a 3x-TBS stimulation protocol at the Schaffer collaterals to measure LTP in the CA1 region (see cross-sectional diagram of the hippocampus in Fig 1A, inset). LTP showed a trend toward enhancement for the N-A β core-treated B6.SJL slices as compared to untreated slice, though it was not significant (Figs 1C&1E). Consistent with previous findings [22], LTP in the 5 $\times$ FAD slices

was substantially reduced compared to that observed for slices from B6.SJL (Figs 1C&1E; 24.2% of control), dropping to near baseline at 60mins post-TBS. By contrast, prestimulation treatment with 500nM N-A β core restored LTP in the 5 $\times$ FAD slices to the level seen for the wild-type slices (Figs 1C&1E; 107% $\pm$ 44% s.d. of control). These findings demonstrate that the N-A β core can reverse LTP deficits induced from prolonged exposure to pathological levels of A β , while modestly enhancing basal synaptic transmission.

The N-A β core reversed full-length A β -linked downregulation of hippocampal AMPA-type glutamate receptors

Regulation of synaptic expression of AMPA-type glutamate receptors (AMPA-Rs) has been shown to underlie LTP [23,24]. As downregulation of AMPA-Rs is linked to the impairment in hippocampal LTP in APP/PS1 mice [15], the impact *in vitro* and *in vivo* of the N-A β core on the regulation of hippocampal AMPA-Rs was assessed.

Utilizing hippocampal neuronal cultures derived from the background wild-type mice, B6.SJL, and treated with exogenous full-length A β (A β ₄₂) for 7 days, the N-A β core was shown to alleviate the downregulation of AMPAR1 (GluA1) transcript expression assessed via qPCR in this *in vitro* A β toxicity model (Fig 2A; 22% $\pm$ 0.8% s.d. of A β ₄₂ condition). There was no significant impact of the N-A β core on the modest downregulation of AMPAR2 (GluA2) transcript expression.

Proteins solubilized from hippocampi isolated from 5 $\times$ FAD mice bilaterally injected with the N-A β core or saline vehicle were assayed for changes in *in vivo* expression of hippocampal AMPAR1 (GluA1) via immunoblot analysis. Total GluA1 levels in the

hippocampi were increased with exposure to the N-A β core as compared to saline-injected controls (Fig 2B; 248% $\pm$ 67% s.d.). The increase in relative pAMPA1 (pS831) in the hippocampi exposed to N-A β core was accounted by the increase in total GluA1 (Fig 2B; 127% $\pm$ 35% s.d.). Together, these findings indicate that reversal of the LTP impairment in the 5 $\times$ FAD APP/PS1 by the N-A β core involves regulation of AMPAR expression.

Structure-function and concentration-dependence of the N-A β core in reversing LTP impairment in hippocampal slices from 5 $\times$ FAD APP/PS1 mice

We also tested for basic concentration-dependence of the N-A β core in reversing LTP impairment in the 5 $\times$ FAD mouse hippocampal slices. Low concentration (<pM) of the N-A β core showed no difference on LTP compared to control 5 $\times$ FAD slices (Fig 1C & 1E; 107% $\pm$ 7% s.d. compared to 5 $\times$ FAD).

Through A β -interacting receptor-linked Ca²⁺ and neurotoxicity assays, we had previously shown that mutating the tyrosine residue in the N-A β core to a serine [Y10S] or mutating the two histidine residues to two alanines [H13A,H14A] reduces activity, indicating these amino acid residues in the N-A β core sequence are essential for activity [13,19]. To confirm the specificity of the N-A β core in reversing LTP impairment in 5 $\times$ FAD hippocampal slices, we tested the reverse-sequence N-A β core [QHHVEY] and an inactive triple mutant [SEVAAQ]. Treatment with the reverse-sequence N-A β core partially restored LTP in the 5 $\times$ FAD hippocampal slices (Figs 1D & 1E). Note that the reverse-sequence peptide contains a di-histidine sequence. By contrast, the inactive triple mutant had no significant effect on LTP in the 5 $\times$ FAD slices (Figs 1D & 1E; 110% $\pm$ 13% s.d.

compared to 5×FAD). It is important to note that there was no change in basal synaptic transmission or a trend toward increasing LTP in the wild-type slices treated with the reverse N-Aβcore (not shown), as seen for the N-Aβcore (Fig 1). Taken together, these results confirm the contribution of the two essential histidine residues to the positive neuromodulatory activity of the N-Aβcore.

