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Neurotrophins Enhance CaMKII Activity and Rescue Amyloid- β -Induced Deficits in Hippocampal Synaptic Plasticity

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Abstract

Amyloid- β ($A\beta$) peptide-induced impairment of hippocampal synaptic plasticity is considered an underlying mechanism for memory loss in the early stages of Alzheimer's disease and its animal models. We previously reported inhibition of long-term potentiation (LTP) and miniature excitatory postsynaptic currents by oligomeric $A\beta_{1-42}$ at hippocampal synapses. While multiple cellular mechanisms could be involved in $A\beta$ -induced synaptic dysfunction, blockade of activity-dependent autophosphorylation of Ca^{2+} and calmodulin-dependent protein kinase II (CaMKII) appeared to be a major component of $A\beta$ action in our studies. The present study further tested this hypothesis and examined the therapeutic potential of trkB receptor-acting neurotrophins in rescuing $A\beta$ -induced synaptic and signaling impairments. As expected, treatment of rat hippocampal slices with $A\beta_{1-42}$ significantly reduced LTP in the Schaffer collateral-CA1 pathway and dentate medial perforant path. LTP-associated CaMKII activation and AMPA receptor phosphorylation were blocked by $A\beta_{1-42}$ at the same concentration that inhibited LTP. $A\beta$ -induced LTP impairment, however, was prevented when slices were co-treated with neurotrophin 4 (NT4). Western blotting and immunohistochemical analyses confirmed that treatment with NT4 or brain-derived neurotrophic factor, another trkB-acting neurotrophin, could oppose $A\beta$ action, enhancing autophosphorylation of CaMKII, and AMPA receptor phosphorylation at a CaMKII-dependent site. These findings support the view that CaMKII is a key synaptic target of $A\beta$ toxicity as well as a potential therapeutic site of neurotrophins for Alzheimer's disease.

Keywords

Amyloid- β ; calcium and calmodulin-dependent protein kinase II; hippocampus; long-term potentiation; neurotrophins

INTRODUCTION

Accumulation of amyloid- β ($A\beta$) peptides in the brain is a crucial step in the pathogenesis of Alzheimer's disease (AD). Earlier studies linked neurotoxic action of $A\beta$ with its insoluble, fibrillar forms deposited in senile plaques in AD brain. Such a link was controversial because of lack of strong correlations between fibrillar $A\beta$ load and the extent of memory loss in AD and transgenic mouse models. Studies in recent years have suggested that soluble oligomeric forms of $A\beta$ may be a major player in causing synaptic dysfunction and memory loss during early AD. Accumulation of various $A\beta$ assemblies, most notably a soluble

dodecamer A β *56, reportedly disrupts cognitive function in A β PP transgenic mice [1]. Both synthetic and naturally existing soluble A β oligomers can bind to synaptic sites in rat hippocampal and cortical cultures [2]. Application of A β oligomers leads to rapid inhibition of hippocampal long-term potentiation (LTP) [3–5], facilitation of long-term depression [6], and suppression of spontaneous synaptic activity in hippocampal and cortical cultures [7–9]. These findings support the view that A β oligomer-induced synaptic dysfunction in brain regions crucial for memory formation and storage, such as hippocampus and cortex, has a causative role in memory loss of early AD [10,11]. While this view has been increasingly accepted in recent years, the exact synaptic targets and key signaling events underlying A β -induced synaptic depression remain to be further elucidated.

We previously reported that acute application of A β _{1–42} strongly inhibited LTP induction and maintenance in the hippocampal Schaffer collateral-CA1 pathway and dentate medial perforant path [12]. Further analyses showed that A β -induced LTP deficits were linked with inhibition of N-methyl-D-aspartate (NMDA) receptor channel currents and activation of the calcineurin-protein phosphatase 1 pathway [13, 14]. A β _{1–42} also blocked LTP-triggered activation of Ca²⁺ and calmodulin-dependent protein kinase II (CaMKII) and subsequent phosphorylation of the α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor at Ser831 of the GluR1 subunit, a CaMKII-dependent site [15]. These findings suggest that multiple Ca²⁺-regulated signaling pathways are involved in mediating A β action at hippocampal synapses. The essential role of CaMKII in long-term synaptic plasticity and cognitive function is well documented. Synaptic activity-triggered Ca²⁺ influx through NM-DA receptor channels can activate CaMKII and promote its autophosphorylation at Thr286, which results in a persistently active form of the kinase that is required for LTP [16]. Activation of a calcineurin-dependent phosphatase pathway, however, can dephosphorylate CaMKII and reduce its activity [17]. It is thus highly plausible that CaMKII is a key synaptic target for A β . By integrating several cellular effects of A β , changes in CaMKII activity could lead to synaptic weakening and impaired synaptic plasticity [18]. Drugs that enhance CaMKII signaling, therefore, should be able to oppose A β action and rescue synaptic and cognitive function. The present study tested these hypotheses and demonstrated that application of trkB-acting neurotrophins, which are known to enhance synaptic plasticity in adult hippocampus [19], could stimulate CaMKII activity and effectively rescue A β -induced LTP deficits at hippocampal synapses.

