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Amyloid beta from axons and dendrites reduces local spine number and plasticity

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Abstract

Excessive synaptic loss is thought to be one of the earliest events in Alzheimer's disease. Amyloid beta (A β), a peptide secreted in an activity-modulated manner by neurons, has been implicated in the pathogenesis of Alzheimer's disease by removing dendritic spines, sites of excitatory synaptic transmission. However, issues regarding the subcellular source of A β , as well as the mechanisms of its production and actions that lead to synaptic loss, remain poorly understood. In rat organotypic slices, we found that acute overproduction of either axonal or dendritic A β reduced spine density and plasticity at nearby (~5–10 μ m) dendrites. The production of A β and its effects on spines were sensitive to blockade of action potentials or nicotinic receptors; the effects of A β (but not its production) were sensitive to NMDA receptor blockade. Notably, only 30–60 min blockade of A β overproduction permitted induction of plasticity. Our results indicate that continuous overproduction of A β at dendrites or axons acts locally to reduce the number and plasticity of synapses.

The early stages of Alzheimer's disease pathogenesis are thought to occur at the synapse, since synapse loss is the best correlate with memory dysfunction¹. Considerable evidence suggests that A β , a secreted proteolytic derivative of amyloid precursor protein (APP), is important for the early 'synaptic failure' that is seen in Alzheimer's disease pathogenesis (reviewed in ref. 2). A β oligomers bind to synaptic sites³ and reduce the density of spines in organotypic hippocampal slice cultures^{4–6}, dissociated cultured neurons^{7–9} and transgenic mouse models^{10–12}. Consistent with these structural abnormalities, neurons treated with A β or that overexpress APP show depressed glutamatergic transmission^{4,7,13–16}.

Given the memory deficits observed in Alzheimer's disease, it is notable that soluble A β oligomers impair long-term potentiation (LTP, a synaptic model of memory)^{11,17,18} and memory^{11,19,20}, which can be ameliorated by treatment with antibody to A β or small

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AUTHOR CONTRIBUTIONS

W.W., L.N.N., H.W.K. and H.H. designed and conducted the experiments and analyzed data. S.S. and R.M. designed experiments and supervised the project.

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molecules that inhibit A β aggregation^{21–24}. Despite the well-studied effect of A β on electrophysiological LTP, its effect on spine structural plasticity, which occurs during LTP^{25–28}, has not been examined. For instance, it is not known if increased production of A β in one neuron will affect structural plasticity in a nearby neuron.

Although A β perturbs synaptic transmission and plasticity, such A β -mediated processes are subject to activity-dependent modulation. The level of A β secretion is controlled by neural activity in brain slices¹³ and *in vivo*²⁹. In humans, regions of the brain with high resting activity are positively correlated with A β plaque load³⁰. In addition to A β production, the effects of A β may also depend on neural activity. For instance, NMDA receptor activation is required for A β -mediated spine loss⁵ and synaptic depression¹³.

To understand the interaction between A β and synaptic function, we sought to identify the subcellular sites from which A β acts. In particular, it has not been clearly established whether the A β that produces synaptic deficits is generated in pre- or postsynaptic compartments. APP and its derivatives, as well as components of the APP-processing enzymes β -secretase and γ -secretase, have been detected in axons and dendrites by biochemical, immunostaining and electron-microscopy studies^{31–37}. However, detecting effects produced from axonally or dendritically produced A β has been challenging. We isolated the sites of increased A β production by selectively expressing APP in pre- or postsynaptic neurons. We used two-photon laser-scanning imaging to monitor the synaptic deficits caused by such dendritic or axonal A β . We found that either dendritic or axonal A β overproduction was sufficient to cause local spine loss and compromise plasticity in the nearby dendrites of neurons that did not overexpress A β . Furthermore, A β -mediated synaptic dysfunction could be pharmacologically ameliorated by blockade of neural activity, NMDA receptors or nicotinic acetylcholine receptors. Our findings indicate that local levels and effects of dendritic and axonal A β can be controlled by activity-modulated mechanisms.