N-Aβcore rescue of Aβ-induced LTP deficits involves PI3 kinase and mTOR

LTP has shown to involve multiple protein kinase and phosphatase pathways [25,26]. As prior evidence implicates the PI3 kinase and mTOR pathways in the regulation of Aβ neurotoxicity [27,28] and in the regulation of LTP [29], we evaluated the roles of PI3 kinase and mTOR in the action of the N-Aβcore in reversing impaired LTP via treatment of the hippocampal slice preparations with selective inhibitors. As shown in Figure 3, application of PI3 kinase inhibitor LY294002 had no impact on LTP in hippocampal slices from control (background B6.SJL) mice or 5×FAD mice. In contrast, application of LY294002 prior to treatment with the N-Aβcore prevented the rescue by the N-Aβcore of LTP in the slices from 5×FAD mice (Fig 3; 94.3% ± 15% s.d. compared to 5×FAD).

Prior inhibition of the PI3 kinase pathway effector mTOR by rapamycin also prevented the rescue by the N-Aβcore of LTP in the slices from 5×FAD mice (Fig 4; 101% ± 20% s.d. compared to 5×FAD). However, rapamycin did reduce LTP in the control B6.SJL slices, to a level similar to that seen for the LTP in 5×FAD slices. The inhibitor had no significant effect on the reduced level of LTP in the 5×FAD slices.

To further probe the mechanism by which the N-A β core regulates the PI3 kinase pathway, the impact of the core peptide on A β -linked regulation of the PI3 kinase and its downstream effectors Akt and mTOR was investigated using mouse hippocampal neuron cultures and mouse hippocampal slices. Treatment of neuron cultures with full-length A β (1-42) was shown to downregulate expression of various PI3 kinase, Akt and mTOR transcripts (Fig 5). Co-treatment with the N-A β core alleviated the A β -induced downregulation of Akt1 and mTOR transcripts (Fig 5).

Elevated levels of A β enhances long-term depression and the N-A β core reverses A β -linked LTD enhancement in hippocampal slices from 5 $\times$ FAD APP/PS1 mice

LTD is an essential component of synaptic plasticity underlying memory processing in the hippocampus, as synapses cycle between LTP and LTD, a process known as synaptic scaling [30]. To date, few studies have examined the effects of pathological levels of soluble A β on LTD induction, and moreover, the results have been mixed. For example, while focusing on NMDA receptor-dependent LTD, administration of synthetic A β led to an enhancement of LTD in some cases [eg. 10,31,32], whereas other studies reported no effect [eg. 33]. Here, we aimed to examine the effects of endogenous soluble A β on LTD in the 5 $\times$ FAD hippocampal slices. Using LFS to induce LTP in the same Schaffer collateral – CA1 pathway in hippocampal slice as that used for LTP, the LTD in slices from the 5 $\times$ FAD mice was more pronounced than that observed for LTD induced in slices from B6.SJL control mice (Figs 6A & 6B; $-32.5\% \pm -5.1\%$ s.d. for 5 $\times$ FAD compared to $-18.1\% \pm -4.9\%$ s.d for the control B6.SJL slices). Interestingly, treatment of 5 $\times$ FAD mouse

slices with the N-A β core prior to and during LFS resulted in a restoration of LTD to the level observed for the B6.SJL slices (Figs 6A & 6B; $-21.4\% \pm 4.5\%$). The N-A β core had no effect on metabotropic glutamate receptor-induced LTD (Fig 7). Taken together, these data suggest that A β plays a role in facilitating LTD and the N-A β core may protect against A β -induced LTD enhancement. The role of the NMDA receptor in A β facilitation of LTD warrants further investigation.

Discussion

Previous studies have established a strong link between the progression of AD and the extent of synaptic dysfunction occurring in the early stages of the disease, prior to the formation of A β plaques and tau neurofibrillary tangles [1,5,34,35]. In AD-like models it has been widely demonstrated that elevated levels of soluble oligomeric A β drive LTP inhibition [3,7,8,36], coupled to downregulation of synaptic AMPARs. By contrast, the link between pathological levels of A β and LTD are less well understood, and as previously noted, investigations of the impact of A β on LTD have had conflicting results.

Low “physiological” levels (pM) of soluble A β have been shown to enhance synaptic plasticity and facilitate hippocampal-based learning and memory [11-13], suggesting a neuromodulatory role of soluble A β at physiological levels. Augmentation of LTP by pM A β correlated with enhanced expression of AMPARs. Similar results were obtained using an N-terminal fragment of A β (1-15) implicated that sequence within A β as accountable for the positive neuromodulatory activity of full-length A β [13]. We wondered whether the N-A β core (10-15), encompassing the essential sequence within

the N-terminal fragment accounting for its positive neuromodulatory activity and its cellular neuroprotective activity against A β neurotoxicity, could itself enhance synaptic plasticity and protect against A β -induced synaptic dysfunction.