MATERIALS AND METHODS

Brain slice preparation and treatment

Hippocampal slices were prepared from 30- to 45-day-old male Sprague-Dawley rats as previously described [12]. Rats were sacrificed under isoflurane anesthesia following a protocol in accordance with PHS Guidelines, reviewed and approved by the Chancellor's Animal Research Committee at University of California, Los Angeles. The brain was removed and sectioned into 500- μ m-thick slices with a vibroslicer. Individual slices were trimmed to remove the surrounding areas of the hippocampal section. After recovering for 1–3 h in a holding chamber containing oxygenated artificial cerebrospinal fluid (aCSF), the hippocampal slices were treated with A β _{1–42} and/or neurotrophins for 30 min and used for electrophysiological recordings, Western blotting, or immunohistochemical analysis.

Electrophysiological recordings

Hippocampal slices were placed in a submerged recording chamber and perfused with 29–30°C oxygenated aCSF at 2–3 ml/min. Field potentials were evoked and recorded in the CA1 region and dentate gyrus using standard extracellular recording techniques as described [12]. In the CA1 region, Schaffer collateral-evoked field excitatory postsynaptic potentials

(fEPSPs) were recorded in the stratum radiatum. In the dentate gyrus, the medial perforant path was stimulated in the middle third of the molecular layer, and evoked population spike and fEPSPs were recorded from the granule cell body and molecular layers. Baseline responses were collected using 0.008 or 0.016 Hz test pulses that yielded 40–50% of the maximal evoked responses in aCSF. The early LTP was induced using a single high-frequency stimulation (HFS) of 100 Hz for 1 s at the same stimulus intensity as used for the baseline. The normal test pulses were resumed after the HFS. LTP was measured 30 or 45 min after HFS in the dentate or CA1 pathways and expressed as percent changes of evoked responses from the average baseline values.

Western blot analysis

Drug- and LTP-induced changes in the level of total CaMKII, phospho-Thr286-CaMKII (p-CaMKII), total GluR1, and phospho-Ser831-GluR1 (p-GluR1) were measured in hippocampal slice tissues following the protocols previously described with some modifications [15,20]. Individual hippocampal slices were quickly frozen on dry ice after treatment and homogenized in ice-cold buffer containing 50 mM Tris/HCl (pH 7.0), 50 mM NaF, 10 mM EGTA, 10 mM EDTA, 80 mM sodium molybdate, 5 mM sodium pyrophosphate, 1 mM sodium orthovanadate, 0.01% Triton X-100 and 4 mM para-nitrophenylphosphate. To stop the activity of proteases and protein phosphatases, the homogenization buffer also contained Protease Inhibitors Complete (Roche Molecular Biochemicals) and Protein Phosphatase Inhibitor Cocktail I and II (Sigma-Aldrich). Aliquots of homogenates were taken to determine protein concentrations using a Bio-Rad Protein Assay Kit. A cold denaturing loading buffer was added to the remaining homogenates, and sample aliquots containing 20 μ g of protein each were electrophoresed on 12% SDS/PAGE gels. The proteins were transferred onto nitrocellulose membranes that were probed overnight at 4°C with various primary antibodies and incubated for 2 h with horseradish peroxidase-conjugated secondary antibodies. The immunoreactive bands were visualized with enhanced chemiluminescence (ECL-plus, GE Healthcare), and signals were quantified with densitometry using ImageQuant TL software (GE Healthcare). For loading controls, all blots were re-probed with a β -actin antibody (Sigma-Aldrich) and the signal value for each band of interest was normalized to that of β -actin. As multiple slices from the same rats were used for different drug treatments, percent changes in the protein of interest due to treatments were calculated relative to aCSF-treated controls run in the same experiments using slices from the same animals.

Immunohistochemistry

After drug treatment, hippocampal slices were fixed using 4% paraformaldehyde overnight, cryoprotected in phosphate-buffered saline (PBS) containing 30% sucrose, and cut into 40 μ m cryostat sections. The sections were washed with PBS and incubated at 24°C for 30 min in a blocking solution containing 20% goat serum, 0.2% Triton X-100, and 0.1% bovine serum albumin in PBS. The brain sections were then probed with the primary antibodies against p-CaMKII and p-GluR1 at 24°C for 3 h, followed by 4°C overnight. After washing, brain sections were further incubated at 24°C for 2 h with Cy3- (1:2000; Jackson Laboratory) and FITC-conjugated (1:1000; Abcam) secondary antibodies. Confocal fluorescent images of double labeling were obtained using a Zeiss LSM 5 Pascal laser scanning microscope with a 5 $\times$ water immersion objective. To control for nonspecific labeling, immunostaining was performed in control slices without adding the primary or secondary antibodies.