RESULTS

Dendritic A β reduces spine density at nearby dendrites

Previous studies have shown that overexpression of APP leads to A β secretion and a loss of spines on neurons overproducing APP^{4,10} that phenocopies quantitatively the effects of exogenous addition of oligomeric A β ^{5,6,9,38} at concentrations reached in the brains of individuals with Alzheimer's disease³⁶. This robust effect of A β on spine density allowed us to use an imaging assay to monitor the effects of local A β . We reasoned that if A β overproduction could cause spine loss in APP-overexpressing neurons, then neighboring neurons close to A β -containing structures may also be exposed to higher levels of secreted A β and therefore lose their spines. We first tested whether A β from dendrites of APP-overexpressing neurons can affect neighboring dendrites that do not overexpress APP. The CA1 region of a hippocampal slice culture was infected with two Sindbis viruses: an enhanced green fluorescent protein (EGFP) virus that did not express APP at a moderate titer to label multiple neurons and a double promoter APP/tomato virus at a very low titer so that only one or a few cells were infected and expressed APP (Fig. 1a). Two-photon laser-scanning microscopy was used to image 50–100- μ m segments of CA1 pyramidal neuron apical dendrites located 150–250 μ m from the cell body 1 d after APP overexpression. EGFP-labeled CA1 dendrites that did not overexpress APP were divided into two groups: a group of dendrites running parallel and staying within 10 μ m of an APP-overexpressing dendrite and a control group consisting of dendrites from the same infected slices, but which were more than 50 μ m away from any APP-overexpressing structures (Fig. 1b). The spine density of EGFP-labeled dendrites more than 50 μ m from APP-overexpressing dendrites was not significantly different from the spine density in slices with no APP overexpression ($P = 0.2$, data not shown). However, both EGFP-labeled dendrites within 10 μ m of APP-

overexpressing dendrites and APP-overexpressing dendrites showed a reduction in spine density (Fig. 1c). The reduction in spine density was blocked by a γ -secretase inhibitor (L685,458; Fig. 1c), indicating that A β from dendrites of APP-overexpressing cells caused spine loss in a population of nearby dendrites. Similar effects were observed when we overexpressed APP in hippocampal neurons *in vivo*; dendrites expressing only EGFP that were within 10 μ m of a dendrite from an APP-overexpressing neuron showed a reduction in spine density (Supplementary Fig. 1).

Neural activity has been shown to be involved in the modulation of A β production and effects^{5,13,29,30}. We examined how activity influenced spine loss from dendritic A β production. Slices were infected with the EGFP virus and the APP virus and cultured with either the sodium channel antagonist tetrodotoxin (TTX), the NMDA receptor antagonist D(-)-2-amino-5-phosphonovaleric acid (AP5), α -bungarotoxin, an antagonist of the α 7 subunit-containing nicotinic acetylcholine receptor (nAChR) or no drugs. We imaged EGFP-labeled and APP-overexpressing dendrites 1 d after APP overexpression and measured spine density. All of the drugs had a protective effect on spine loss mediated by dendritic A β production (Fig. 1c). These results indicate that spine loss resulting from 1 d of dendritic A β overproduction can be reduced by blockade of action potentials, nAChRs or NMDA receptors.