In accordance with previous findings [36], we found that LTP was near absent in the hippocampal slice model from APP/PS1 5 $\times$ FAD transgenic mice, previously shown to be accounted by elevated A β in the brains of the transgenic model mice [20,22]. Treatment here with the N-A β core reversed this deficit back to the LTP observed in slices from the background B6.SJL mice. Treatment of 5 $\times$ FAD slices with the N-A β core trended towards LTP enhancement, suggesting that the reversal of the LTP deficits in the 5 $\times$ FAD slices by the N-A β core was not solely due to competitive binding for target receptors and may possibly involve activation of a neuroprotective pathway that enhances synaptic plasticity [see 37]. Here, we identified the PI3 kinase/Akt/mTOR in the reversal of LTP deficits in 5 $\times$ FAD slices by the N-A β core as a primary pathway which has been shown to be a key link to long-term memory [38]. Other downstream pathways engaged by the N-A β core are not yet definitely identified, but we suspect that key players involved in synaptic modulation are affected, such as regulation of CREB, PKA, and/or CAMKII or downregulation of calcineurin and/or PP1, subsequently altering AMPA receptor trafficking to the synapses [23,24,39,40], consistent with the observed reversal of downregulation of hippocampal AMPARs by the N-A β core in 5 $\times$ FAD mouse hippocampus. Additionally, the enhancement of the basal synaptic transmission with the treatment of the N-A β core suggests a receptor-linked influx of Ca²⁺, which further supports the idea that the N-A β core activates an alternative neuroprotective pathway that

enhances synaptic plasticity, consistent with results for neuroprotection by the N-A β core in A β -triggered neurotoxicity [19].

Previously, it has been shown that BDNF enhances basal synaptic transmission [41], therefore, we suspect that the N-A β core may be upregulating BDNF expression, possibly through a Ca²⁺-dependent increase in CREB activation and/or expression [42,43]. Another possibility is that the N-A β core-induced Ca²⁺ influx could also regulate BDNF release at the synapses, thus, enhancing baseline synaptic transmission and ultimately LTP. It would be interesting to examine the effect of the basal synaptic transmission by the N-A β core long-term, and whether the enhancement of LTP observed required changes in baseline transmission prior tetanic stimulation.

In the context of synaptic plasticity, LTD is necessary for neural homeostasis. NMDA receptor-dependent LTD often involves internalization of AMPA receptors via a caspase-dependent pathway [16,44]. To date, however, there is limited understanding in regard to the effects of pathological A β on LTD, where some groups show that synthetic A β enhances LTD [31,32,45] and others show no effect [33,46], though the differential action of A β may have resulted from recording in different subregions of the hippocampus (eg. CA1 vs. dentate gyrus). Here, we found that high concentrations of endogenous soluble A β shown to be present in the brains of 5 $\times$ FAD mice resulted in enhanced LTD in hippocampal slices, and treatment with the N-A β core prior to and during the LFS induction of LTD reverses this enhancement. Interestingly, Hu *et. al.* found that applying synthetic soluble A β prior to LFS did not affect the early phase of LFS-induced LTD (<2h post LFS), but facilitated the late phase (3-5h post LFS) [45], thus, possibly accounting for different findings. It is important to note that late phase LTP and LTD require new

protein synthesis, and mTOR is linked to the regulation of protein synthesis [47]. Indeed, the reversal by the N-A β core of LTP deficit in the 5 $\times$ FAD slices was dependent upon mTOR. As LTD and LTP work in concert to allow for reversible synaptic plasticity and synaptic scaling, the LFS-induced enhancement of LTD in the 5 $\times$ FAD slices could affect subsequent LTP, and this may be another reason why an LTP deficit was observed in the 5 $\times$ FAD slices compared to wild-type preparations.

Although NMDA and AMPA receptors are involved in different aspects of LTP and LTD, and their expression is affected by elevated A β , metabotropic glutamate receptors (mGluRs) have also been implicated in A β -induced synaptic dysfunction [9,45,48,49]. Interestingly, NMDA-independent, mGluR-induced LTD was found to be unaffected by the N-A β core. While mGluRs have been linked via cellular prion to A β -induced cellular toxicity [49], our findings support a divergence in the A β -linked signaling pathways affected by the N-A β core in synaptic plasticity. Further work is needed to elucidate the detailed molecular mechanisms involved in N-A β core protection or reversal of A β -linked synaptic dysfunction, including caspase-dependent intracellular pathways leading to the regulation of AMPA receptors, and eventual synaptic loss in AD.