Drugs and primary antibodies

A β _{1–42} was purchased from Bachem (Torrance, CA) and prepared as previously described to yield oligomeric A β [9,15]. Brain-derived neurotrophic factor (BD-NF) and neurotrophin 4

(NT4) were obtained from Regeneron and Sigma-Aldrich. Drugs were diluted with aCSF to the desired final concentrations immediately before application. A monoclonal phosphorylation-site specific antibody recognizing phospho-Thr286- α CaMKII was obtained from Upstate (Lake Placid, NY) and used for Western blot (1:1000) or immunohistochemical analysis (1:100). A polyclonal phospho-specific antibody against phospho-Ser831-GluR1 was obtained from Abcam (Cambridge, UK) and used for Western blot (1:1000) or immunohistochemical analysis (1:200). The antibodies for total CaMKII (Upstate) and total GluR1 (Abcam) were used for Western blot analysis at 1:2000.

Statistical analysis

Data are presented as group means $\pm$ SE. One-way ANOVA was applied to test for overall statistical significance across multiple group means, followed by Bonferroni *post hoc* test for pairwise mean comparisons. Student's *t*-test was used in LTP experiments for two-group comparisons. Statistical significance was defined as $p < 0.05$.

RESULTS

Neurotrophin rescues $A\beta$ -induced LTP deficits

We first examined whether exogenously applied neurotrophin could affect synaptic plasticity and rescue impaired LTP in $A\beta$ -treated hippocampal slices. As shown in Fig. 1, a single HFS induced early LTP in the Schaffer collateral-CA1 pathway of control slices, increasing the EPSP slope to $176 \pm 8\%$ of the baseline at 45 min after HFS ($n = 23$). The CA1 LTP was significantly reduced in slices pretreated with $1 \mu\text{M } A\beta_{1-42}$ for 30 min ($137 \pm 4\%$, $n = 27$, $p < 0.01$ compared to control LTP). NT4 pretreatment alone (100 ng/ml, 30 min) caused no evident changes in either baseline synaptic responses or early LTP ($167 \pm 9\%$, $n = 9$). However, when slices were co-treated with $A\beta$ and NT4, the subsequent LTP ($165 \pm 9\%$, $n = 11$) was significantly higher than that in slices treated with $A\beta$ alone ($p < 0.01$) and indistinguishable from control LTP (Fig. 1A, 1B). Similarly, in the dentate medial perforant path (Fig. 1C), early LTP induced in $A\beta$ -treated slices ($113 \pm 5\%$, $n = 15$) was significantly smaller than that in control slices ($132 \pm 5\%$, $n = 12$, $p < 0.05$). NT4 treatment did not alter dentate LTP by itself ($130 \pm 5\%$, $n = 17$) but prevented LTP inhibition by $A\beta$ when co-applied. The synaptic responses recorded 30 min after HFS in slices treated with both NT4 and $A\beta$ ($134 \pm 6\%$, $n = 19$) was significantly higher than that in slices treated with $A\beta$ alone and indistinguishable from the control LTP. Thus, when it did not affect LTP under control conditions, NT4 could rescue $A\beta$ -induced LTP deficits in two major hippocampal pathways.

Neurotrophins enhance CaMKII and AMPA receptor phosphorylation

Next, we determined whether neurotrophin treatment could enhance CaMKII autophosphorylation and function in hippocampal neurons. Hippocampal slices were incubated in oxygenated aCSF containing 50 or 100 ng/ml BDNF or NT4 for 30 min and immediately processed for Western blot analysis. As shown in Fig. 2A, while neither affected the level of total CaMKII, both BDNF and NT4 enhanced CaMKII autophosphorylation in a dose-dependent manner. The p-CaMKII level was increased by 68–130% after NT4 treatment and by 97–143% after BDNF treatment, compared to the level in control slices treated with drug-free aCSF ($n = 15$ for all groups, $p < 0.05$ or 0.01). Consistent with upregulation of CaMKII activity, CaMKII-dependent phosphorylation of AMPA receptors at Ser831-GluR1 was dose-dependently increased in BDNF- or NT4-treated slices (Fig. 2B). The p-GluR1 level was increased by 80–131% and 89–172%, respectively, after NT4 or BDNF treatments ($n = 15$, $p < 0.05$ or 0.01 compared to the controls). No significant changes in the total GluR1 level were detected. Immunohistochemical analysis using phosphospecific antibodies showed increased

fluorescent labeling for p-CaMKII and p-GluR1 in the CA1 pyramidal cell layer, dentate granule cell layer and respective dendritic layers (Fig. 3).