Axonal A β reduces spine density at nearby dendrites

We next examined whether the axonal compartment can also be a source of A β . To specifically examine the effect of axons, we infected CA3 neurons with the APP/tomato virus and CA1 neurons with the EGFP virus (Fig. 2a). We examined CA1 regions far (>200 μ m) from the CA3 dendritic region that contained axonal projections from CA3 pyramidal neurons. Thus, axons from APP virus-infected CA3 neurons were the only APP-overexpressing structures in the CA1 regions that we examined. In each slice, we were able to find areas in the CA1 stratum radiatum containing many APP-overexpressing axons (more than ten APP-overexpressing boutons per 5,000 μ m³, near APP axons) as well as areas containing very few or no APP-overexpressing axons (less than one APP-overexpressing bouton per 5,000 μ m³, far from APP axons; Fig. 2b). After 2 d of infection, the spine density of EGFP dendrites in APP virus-infected slices, but far from APP-labeled axons was not significantly different from the spine density of EGFP dendrites in slices with no APP overexpression ($P = 0.8$, data not shown). However, EGFP-labeled dendrites near APP axons showed reduced spine density compared with the EGFP group far from APP axons (Fig. 2c). The spine reduction by axonally expressed APP was blocked by γ -secretase inhibition, indicating that A β from axons reduced local spine density.

To examine whether neural activity can modulate spine loss from axonal A β , we infected slices with the EGFP and APP/tomato viruses and maintained them in TTX, AP5 or α -bungarotoxin. All three drugs prevented axonal A β -mediated spine loss in EGFP-labeled dendrites near APP axons (Fig. 2c). These results indicate that spine loss from axonal A β is subject to activity-dependent modulation.

Pharmacological control of production and effects of A β

Although blockade of action potentials, nAChR or NMDA receptors affected A β -mediated spine loss, it is not clear whether these treatments regulate A β production or the effects of A β on dendritic spines. We wished to determine whether AP5, TTX or α -bungarotoxin had an effect on A β production. The CA1 region of hippocampal slice cultures was infected with APP virus and incubated in normal medium or in medium containing AP5, TTX, α -bungarotoxin or L685,458 for 24 h. Both the infected slices and slice culture media were harvested at the end of the drug treatment. A β was measured in the media by ELISA and

full-length APP was measured in slices by western blotting. Secreted A β levels were compared across samples by normalizing secreted A β by levels of APP expression. Treatment of slices with TTX, α -bungarotoxin or L685,458 led to reduced A β levels in the medium of APP virus-infected slices (Fig. 3a,b). Notably, AP5 had no effect on secretion of A β .

We found that action potentials and nAChR were able to regulate A β secretion; however, our results do not exclude the possibility that they can also regulate the effect of A β on dendritic spines. To address this, we added synthetic A β 42, prepared in a manner that promotes formation of oligomeric structures (see Online Methods), to the media of EGFP virus-infected slice cultures for 24 h in the presence of TTX, α -bungarotoxin or AP5. Neither TTX nor α -bungarotoxin prevented A β -mediated spine loss (Fig. 4), indicating that action potentials and nAChRs regulate A β secretion, but not the effect of A β on spine loss. Although AP5 had no effect on A β secretion (Fig. 3a,b), AP5-treated neurons had normal spine density in the presence of synthetic A β (Fig. 4), indicating that NMDA receptor blockade modulated the effect of A β on spine density, but not secretion of A β . Together, these results indicate that different forms of activity can modulate A β -mediated spine loss through the regulation of A β secretion and by regulating the effects of A β on dendritic spines.

A β reduces spine structural plasticity

These results indicate that a substantial fraction of dendritic spines are lost over time in the presence of A β from dendrites or axons. But what is the effect of A β on the plasticity of the remaining spines? We next examined the structural plasticity of spines in the presence of A β . LTP and a long-lasting spine enlargement can be reliably induced in slices by a brief bath application of a solution that favors NMDA receptor activation and global neuronal bursting^{25,39}. Using this chemical LTP (cLTP) induction protocol, we examined spine enlargement in neurons that had been transfected with both APP and the cytoplasmic marker dsRed by biolistics transfection for 3 d. Images of spines were taken at multiple time points before, during and after cLTP induction. The volume of each spine at each time point was measured and normalized to the baseline value before cLTP induction. EGFP-transfected cells had stable cLTP-induced spine enlargement for at least 70 min after induction. However, APP-transfected neurons showed less cLTP-induced spine enlargement (Fig. 5a,b), indicating that structural plasticity was impaired in the APP-overexpressing cells. This compromised plasticity was reversible, as addition of L685,458 for 30 min before and during cLTP induction rescued the spine enlargement deficit in APP-overexpressing neurons (Fig. 5b). This indicates that continuous A β production is necessary to impair structural plasticity.