Conclusions

The essential core hexapeptide sequence, YEVHHQ, or N-A β core, within the neuroprotective N-terminal fragment of A β was able to effectively, selectively and potently reverse deficits in synaptic plasticity in hippocampal slices from adult APP/PS1 5 $\times$ FAD transgenic mice. Attenuated LTP and enhanced LTD observed in the slices from 5 $\times$ FAD mice were both rescued by the N-A β core, returning LTP and LTD back to the levels found

for hippocampal slices from background control (B6.SJL) mice. The involvement of the PI3 kinase pathway, specifically, mTOR in the protective action of the N-A β core against FAD-linked deficits in synaptic plasticity indicates possible connection between N-A β core-linked signaling and the translation machinery at hippocampal synaptic sites (dendritic spines). It would thus be of interest to examine the specific signaling pathways engaged by N-A β core at hippocampal sites, especially key downstream elements in translational control such as S6 kinase, a prominent substrate for mTORC1 in neuronal systems.

Author Contributions

Conceptualization: Kelly H. Forest and Robert A. Nichols

Data curation: Kelly H. Forest, Ruth Taketa, Komal Arora

Formal analysis: Kelly H. Forest, Ruth Taketa, Komal Arora, Cedimir Todorovic

Funding acquisition: Robert A. Nichols

Methodology: Kelly H. Forest, Ruth Taketa, Komal Arora, Cedimir Todorovic

Investigation: Kelly H. Forest, Ruth Taketa, Komal Arora

Supervision: Robert A. Nichols

Validation: Kelly H. Forest

Writing – original draft: Kelly Forest and Robert A. Nichols

Writing – review & editing: Kelly Forest, Ruth Taketa, Cedimir Todorovic, Robert A. Nichols

Acknowledgments

We thank Dr. Naghum Alfulaij for advice. We thank Ms. Ann Hashimoto for mouse breeding and husbandry. We thank Dr. Tessi Sherrin for help with the hippocampal microinjections.

References

1. Scheff SW, Price DA, Schmitt FA, Mufson EJ. Hippocampal synaptic loss in early Alzheimer's disease and mild cognitive impairment. *Neurobiol Aging*. 2006; 27:1372–84.
2. Reddy PH, Mani G, Park BS, Jacques J, Murdoch G, Whetsell W, et al. Differential loss of synaptic proteins in Alzheimer's disease: implications for synaptic dysfunction. *J Alzheimers Dis*. 2005; 7:103-17.
3. Walsh DM, Klyubin I, Fadeeva JV, Cullen WK, Anwyl R, Wolfe MS, et al. Naturally secreted oligomers of amyloid β protein potently inhibit hippocampal long-term potentiation in vivo. *Nature* 2002; 416:535–9.
4. Glabe CC. Amyloid accumulation and pathogenesis of Alzheimer's disease: significance of monomeric, oligomeric and fibrillar A β . In: Harris JR, Fahrenholz F, editors. *Alzheimer's disease: Cellular and molecular aspects of amyloid β* . Boston: Springer; 2005. pp. 167–177.
5. Shankar GM, Walsh DM. Alzheimer's disease: synaptic dysfunction and A β . *Mol Neurodegener*. 2009; 4:48.
6. Oddo S, Caccamo A, Shepherd JD, Murphy MP, Golde TE, Kaye R, et al. Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular A β and synaptic dysfunction. *Neuron*. 2003; 39:409–21.
7. Cullen WK, Suh YH, Anwyl R, Rowan MJ. Block of LTP in rat hippocampus in vivo by β -amyloid precursor protein fragments. *Neuroreport*. 1997; 8:3213–7.
8. Chapman PF, White GL, Jones MW, Cooper-Blacketer D, Marshall VJ, Irizarry M, et al. Impaired synaptic plasticity and learning in aged amyloid precursor protein transgenic mice. *Nat Neurosci*. 1999; 2:271-6.
9. Chen X, Lin R, Chang L, Xu S, Wei X, Zhang J, et al. (2013) Enhancement of long-term depression by soluble amyloid β protein in rat hippocampus is mediated by metabotropic glutamate receptor and involves activation of p38MAPK, STEP and caspase-3. *Neuroscience*. 2013; 253:435–43.
10. Li S, Hong S, Shepardson NE, Walsh DM, Shankar GM, Selkoe D. Soluble oligomers of amyloid β -protein facilitate hippocampal long-term depression by disrupting neuronal glutamate uptake. *Neuron*. 2009; 62:788–801.
11. Puzzo D, Privitera L, Leznik E, Fà M, Staniszewski A, Palmeri A, et al. Picomolar amyloid- β positively modulates synaptic plasticity and memory in hippocampus. *J Neurosci*. 2008; 28:14537–45.