Neurotrophins facilitate LTP-induced CaMKII activation and AMPA receptor phosphorylation

Last, we examined whether neurotrophins can oppose $A\beta$ action, promoting LTP-associated CaMKII and GluR1 phosphorylation. Hippocampal slices were pretreated with neurotrophin alone or combined with $A\beta$ for 30 min, received a single HFS to induce dentate LTP, and harvested at 15 min after HFS. The dentate area of the slices was quickly dissected out, frozen on dry ice, and processed for Western blot analyses. To determine drug-induced changes in basal phosphorylation without induction of LTP, additional slices from the same animals were used as paired controls that received matching drug treatment but not HFS. As shown in Fig. 4, $A\beta_{1-42}$ treatment ($1 \mu\text{M}$) did not affect the basal level of p-CaMKII and p-GluR1, but blocked HFS-induced increases in CaMKII and GluR1 phosphorylation. Neurotrophin treatment (100 ng/ml) increased p-CaMKII and p-GluR1 levels and prevented their inhibition by $A\beta$ during LTP. In comparison, NT4 appeared to induce less basal phosphorylation than BD-NF, which allowed further increases in phosphorylation by HFS relative to paired, unstimulated slices (Fig. 4A). We therefore further focused on the effect of NT4 on LTP-associated CaMKII and GluR1 phosphorylation in the presence or absence of $A\beta$ (Fig. 4B, 4C). Following the HFS, the p-CaMKII level in slices pretreated with both NT4 and $A\beta$ increased to $163 \pm 12\%$ of the control level, which was significantly higher than the post-HFS level in slices treated with $A\beta$ alone ($104 \pm 13\%$, $p < 0.01$) and comparable to the post-HFS level in slices treated with aCSF or NT4 alone ($146 \pm 10\%$ or $169 \pm 11\%$, $p > 0.05$ for both comparisons). In slices not subject to HFS, NT4 treatment significantly elevated the basal p-CaMKII level ($131 \pm 9\%$ of the controls, $p < 0.05$) and this effect was not blocked by co-treatment with $A\beta$ (Fig. 4B). Similar patterns of changes in p-GluR1 levels were induced by NT4 and/or $A\beta$ under basal condition and during LTP (Fig. 4C).

DISCUSSION

TrkB-acting neurotrophins play a crucial role in hippocampal synaptic plasticity in the adult brain. Targeted deletion of the gene for BDNF [21,22] or trkB receptors [23] in mice results in severe impairments in early and late LTP at CA1 synapses and behavioral deficits in hippocampus-dependent tasks. Exogenously applied BDNF, on the other hand, restores hippocampal LTP in brain slices derived from BDNF knockout mice [24] and other mutant mice lacking gene products required for activity-dependent synaptic plasticity [25,26]. Although not as well-known as BDNF, NT4 has also been implicated in maintaining hippocampal synaptic function. The NT4 knockout mice display normal basal synaptic transmission and short-term synaptic plasticity but impaired late LTP at hippocampal CA1 synapses and show long-term memory deficits during contextual fear conditioning [27]. When replacing the BDNF coding sequence with the NT4 sequence in a knock-in mouse, expression of NT4 completely rescues BD-NF deletion-induced morphological and biochemical phenotypes and enhances formation of hippocampal synapses [28]. In the present study, application of exogenous NT4 did not affect hippocampal basal synaptic transmission and early LTP, but it was able to prevent LTP deficits induced by $A\beta$ when co-applied. This LTP-rescuing action of NT4 was reminiscent to that previously reported for exogenously applied BDNF. These findings support the notion that trkB-acting neurotrophins not only are involved in maintaining normal synaptic function, but can also prevent or alleviate neurodegenerative disease-related synaptic dysfunction. Furthermore, BDNF and NT4 strongly enhanced CaMKII autophosphorylation, a signaling event

stimulated by LTP but suppressed by $A\beta$. These results are consistent with the proposed role of CaMKII as a key regulatory target for both $A\beta$ toxicity and neurotrophin rescue.