To further test the acute effect of A β on structural plasticity, we briefly added synthetic oligomeric A β (1–42) to EGFP-transfected neurons 1 h before and during cLTP induction. Compared with the untreated control, oligomeric A β (1–42)-treated neurons showed less spine enlargement after induction (Fig. 5a,b). Thus, short exposure of wild-type neurons to synthetic oligomeric A β is sufficient to impair cLTP-induced spine enlargement.

Given the modulatory roles of NMDAR and nAChR in A β -mediated spine loss, we examined their involvement in cLTP-induced spine enlargement in neurons overexpressing APP. To block NMDAR activation, we added AP5 1 h before cLTP induction, briefly removing it during cLTP induction (as NMDAR activity is required for cLTP induction) and replacing it after cLTP induction. This treatment led to normal spine enlargement in APP-overexpressing neurons (Fig. 5c), indicating that an AP5-sensitive process is continuously activated by APP overexpression to prevent cLTP-induced spine plasticity.

Blockade of nAChR was achieved by the addition of α -bungarotoxin 1 h before cLTP induction. Although the subsequent cLTP induction and expression was performed in solutions free of α -bungarotoxin, nAChRs were likely blocked as a result of the very slow off-rate of α -bungarotoxin⁴⁰. Following treatment with α -bungarotoxin, spines from APP-overexpressing neurons showed more spine enlargement after cLTP induction (Fig. 5d). These results indicate that blockade of NMDAR and nAChR can substantially reduce the A β -mediated deficit in cLTP-induced spine enlargement.

Axonal and dendritic A β reduces plasticity at nearby dendrites

The effects of axonal A β on spine plasticity were examined in a population of spines close to APP-overexpressing axons. EGFP and APP/dsRed were expressed in the CA1 and CA3 regions, respectively. We first conducted a control experiment to determine whether slices with APP-overexpressing CA3 neurons would exhibit similar synchronous bursting during cLTP induction as slices without APP-overexpressing CA3 neurons. This control experiment was necessary, as APP-overexpression can produce synaptic depression¹³ and synaptic activity is important for synchronizing CA3 bursting. Cell-attached recordings were performed simultaneously in pairs of CA1 and CA3 neurons before and during cLTP induction. Compared with uninfected controls, slices infected with EGFP and APP viruses showed similar synchronous bursting in both CA1 and CA3 neurons (Fig. 6a and Supplementary Fig. 2). This indicates that the APP overexpression in the CA3 region did not inhibit the neuronal bursting induced by the cLTP protocol. Indeed, normal spine enlargement was observed in EGFP-labeled CA1 cells far from APP-overexpressing axons (>50 μ m) in the infected slices (Fig. 6b,c). However, EGFP-labeled spines within 3 μ m of APP-overexpressing axons showed a reduction in spine enlargement (Fig. 6b,c). This indicates that axonal A β impairs nearby spine plasticity.

To test whether dendritic A β affects plasticity in neighboring spines, we examined cLTP-induced spine enlargement in spines close to APP-overexpressing dendrites. The CA1 region of a hippocampal slice culture was infected with the EGFP and APP viruses. EGFP-labeled spines within 3 μ m of an APP-overexpressing dendrite were grouped as spines close to APP dendrites (Fig. 7a). Spines more than 50 μ m away from any APP-overexpressing structure were used as a control group. Following cLTP induction, spines near APP-overexpressing dendrites showed reduced enlargement compared with the control group (Fig. 7b), indicating that A β from APP-overexpressing dendrites reduces spine plasticity at nearby dendrites.