12. Puzzo D, Privitera L, Palmeri A. Hormetic effect of amyloid- β peptide in synaptic plasticity and memory. *Neurobiol Aging*. 2012; 33:1484.e15-24.
13. Lawrence JLM, Tong M, Alfulaij N, Sherrin T, Contarino M, White MM, et al. Regulation of presynaptic Ca^{2+} , synaptic plasticity and contextual fear conditioning by a N-terminal β -amyloid fragment. *J Neurosci*. 2014; 34:14210–18.
14. Snyder EM, Nong Y, Almeida CG, Paul S, Moran T, Choi EY, et al. Regulation of NMDA receptor trafficking by amyloid- β . *Nat Neurosci*. 2005; 8:1051-8.
15. Hsieh H, Boehm J, Sato C, Iwatsubo T, Tomita T, Sisodia S, et al. AMPAR removal underlies A β -induced synaptic depression and dendritic spine loss. *Neuron*. 2006; 52:831–43.
16. D'Amelio M, Cavallucci V, Middei S, Marchetti C, Pacioni S, Ferri A, et al. Caspase-3 triggers early synaptic dysfunction in a mouse model of Alzheimer's disease. *Nat Neurosci*. 2011; 14:69-76.
17. Portelius E, Price E, Brinkmalm G, Stieler M., Olsson M, Persson R, et al. A novel pathway for amyloid precursor processing. *Neurobiol Aging*. 2011; 32:1090-8.
18. Morrissey JA, Mockett BG, Singh A, Kweon D, Ohline SM, Tate WP et al. A C-terminal peptide from secreted amyloid precursor protein- α enhances long-term potentiation in rats and a transgenic mouse model of Alzheimer's disease. *Neuropharmacol*. 2019; 157:107670
19. Forest KH, Alfulaij N, Arora K, Taketa R, Sherrin T, Todorovic C et al. Protection against β -amyloid neurotoxicity by a non-toxic endogenous N-terminal β -amyloid fragment and its active hexapeptide core sequence. *J Neurochem*. 2018; 144:201-17.
20. Oakley H, Cole SL, Logan S, Maus E, Shao P, Craft J et al. Intraneuronal β -amyloid aggregates, neurodegeneration, and neuron loss in transgenic mice with five familial Alzheimer's disease mutations: potential factors in amyloid plaque formation. *J Neurosci*. 2006; 26:10129-40.
21. Cheng Q, Yakel JL. Activation of $\alpha 7$ nicotinic acetylcholine receptors increases intracellular cAMP levels via activation of AC1 in hippocampal neurons. *Neuropharmacol*. 2015; 95:405-14.
22. Crouzin N, Baranger K, Cavalier M, Marchalant Y, Cohen-Solal C, Roman FS et al. Area-specific alterations of synaptic plasticity in the 5XFAD mouse model of Alzheimer's disease: dissociation between somatosensory cortex and hippocampus. *PLoS ONE*. 2013; 8:e74667.

23. Makino H, Malinow R. AMPA receptor incorporation into synapses during LTP: the role of lateral movement and exocytosis. *Neuron*. 2009; 64:381-90.
24. Huganir RL, Nicoll RA. AMPARs and synaptic plasticity: the last 25 years. *Neuron*. 2013; 80:704-17.
25. Kennedy MB. Synaptic signaling in learning and memory. *Cold Spring Harb Perspect Biol*. 2013; 8:a016824.
26. Thomas GM, Huganir RL. MAPK cascade signalling and synaptic plasticity. *Nat Rev Neurosci*. 2004; 5:173-83.
27. Lee K-Y, Koh S-H, Noh MY, Kim SH, Lee YJ. Phosphatidylinositol-3-kinase activation blocks amyloid beta-induced neurotoxicity. *Toxicol*. 2008; 243:43-50.
28. Lafay-Chebassier C, Paccalin M, Page G, Barc-Pain S, Perault-Pochat MC, Gil R et al. mTOR/p70S6k signaling alteration by A β exposure as well as in APP-PS1 transgenic models and in patients with Alzheimer's disease. *J Neurochem*. 2005; 94:215-25.
29. Hoeffler CA, Klann E. mTOR signaling: At the crossroads of plasticity, memory and disease. *Trends Neurosci*. 2010; 33:67-75.
30. Turrigiano GG. Nagerl UV, Eberhorn N, Cambridge SB, Bonhoeffer T. Bidirectional activity-dependent morphological plasticity in hippocampal neurons. *Neuron*. 2004; 44:759-67.
31. Kim J-H, Anwyl R, Suh Y-H, Djamgoz MBA, Rowan MJ. Use-dependent effects of amyloidogenic fragments of β -amyloid precursor protein on synaptic plasticity in rat hippocampus *in vivo*. *J Neurosci*. 2001; 21:1327-33.
32. Shankar GM, Li S, Mehta TH, Garcia-Munoz A, Shepardson NE, Smith I, et al. Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nat Med*. 2008; 14:837-42.
33. Wang H-W, Pasternak JF, Kuo H, Ristic H, Lambert MP, Chromy B, et al. Soluble oligomers of β amyloid (1-42) inhibit long-term potentiation but not long-term depression in rat dentate gyrus. *Brain Res*. 2002; 924:133-40.
34. Terry RD, Masliah E, Salmon DP, Butters N, DeTeresa R, Hill R et al. Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. *Ann Neurol*. 1991; 30:572-80.
35. Koffie RM, Hyman BT, Spires-Jones TL. Alzheimer's disease: synapses gone cold. *Mol Neurodegener*. 2011; 6:63.