Previous studies have indicated that LTP enhancement by BDNF can be mediated at presynaptic sites by increasing glutamate release [29]. Evidence also exists for postsynaptic action of BDNF which evokes depolarization and Ca^{2+} transients in dendrites and spines of the postsynaptic neuron [30]. More recently, it has been shown that application of BDNF simultaneously stimulates pre- and postsynaptic NMDA receptors in hippocampal slices [31]. As membrane depolarization and subsequent Ca^{2+} influx through NM-DA receptor channels is the major driving force for CaMKII autophosphorylation, these actions of neurotrophins could readily lead to enhanced CaMKII autophosphorylation as seen in this study, and the resulting CaMKII activation is likely to occur at both pre-and postsynaptic sites. Autophosphorylation at Thr286 is known to render persistent, Ca^{2+} - and calmodulin-independent CaMKII activity and promote translocation of the kinase to the postsynaptic density [32]. This process enables CaMKII to modulate multiple synaptic proteins in a highly efficient manner and hence enhances LTP. At the presynaptic site, CaMKII-mediated phosphorylation of synapsin 1 promotes its dissociation from synaptic vesicles, causing increased neurotransmitter release [33]. At the postsynaptic site, the best known substrate of CaMKII is the AMPA type of glutamate receptors. CaMKII-induced phosphorylation of AMPA receptors at Ser831-GluR1 increases channel conductance of the receptor [34], which can directly contribute to LTP expression. Consistent with this scenario, our results showed significant increases in GluR1 phosphorylation after neurotrophin treatments, in parallel with enhanced CaMKII autophosphorylation and LTP rescue. Activation of postsynaptic CaMKII is also known to trigger rapid delivery of AMPA receptors from non-synaptic sites into the synapses [35] and promote activity-dependent growth of dendritic spines and formation of new synapses [36]. In contrast, $A\beta$ reportedly reduces the number of synaptic AMPA receptors [37,38], blocks activity-dependent AMPA receptor phosphorylation [15], and may cause loss of dendritic spines in the hippocampus [39]. Taken together, CaMKII activity and $A\beta$ have opposing effects in regulating multiple aspects of synaptic function. The substantial enhancement of CaMKII activity by neurotrophins could therefore well explain their LTP-rescuing effect in the presence of $A\beta$. It should be mentioned, however, our study does not exclude the possibility that neurotrophins can act through other signaling pathways to rescue synaptic function. Through trkB receptors, neurotrophins can activate multiple signaling cascades, including phosphoinositide 3-kinases, phospholipase C, and mitogen-activated protein kinases (MAPK) [40]. Future studies are needed to determine the involvement of these pathways in the protective effect of neurotrophins.

The reversal of $A\beta$ -induced synaptic and signaling impairments by BDNF or NT4 indicates therapeutic potentials of trkB-acting neurotrophins for AD. There is ample evidence showing rapid regulation of neurotrophin synthesis by neuronal activity and release of endogenous neurotrophins in an activity-dependent manner from neuronal dendrites [19]. The increased neurotrophin level at synapses provides strong support to synaptic plasticity [41] and protects neurons from excitotoxic stress and oxidative insults [42]. These properties of neurotrophins, combined with the finding that BDNF expression is significantly reduced in the hippocampus and frontal cortex of AD brain [43,44], make neurotrophin-based strategies attractive candidates for AD treatment. Previous studies have investigated the protective role of neurotrophins in AD, focusing to large extent on their ability to improve neuronal cell survival against $A\beta$ toxicity [45]. Further studies, however, are in need to determine whether and how neurotrophins can reverse $A\beta$ -induced synaptic dysfunction via regulation of key signaling pathways involved in synaptic modulation, such as CaMKII and MAPK. Elucidation of such mechanisms is particularly relevant for treatment of early AD in which synaptic and cognitive impairments may be developed prior to synaptic loss and neuronal

cell death. Promising new approaches that upregulate neurotrophin signaling, such as development of functional mimetics of neurotrophins capable of penetrating blood-brain-barrier [46] and utilization of neural stem cell-derived BDNF [47], have provided valuable tools for further investigation in this area.