These results predict that the further away the spines are from the A β production site, the less impaired their structural plasticity will be. To address this, we grouped EGFP-labeled spines on the basis of their distance from APP-overexpressing dendrites and compared them for volume change after cLTP (Fig. 7c). For spines within 3 μ m of APP dendrites or those 3–6 μ m from APP dendrites, spine enlargement after cLTP was reduced compared with EGFP spines far away from APP dendrites (>50 μ m) (Fig. 7c). However, normal spine plasticity was observed in the group of spines 6–20 μ m from APP dendrites (Fig. 7c), which suggests that the effective distance of dendritic A β on spine plasticity lies somewhere around 5–10 μ m from the APP-expressing dendrites in our experimental system. A similar distance-dependent effect was not attempted for experiments in which presynaptic CA3 axons were driven to overexpress APP, as large groups of axons were generally infected in any region, making it impossible to determine the distance from source to spine. The observed effect on spine plasticity (Figs. 4–7) was not a result of a difference in initial spine size among groups, as similar spine size distributions were observed among them (Supplementary Fig. 3).

DISCUSSION

Numerous studies indicate that A β peptides are important for initiating the pathogenesis of Alzheimer's disease². The mechanisms by which this occurs are not known, although recent studies have indicated that A β can reduce synaptic transmission and lead to the loss of synapses^{4,5,16}. Although A β is produced in vesicles from the secretory and endosomal system (reviewed in ref. 41), it has not been clear whether the A β that produces synaptic deficits originates from pre- or postsynaptic compartments. Previous studies found that APP can be transported anterogradely in axons^{32,33,35} and that A β can be made in axonal terminal fields²⁹; however, these studies did not demonstrate synaptic defects from axonally released A β . Other studies have shown that surgical lesions of axons decreased A β -rich plaques in axonal terminal regions of APP transgenic animals^{31,42}. However, these ablations should also reduce postsynaptic depolarization, which could reduce dendritic A β . Indeed, some studies have shown that overexpression of APP in dendritic compartments can reduce synaptic transmission in nearby neurons that do not overexpress APP, suggesting that dendritic A β can affect nearby synapses¹³.

We found that overexpression of APP in either dendritic or axonal compartments led to a reduction in spine density and plasticity in nearby neurons. This effect is relevant to Alzheimer's disease, as A β application at levels reached in the brains of individuals with Alzheimer's disease³⁶ produced a similar reduction in spine density and plasticity⁵ that could similarly be prevented by AP5. This effect was likely a result of secreted A β , as blockade of A β secretion by a γ -secretase inhibitor blocked spine reduction and impaired plasticity by APP overexpression. Furthermore, expression of APP(MV), a mutant form of APP that does not produce A β , but makes other cleavage products of APP, failed to produce a reduction in spine density⁴. It is possible that production of A β in axons or dendrites leads to secretion of additional toxic substances or prevents normal synaptic function and thereby leads to the local effects that we observed. Identifying the local target of A β that leads to reduction of spines and their plasticity would help to elucidate the mediators of these synaptic effects. Our findings indicate that A β from axonal or dendritic compartments can lead to a loss of synaptic structure and function. However, we employed acute production or delivery of A β . Thus, the effects that we observed may not be entirely representative of events that occur in a chronic condition such as Alzheimer's disease. Nevertheless, the effects that we observed in spine reduction were similar in magnitude and in pharmacological sensitivity to effects produced by concentrations of A β that are estimated to be present in the brains of individuals with Alzheimer's disease^{36,43}. Our results do not provide information regarding the source of A β in nonpathological conditions, where lower levels are likely produced. It is noteworthy that in Alzheimer's disease regions in the brain that are synaptically connected (for example, entorhinal cortex and hippocampus) can be affected in temporally linked manner. Our findings suggest that neurons in entorhinal cortex with increased A β production would affect the entorhinal cortex through its A β -rich dendrites and the dentate gyrus through its A β -rich axons.