36. Kimura R, Ohno M. Impairments in remote memory stabilization precede hippocampal synaptic and cognitive failures in 5XFAD Alzheimer mouse model. *Neurobiol Dis.* 2009; 33:229–35.
37. Forest KH, Nichols RA. Assessing neuroprotective agents for A β -induced neurotoxicity. *Trends Mol Med.* 2019; 25:685-95.
38. Bekinschtein P, Katche C, Slipczuk LN, Igaz LM, Cammarota M, Izquierdo I et al. mTOR signaling in the hippocampus is necessary for memory formation. *Neurobiol Learn Mem.* 2007; 87:303-7.
39. Derkach VA, Oh MC, Guire ES, Soderling TR. Regulatory mechanisms of AMPA receptors in synaptic plasticity. *Nat Rev Neurosci.* 2007; 8:101-13.
40. Herring BE, Nicoll RA. Long-term potentiation: from CaMKII to AMPA receptor trafficking. *Annu Rev Physiol.* 2016; 78:351–65.
41. Patterson SL, Abel T, Deuel TA, Martin KC, Rose JC, Kandel ER. Recombinant BDNF rescues deficits in basal synaptic transmission and hippocampal LTP in BDNF knockout mice. *Neuron.* 1996; 16:1137–45.
42. Tao X, Finkbeiner S, Arnold DB, Shaywitz AJ, Greenberg ME. Ca²⁺ influx regulates *BDNF* transcription by a CREB family transcription factor-dependent mechanism. *Neuron.* 1998; 20:709–26.
43. Lu B. BDNF and activity-dependent synaptic modulation. *Learn Mem.* 2003; 10:86–98.
44. Li Z, Sheng M. Caspases in synaptic plasticity. *Mol Brain.* 2012; 5:15.
45. Hu N-W, Nicoll AJ, Zhang D, Mably AJ, O'Malley T, Purro SA, et al. mGlu5 receptors and cellular prion protein mediate amyloid- β -facilitated synaptic long-term depression *in vivo*. *Nat Commun.* 2014; 5:3374.
46. Raymond CR, Ireland DR, Abraham WC. NMDA receptor regulation by amyloid- β does not account for its inhibition of LTP in rat hippocampus. *Brain Res.* 2003; 968:263–72.
47. Wang X, Proud CG. The mTOR pathway in the control of protein synthesis. *Physiol.* 2006; 21:362-69.
48. Wang Q, Walsh DM, Rowan MJ, Selkoe DJ, Anwyl R. Block of long-term potentiation by naturally secreted and synthetic amyloid β -peptide in hippocampal slices is mediated via activation of the kinases c-Jun N-terminal kinase, cyclin-dependent kinase 5, and p38 mitogen-activated protein kinase as well as metabotropic glutamate receptor type 5. *J Neurosci.* 2004; 24:3370–8.

49. Haas LT, Salazar SV, Kostylev MA, Um JW, Kaufman AC, Strittmatter SM.
Metabotropic glutamate receptor 5 couples cellular prion protein to intracellular signaling in Alzheimer's disease. Brain. 2016; 139:526-46.