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References

1. Lesne S, Koh MT, Kotilinek L, Kaye R, Glabe CG, Yang A, Gallagher M, Ashe KH. A specific amyloid-beta protein assembly in the brain impairs memory. *Nature*. 2006; 440:352–357. [PubMed: 16541076]
2. Lacor PN, Buniel MC, Chang L, Fernandez SJ, Gong Y, Viola KL, Lambert MP, Velasco PT, Bigio EH, Finch CE, Krafft GA, Klein WL. Synaptic targeting by Alzheimer's-related amyloid beta oligomers. *J Neurosci*. 2004; 24:10191–10200. [PubMed: 15537891]
3. Lambert MP, Barlow AK, Chromy BA, Edwards C, Freed R, Liosatos M, Morgan TE, Rozovsky I, Trommer B, Viola KL, Wals P, Zhang C, Finch CE, Krafft GA, Klein WL. Diffusible, nonfibrillar ligands derived from A β 1-42 are potent central nervous system neurotoxins. *Proc Natl Acad Sci U S A*. 1998; 95:6448–6453. [PubMed: 9600986]
4. Walsh DM, Klyubin I, Fadeeva JV, Cullen WK, Anwyl R, Wolfe MS, Rowan MJ, Selkoe DJ. Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation *in vivo*. *Nature*. 2002; 416:535–539. [PubMed: 11932745]
5. Shankar GM, Li S, Mehta TH, Garcia-Munoz A, Shepardson NE, Smith I, Brett FM, Farrell MA, Rowan MJ, Lemere CA, Regan CM, Walsh DM, Sabatini BL, Selkoe DJ. Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nat Med*. 2008; 14:837–842. [PubMed: 18568035]
6. Li S, Hong S, Shepardson NE, Walsh DM, Shankar GM, Selkoe D. Soluble oligomers of amyloid beta protein facilitate hippocampal long-term depression by disrupting neuronal glutamate uptake. *Neuron*. 2009; 62:788–801. [PubMed: 19555648]
7. Nimmrich V, Grimm C, Draguhn A, Barghorn S, Lehmann A, Schoemaker H, Hillen H, Gross G, Ebert U, Bruehl C. Amyloid beta oligomers (A beta(1-42) globulomer) suppress spontaneous synaptic activity by inhibition of P/Q-type calcium currents. *J Neurosci*. 2008; 28:788–797. [PubMed: 18216187]
8. Gortz P, Opatz J, Siebler M, Funke SA, Willbold D, Lange-Asschenfeldt C. Transient reduction of spontaneous neuronal network activity by sublethal amyloid beta (1-42) peptide concentrations. *J Neural Transm*. 2009; 116:351–355. [PubMed: 19214376]
9. Fradinger EA, Monien BH, Urbanc B, Lomakin A, Tan M, Li H, Spring SM, Condrum MM, Cruz L, Xie CW, Benedek GB, Bitan G. C-terminal peptides coassemble into A β 1-42 oligomers and protect neurons against A β 1-42-induced neurotoxicity. *Proc Natl Acad Sci U S A*. 2008; 105:14175–14180. [PubMed: 18779585]
10. Selkoe DJ. Soluble oligomers of the amyloid beta-protein impair synaptic plasticity and behavior. *Behav Brain Res*. 2008; 192:106–113. [PubMed: 18359102]
11. Venkitaramani DV, Chin J, Netzer WJ, Gouras GK, Lesne S, Malinow R, Lombroso PJ. Beta-amyloid modulation of synaptic transmission and plasticity. *J Neurosci*. 2007; 27:11832–11837. [PubMed: 17978019]
12. Chen QS, Kagan BL, Hirakura Y, Xie CW. Impairment of hippocampal long-term potentiation by Alzheimer amyloid b-peptides. *J Neurosci Res*. 2000; 60:65–72. [PubMed: 10723069]
13. Chen QS, Wei WZ, Shimahara T, Xie CW. Alzheimer amyloid b-peptide inhibits the late phase of long-term potentiation through calcineurin-dependent mechanisms in the hippocampal dentate gyrus. *Neurobiol Learn Mem*. 2002; 77:354–371. [PubMed: 11991763]

14. Knobloch M, Farinelli M, Konietzko U, Nitsch RM, Mansuy IM. Abeta oligomer-mediated long-term potentiation impairment involves protein phosphatase 1-dependent mechanisms. *J Neurosci*. 2007; 27:7648–7653. [PubMed: 17634359]
15. Zhao D, Watson JB, Xie CW. Amyloid beta prevents activation of calcium/calmodulin-dependent protein kinase II and AMPA receptor phosphorylation during hippocampal long-term potentiation. *J Neurophysiol*. 2004; 92:2853–2858. [PubMed: 15212428]
16. Silva AJ, Paylor R, Wehner JM, Tonegawa S. Impaired spatial learning in alpha-calcium-calmodulin kinase II mutant mice. *Science*. 1992; 257:206–210. [PubMed: 1321493]
17. Blitzer RD, Connor JH, Brown GP, Wong T, Shenolikar S, Iyengar R, Landau EM. Gating of CaMKII by cAMP-regulated protein phosphatase activity during LTP. *Science*. 1998; 280:1940–1942. [PubMed: 9632393]
18. Xie CW. Calcium-regulated signaling pathways: role in amyloid beta-induced synaptic dysfunction. *Neuromolecular Med*. 2004; 6:53–64. [PubMed: 15781976]
19. Lu B. BDNF and activity-dependent synaptic modulation. *Learn Mem*. 2003; 10:86–98. [PubMed: 12663747]
20. Wang LM, Dragich JM, Kudo T, Odom IH, Welsh DK, O'Dell TJ, Colwell CS. Expression of the circadian clock gene *Period2* in the hippocampus: possible implications for synaptic plasticity and learned behaviour. *ASN Neuro*. 2009; 1:e00012. [PubMed: 19570032]
21. Korte M, Carroll P, Wolf E, Brem G, Thoenen H, Bonhoeffer T. Hippocampal long-term potentiation is impaired in mice lacking brain-derived neurotrophic factor. *Proc Natl Acad Sci U S A*. 1995; 92:8856–8860. [PubMed: 7568031]
22. Korte M, Kang H, Bonhoeffer T, Schuman E. A role for BDNF in the late-phase of hippocampal long-term potentiation. *Neuropharmacology*. 1998; 37:553–559. [PubMed: 9704996]
23. Vyssotski AL, Dell'Omo G, Poletaeva, Vyssotski DL, Minichiello L, Klein R, Wolfer DP, Lipp HP. Long-term monitoring of hippocampus-dependent behavior in naturalistic settings: mutant mice lacking neurotrophin receptor TrkB in the forebrain show spatial learning but impaired behavioral flexibility. *Hippocampus*. 2002; 12:27–38. [PubMed: 11918285]
24. 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; 16:1137–1145. [PubMed: 8663990]
25. Muller D, Djebbara-Hannas Z, Jourdain P, Vutsits L, Durbec P, Rougon G, Kiss JZ. Brain-derived neurotrophic factor restores long-term potentiation in polysialic acid-neural cell adhesion molecule-deficient hippocampus. *Proc Natl Acad Sci U S A*. 2000; 97:4315–4320. [PubMed: 10760298]
26. Pang PT, Teng HK, Zaitsev E, Woo NT, Sakata K, Zhen S, Teng KK, Yung WH, Hempstead BL, Lu B. Cleavage of proBDNF by tPA/plasmin is essential for long-term hippocampal plasticity. *Science*. 2004; 306:487–491. [PubMed: 15486301]
27. Xie CW, Sayah D, Chen QS, Wei WZ, Smith D, Liu X. Deficient long-term memory and long-lasting long-term potentiation in mice with a targeted deletion of neurotrophin-4 gene. *Proc Natl Acad Sci U S A*. 2000; 97:8116–8121. [PubMed: 10869436]
28. Fan G, Egles C, Sun Y, Minichiello L, Renger JJ, Klein R, Liu G, Jaenisch R. Knocking the NT4 gene into the BDNF locus rescues BDNF deficient mice and reveals distinct NT4 and BDNF activities. *Nat Neurosci*. 2000; 3:350–357. [PubMed: 10725924]
29. Gottschalk W, Pozzo-Miller LD, Figurov A, Lu B. Presynaptic modulation of synaptic transmission and plasticity by brain-derived neurotrophic factor in the developing hippocampus. *J Neurosci*. 1998; 18:6830–6839. [PubMed: 9712654]
30. Kovalchuk Y, Hanse E, Kafitz KW, Konnerth A. Postsynaptic Induction of BDNF-Mediated Long-Term Potentiation. *Science*. 2002; 295:1729–1734. [PubMed: 11872844]
31. Madara JC, Levine ES. Presynaptic and postsynaptic NMDA receptors mediate distinct effects of brain-derived neurotrophic factor on synaptic transmission. *J Neurophysiol*. 2008; 100:3175–3184. [PubMed: 18922945]
32. Strack S, Choi S, Lovinger DM, Colbran RJ. Translocation of autophosphorylated calcium/calmodulin-dependent protein kinase II to the postsynaptic density. *J Biol Chem*. 1997; 272:13467–13470. [PubMed: 9153188]