Blockade of nAChRs with α -bungarotoxin led to a reduction of A β secretion from APP-overexpressing neurons. $\alpha 7$ -containing nAChRs are present at synaptic and extrasynaptic sites on pre- and postsynaptic compartments, as well as in the cell bodies of hippocampal pyramidal neurons^{44,45}. However, organotypic hippocampal slices have few or no cholinergic neurons. Therefore, there is probably a ligand other than acetylcholine acting on nAChRs that is blocked by α -bungarotoxin. One possibility is that A β acts on nAChRs⁴⁶ that are close to the site of A β release, leading to increased intracellular calcium⁴⁷, which leads to an increase in A β production and/or secretion. That is, A β could be part of a positive feedback loop that is mediated by nAChR activation and leads to increased A β secretion. Blockade of nAChRs would decrease A β secretion and reduce the effects of APP

overexpression on spine density and plasticity. Additional protective mechanisms by which α -bungarotoxin acts cannot be ruled out. It is notable that a widely used treatment strategy for Alzheimer's disease is to increase brain acetylcholine levels (reviewed in ref. 48). Our results suggest that this may lead to increase A β secretion and would therefore be detrimental. Indeed the long-term effect of enhancing acetylcholine levels on Alzheimer's disease progress is still in question⁴⁸.

Blockade of NMDA receptors produced effects different from blockade of nAChRs. AP5 did not reduce the secretion of A β . However, AP5 did prevent spine loss in cells overexpressing APP, on nearby cells and in slices exposed to exogenous A β . Thus, NMDA receptor activation is required for A β to exert its effects on spines, consistent with recent results⁵. It is noteworthy that the protective effects of AP5 or a γ -secretase inhibitor on spine plasticity in cells overexpressing APP could be seen with only 1 h pretreatment of slices with the drug. Apparently, the effects of A β during this period are important for its effects on plasticity and they require NMDA receptor activity. This effect is reminiscent of findings that mild activation of NMDA receptors before induction can block LTP⁴⁹. It is not clear whether A β produces NMDA receptor potentiation⁵⁰ or weakening^{5,14}, either of which could potentially mediate this effect.

In conclusion, we found that overproduction of axonal or dendritic A β can lead to a decrease in spine number and plasticity. The effects of A β were local and could be modulated by action potentials, nAChR and NMDA receptors. Thus, neurons that overproduce A β will control the gain of inputs and outputs to nearby neurons that do not overproduce A β . Although this could be a protective physiological mechanism, in excess, it could substantially reduce the function of a circuit.

METHODS

Methods and any associated references are available in the online version of the paper at <http://www.nature.com/natureneuroscience/>.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Dendritic A β reduces local spine density in an activity-dependent manner. **(a)** Viral-infected CA1 region of a hippocampal slice culture showing many EGFP-expressing and a few APP/tomato-expressing neurons. Scale bar represents 50 μm . **(b)** Apical dendrites of CA1 pyramidal neurons expressing EGFP or APP/tomato as in **a**. Top, an EGFP-labeled dendritic segment >50 μm away from APP-labeled structures. Bottom, an EGFP-labeled dendritic segment that is within 10 μm of an APP-labeled dendrite. Scale bar represents 5 μm . **(c)** Bar graph of spine density in EGFP neurons near or far from APP dendrites, and APP-expressing neurons in normal medium or medium containing TTX, AP5, α -bungarotoxin or L685,458 (no treatment (14 slices): 21, 14 and 17 dendrites for >50 μm from APP dendrite, <10 μm from APP dendrites and APP-expressing cells, respectively; TTX (13 slices): 18, 14 and 25; AP5 (10 slices): 15, 13 and 18; α -bungarotoxin (15 slices): 13, 15 and 19; L685,458 (13 slices): 19, 13 and 11). Error bars represent s.e.m. Two-way ANOVA showed significant drug $\times$ APP interaction ($F = 4.38$, $P < 0.001$; t test, * $P < 0.0001$, ** $P = 0.002$).

Figure 2.