Figure Legends

Fig. 1. The N-A β core reverses A β -induced synaptic impairment in APP/PSEN1 mutant mouse hippocampal slices. Input/Output curves (**A**) plotting average fEPSP slopes versus stimulus strength before treatment and baseline recordings (**B**) during drug treatment versus aCSF perfusion alone prior to stimulation for: control aCSF in B6.SJL slices (black, $n=5$), 5 $\times$ FAD slices (red, $n=4$), 500nM N-A β core in B6.SJL slices (grey, $n=3$) and 5 $\times$ FAD slices (blue, $n=4$), plus 500fM N-A β core in 5 $\times$ FAD slices (cyan, $n=5$). No statistically significant differences in the input/output curves (**A**) were found ($p>0.05$, one-way repeated measures ANOVA). Averaged (last 10mins**) baseline data (**B**) were analyzed by one-way ANOVA ($F_{(4,16)} = 7.53$, $p=0.0013$). Bonferroni post hoc tests revealed significant enhancement of baseline fEPSPs by 500nM N-A β core on B6.SJL slices compared to aCSF-perfused B6.SJL slices ($p=0.006$), aCSF perfused 5 $\times$ FAD slices ($p=0.002$), and by 500fM N-A β core on 5 $\times$ FAD slices ($p=0.038$). **C** TBS-induced LTP for B6.SJL slices perfused with control aCSF (black, $N=5$) or 500nM N-A β core (grey, $N=3$) and 5 $\times$ FAD slices with aCSF (red, $N=4$), 500nM N-A β core (blue, $N=4$) and 500fM N-A β core (cyan, $N=5$). Inset in **c**, diagram of hippocampal slice stimulation of the Schaffer collaterals (Stim) and recording fEPSPs in CA1 (Record). **D** TBS-induced LTP for 5 $\times$ FAD slices treated with 500nM [SEVAAQ] (green, $n=5$) or 500nM [QHHEVEY] (brown, $n=3$) substituted N-A β core peptides, and, as a reference, B6.SJL perfused with control aCSF (gray circles, $n=5$, data from **C**). **E** Quantification of average fEPSP slope values for 50-60 min postTBS. All groups shown in panels in **C** and **D** were analyzed by one-way ANOVA ($F_{(6,21)} = 11.33$, $p<0.0001$). Bonferroni post hoc tests: differences found for 5 $\times$ FAD slices ($p=0.0012$), 500fM N-A β core in 5 $\times$ FAD slices ($p=0.0036$), and 500nM [SEVAAQ] in 5 $\times$ FAD slices ($p=0.041$) when compared to aCSF-perfused (control) B6.SJL slices. Application of 500nM N-A β core in 5 $\times$ FAD slices reversed the LTP deficit in the 5 $\times$ FAD slices ($p<0.0005$). Horizontal bars in **C** and **D** indicate the period (20min) of drug delivery for the color-coded conditions as indicated. Arrows indicate timing of TBS in **C** and **D**. Color-coded insets showing examples of fEPSPs (baseline vs. LTP). All data are means $\pm$ s.e.m. N values represent independent experiments (1 slice/mouse). Inset calibration: horizontal, 10ms; vertical, 0.4 mV. * $p<0.05$; ** $p<0.005$, *** $p<0.0005$.

Fig. 2. N-A β Core normalizes AMPAR1 expression in an *in vitro* A β neurotoxicity model of primary hippocampal neurons and *in vivo* in hippocampi of 5 $\times$ FAD APP/PS1 mice. **A** Normalized average expression of AMPA receptor GluA1 and GluA2 transcripts in B6.SJL mouse hippocampal neuron cultures exposed to full-length A β_{1-42} (A β_{42}), the N- A β core (A β_{10-15}) or the combination (combo), as measured via qPCR. Data are means $\pm$ s.d. $N=3$ cultures for each condition. *** $p=0.000001$ (Bonferroni post hoc tests for one-way ANOVA comparison between A β_{42} and A β_{10-15}). There was no statistical difference (ns) between expression of GluA1 for A β_{42} and A β_{42} plus A β_{10-15} (combo), or for any condition for GluA2. **B** Expression of pAMPA1 (pGluA1) or total AMPAR1 (GluA1) in the hippocampi of 5 $\times$ FAD mice injected with sterile saline or N-A β core (Core), as measured using western immunoblot. $N=4$ mice for each condition. Total GluA1: $p=0.247$; pGluA1: $p=0.252$. (Student's t -tests for comparison of saline vs. N-A β core).

Fig. 3. N-A β core protection of A β -induced synaptic dysfunction involves the PI3 kinase pathway. **A** TBS-induced LTP for B6.SJL slices perfused with control aCSF (black) or 100 μ M LY294002 (grey) and for 5 $\times$ FAD slices with aCSF (red), 500nM N-A β core (light blue) or 500nM N-A β core plus 100 μ M LY294002 (dark blue). Color-coded inserts showing examples of fEPSPs (baseline vs. LTP). **B** Quantification of average fEPSP slope values for 50-60 min post-TBS (one-way ANOVA: $F_{(4,20)}=13.63$, $p<0.0001$). Bonferroni post hoc tests showed that rescue of the LTP deficit in the 500nM N-A β core-treated 5XFAD slices (** $p=0.004$, 500nM N-A β core 5XFAD vs 5XFAD; $p<0.05$, vs aCSF B6.SJL) was blocked by 100 μ M LY294002 pretreatment (** $p=0.0002$, 100 μ M LY294002 + 500nM N-A β core vs. aCSF B6.SJL). Arrows indicate timing of TBS in **A**. All data are expressed as means $\pm$ s.e.m. $N=4$ for each condition (1 slice/mouse). Inset calibration: horizontal, 10ms; vertical, 0.4 mV. ** $p<0.005$.