33. Benfenati F, Valtorta F, Rubenstein JL, Gorelick FS, Greengard P, Czernik AJ. Synaptic vesicle-associated Ca²⁺/calmodulin-dependent protein kinase II is a binding protein for synapsin I. *Nature*. 1992; 359:417–420. [PubMed: 1328883]
34. Derkach V, Barria A, Soderling TR. Ca²⁺/calmodulin-kinase II enhances channel conductance of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate type glutamate receptors. *Proc Natl Acad Sci U S A*. 1999; 96:3269–3274. [PubMed: 10077673]
35. Hayashi Y, Shi SH, Esteban JA, Piccini A, Poncer JC, Malinow R. Driving AMPA receptors into synapses by LTP and CaMKII: requirement for GluR1 and PDZ domain interaction. *Science*. 2000; 287:2262–2267. [PubMed: 10731148]
36. Jourdain P, Fukunaga K, Muller D. Calcium/calmodulin-dependent protein kinase II contributes to activity-dependent filopodia growth and spine formation. *J Neurosci*. 2003; 23:10645–10649. [PubMed: 14627649]
37. Hsieh H, Boehm J, Sato C, Iwatsubo T, Tomita T, Sisodia S, Malinow R. AMPAR removal underlies Abeta-induced synaptic depression and dendritic spine loss. *Neuron*. 2006; 52:831–843. [PubMed: 17145504]
38. Gu Z, Liu W, Yan Z. beta-Amyloid impairs AMPA receptor trafficking and function by reducing Ca²⁺/calmodulin-dependent protein kinase II synaptic distribution. *J Biol Chem*. 2009; 284:10639–10649. [PubMed: 19240035]
39. Lanz TA, Carter DB, Merchant KM. Dendritic spine loss in the hippocampus of young PDAPP and Tg2576 mice and its prevention by the ApoE2 genotype. *Neurobiol Dis*. 2003; 13:246–253. [PubMed: 12901839]
40. Reichardt LF. Neurotrophin-regulated signalling pathways. *Philos Trans R Soc Lond B Biol Sci*. 2006; 361:1545–1564. [PubMed: 16939974]
41. Thoenen H. Neurotrophins and neuronal plasticity. *Science*. 1995; 270:593–598. [PubMed: 7570017]
42. Guo ZH, Mattson MP. Neurotrophic factors protect cortical synaptic terminals against amyloid and oxidative stress-induced impairment of glucose transport, glutamate transport and mitochondrial function. *Cereb Cortex*. 2000; 10:50–57. [PubMed: 10639395]
43. Ferrer I, Marin C, Rey MJ, Ribalta T, Goutan E, Blanco R, Tolosa E, Marti E. BDNF and full-length and truncated TrkB expression in Alzheimer disease: Implications in therapeutic strategies. *J Neuropathol Exp Neurol*. 1999; 58:729–739. [PubMed: 10411343]
44. Phillips HS, Hains JM, Armanini M, Laramie GR, Johnson SA, Winslow JW. BDNF mRNA is decreased in the hippocampus of individuals with Alzheimer's disease. *Neuron*. 1991; 7:695–702. [PubMed: 1742020]
45. Tapia-Arancibia L, Aliaga E, Silhol M, Arancibia S. New insights into brain BDNF function in normal aging and Alzheimer disease. *Brain Res Rev*. 2008; 59:201–220. [PubMed: 18708092]
46. Jang SW, Liu X, Yepes M, Shepherd KR, Miller GW, Liu Y, Wilson WD, Xiao G, Blanche B, Sun YE, Ye K. A selective TrkB agonist with potent neurotrophic activities by 7,8-dihydroxyflavone. *Proc Natl Acad Sci U S A*. 2010; 107:2687–2692. [PubMed: 20133810]
47. Blurton-Jones M, Kitazawa M, Martinez-Coria H, Castello NA, Muller FJ, Loring JF, Yamasaki TR, Poon WW, Green KN, LaFerla FM. Neural stem cells improve cognition via BDNF in a transgenic model of Alzheimer disease. *Proc Natl Acad Sci U S A*. 2009; 106:13594–13599. [PubMed: 19633196]