Fig. 4. N-A β core protection of A β -induced synaptic dysfunction involves mTOR downstream in the PI3 kinase pathway. **A** TBS-induced LTP for B6.SJL slices perfused with control aCSF (black) or 500nM rapamycin (grey). **B** TBS-induced LTP for

5×FAD slices with aCSF (red), 500nM rapamycin (white circle), 500nM N-A β core (cyan) or 500nM N-A β core plus 500nM rapamycin (blue). **C** Quantification of average fEPSP slope values for 50-60 min post-TBS (one-way ANOVA: $F_{(5,19)} = 8.85$, $p = 0.0002$). B6.SJL slices incubated in 500nM rapamycin (20 min) exhibited reduced LTP when compared to aCSF-treated B6.SJL control slices (Bonferroni post hoc test, $*p = 0.038$). Rescue of LTP deficit following 20 min 500nM N-A β core treatment in 5XFAD slices was abolished with 500nM rapamycin pretreatment (40 min) ($**p = 0.0097$). Arrows indicate timing of TBS in **A** and **B**. All recording data (**A**, **B**) are means $\pm$ s.e.m. Averaged data for 50-60 min post-tetanus (**c**) are means $\pm$ s.d. $N=4$ for each condition (1 slice/mouse). Color-coded insets show examples of fEPSPs (baseline vs. LTP). Inset calibration: horizontal, 10ms; vertical, 0.4 mV $*p < 0.05$; $**p < 0.005$.

Fig. 5. Impact of N-A β core on regulation of various PI3 kinase, Akt and mTOR transcripts by A β . Primary mouse hippocampal neuron cultures were treated with 1 μ M full-length A β_{42} , 1 μ M A β_{10-15} (N-A β core) or the combination (combo). Extracted RNA was subjected to qPCR, probing for Class 1 PI3 kinase catalytic subunits (CA and CB), Akt1 and Akt2 and mTOR. Data are expressed as averaged fold-change over untreated controls, after normalizing for GAPDH expression. Bars are means $\pm$ s.d. $*p = 0.00063$; $**p = 0.000002$; $***p = 0.0001$; $\#p = 0.000001$ (Bonferroni post hoc tests for one-way ANOVA comparisons, as shown). For each condition, $N=3$ cultures.

Fig. 6. The N-A β core reverses endogenous A β enhancement of LTD. **A** LFS-induced LTD in hippocampal slices from B6.SJL perfused with control aCSF (black) and 5×FAD slices perfused with aCSF (red) or 500nM N-A β core (blue). Color-coded insets show examples of fEPSPs (baseline vs. LTD). **B** Quantification of average fEPSP slope values for 50-60 min post-LFS (one-way ANOVA $F_{(2,9)} = 13.03$, $p = 0.0022$). Bonferroni post hoc tests: 5×FAD slices displayed significantly more pronounced LTD as compared to control aCSF B6.SJL slices ($*p = 0.012$). 500nM N-A β core in 5XFAD slices returned LTD to the level observed in control aCSF-perfused B6.SJL slices ($p > 0.99$). Data are mean $\pm$ s.e.m. $N=4$ for each condition (1 slice/mouse). Inset calibration: horizontal, 10ms; vertical, 0.4 mV. $*p < 0.05$; $**p < 0.005$.

Fig. 7. N-A β Core has no impact on NMDA-independent, metabotropic glutamate receptor-induced LTD deficits. NMDA-independent LTD was induced by treatment with bath application of 100 μ M 3,5-dihydroxyphenylglycine (DHPG), a metabotropic glutamate receptor (mGluR) group I agonist. **A** DHPG-induced LTD in hippocampal slices from B6.SJL mice perfused with control aCSF (black) or 500nM N-A β core (grey) and 5 $\times$ FAD slices perfused with aCSF (red) or 500nM N-A β core (blue). Color-coded insets show examples of fEPSPs (baseline vs. LTD). Inset calibration: horizontal, 10ms; vertical, 0.4 mV. **B** Quantification of average fEPSP slope values for 50-60 min post-LFS ($p < 0.05$, two-way ANOVA). Data are means $\pm$ s.e.m. $N=4$ for each condition (1 slice/mouse).

Figure 1

A **B**

Figure 3

Figure 4

Primary neurons

Fold change (Normalized to GAPDH)

Figure 5

A **B**

Figure 6

A **B**

Figure 7

Figure 2