Fig. 1. Neurotrophin 4 (NT4) prevents amyloid- β ($A\beta$)-induced LTP deficits in the Schaffer collateral-CA1 pathway (A, B) and dentate medial perforant path (C) of rat hippocampal slices. Slices were pretreated with $1 \mu\text{M}$ $A\beta_{1-42}$ for 30 min in the absence or presence of NT4 (100 ng/ml) and received a single HFS for LTP induction. Note inhibition of LTP by $A\beta$ in both pathways and reversal of such inhibition by NT4 co-treatment. NT4 treatment alone did not affect LTP. $n = 9-27$ for each group. Representative CA1 fEPSP recordings before (1) and after (2) HFS from the same slices are superimposed and shown in (A). Calibration bars: 1 mV/5 ms.

Fig. 2. Neurotrophins enhance CaMKII autophosphorylation (A) and phosphorylation of GluR1 subunits of AMPA receptors (B). Hippocampal slices were treated for 30 min in aCSF containing NT4 or BDNF (50 or 100 ng/ml as labeled) and processed for Western blotting. The representative images (top) and group data (bottom) show dose-dependent increases in p-CaMKII and p-GluR1 levels after neurotrophin treatment, with little changes in total CaMKII or GluR1 levels. Changes in the protein levels were calculated relative to that of control slices treated with drug-free aCSF. $n = 15$ for each group. * and **, $p < 0.05$ and 0.01, respectively, compared to the controls.

Fig. 3.

Immunohistochemical analysis of neurotrophin-induced CaMKII and GluR1 phosphorylation. Hippocampal slices were treated with aCSF (Control), BDNF, or NT4 (100 ng/ml) for 30 min and processed for immunoreactive double labeling of p-CaMKII (top) and p-GluR1 (bottom) using phosphospecific antibodies. Confocal fluorescent images were obtained using a Zeiss LSM 5 Pascal laser scanning microscope with a 5× water immersion objective. Calibration bar: 400 μ m.

Fig. 4. Neurotrophins prevent inhibition by $A\beta_{1-42}$ of LTP-induced CaMKII and GluR1 phosphorylation in the dentate gyrus. A) Representative western blot images. Multiple slices from the same rats were used for different treatments in a single set of experiment as labeled. Note markedly increased p-CaMKII and p-GluR1 levels in slices pretreated with BDNF or NT4 (100 ng/ml), and lack of inhibition by $A\beta_{1-42}$ (1 μ M) of HFS-induced phosphorylation when slices were co-treated with neurotrophins. B and C) Phosphorylation changes in slices pretreated with $A\beta$, NT4, or both. LTP-induced p-CaMKII and p-GluR1 increases were measured 15 min after HFS. The basal phosphorylation levels were determined in paired slices that received matching treatment except no HFS. All data are expressed as percentages of the basal level measured in aCSF-treated slices (control). $n = 19$ for each group. # $p < 0.05$ compared to the basal level of aCSF-treated slices. * and **, $p < 0.05$ and 0.01 , respectively, compared to the post-HFS level in $A\beta$ -treated slices. Except in slices treated with $A\beta$ alone, HFS induced significant increases in p-CaMKII and p-GluR1 levels from the paired basal levels.