Axonal A β reduces local spine density in an activity-dependent manner. **(a)** Tiled two-photon laser-scanning microscopy images of a hippocampal slice culture infected with EGFP virus in the CA1 region and APP/tomato virus in the CA3 region. Scale bar represents 300 μm . **(b)** Images from CA1 stratum radiatum region showing a control region containing EGFP-labeled apical dendrites and <1 APP bouton per 5,000 μm^3 (left) or a region containing EGFP-labeled apical dendrites and >10 APP boutons per 5,000 μm^3 (right). Scale bar represents 10 μm . **(c)** Bar graph of spine density of EGFP neurons in regions containing <1 APP bouton per 5,000 μm^3 and in regions containing >10 APP boutons per 5,000 μm^3 . Slices were maintained in normal medium or in medium containing TTX, AP5, α -bungarotoxin or L685,458, as indicated (no treatment (21 slices): 21 and 20 dendrites for <1 APP bouton per 5,000 μm^3 and >10 APP boutons per 5,000 μm^3 ; TTX (10 slices): 20 and

15; AP5 (12 slices): 24 and 17; α -bungarotoxin (10 slices): 20 and 39; L685,458 (10 slices): 15 and 11). Two-way ANOVA showed significant drug $\times$ APP interaction ($F = 2.77$, $P = 0.005$; t test, $*P < 0.0001$). Similar effects, although less robust, were seen after 1 d of APP overexpression (data not shown). Error bars represent s.e.m.

Figure 3.

Aβ secretion is sensitive to blockade of action potentials or nAChRs, but not to blockade of NMDA receptors. **(a)** The CA1 region of hippocampal slice cultures was infected with APP and incubated in normal media (control) or in media containing AP5, TTX, α-bungarotoxin or L685,458. Secreted Aβ (1–42) levels were normalized to that of the control group (control, 8 membranes; AP5, 8 membranes; TTX, 4 membranes; α-bungarotoxin, 5 membranes; L685,458, 5 membranes; ANOVA, $F = 4.2$, $P = 0.009$; t test, $* P < 0.002$). **(b)** Secreted Aβ (1–40) levels were analyzed as in **a** (control, 8 membranes; AP5, 9 membranes; TTX, 5 membranes; α-bungarotoxin, 5 membranes; L685,458, 5 membranes; ANOVA, $F = 7.54$, $P = 0.0004$; t test between control and drug-treated groups, $* P < 0.01$). Error bars represent s.e.m.

Figure 4.

Synthetic A β -induced spine loss can be rescued by blockade of NMDA receptors, but not by blockade of action potentials or nAChRs. The CA1 region of hippocampal slice cultures was infected with EGFP virus and incubated in normal medium or in medium containing A β (1–42) and AP5, TTX or α -bungarotoxin as indicated. Spine density was measured after 24 h of infection and drug treatment (no treatment: 17 dendrites, 9 slices; A β 42: 24 dendrites, 10 slices; A β 42 + AP5: 15 dendrites, 9 slices; A β 42 + TTX: 13 dendrites, 7 slices; A β 42 + α -bungarotoxin: 14 dendrites, 7 slices; ANOVA, $F = 7.27$, $P < 0.0001$; t test between no treatment and drug-treated groups, $*P < 0.002$). Error bars represent s.e.m.

Figure 5.

Acute overproduction of Aβ reduces spine structural plasticity by NMDAR- and nAChR-dependent mechanisms. **(a)** Spine volumes from neurons transfected with EGFP or APP/dsRed before and after cLTP induction. Aβ (1-42) or L685,458 were applied as indicated. The signals from the cytoplasmic markers are displayed. Scale bar represents 1 μm. **(b)** Acute overproduction of Aβ reduced spine structural plasticity. The time of cLTP induction, Aβ (1-42) or L685,458 application is indicated at the top of the graph (APP: $n = 166$ spines, 5 slices; APP + L685,458: $n = 312$ spines, 8 slices; EGFP: $n = 247$ spines, 6 slices; EGFP + Aβ (1-42): $n = 275$ spines, 6 slices; ANOVA, $F = 7.99$, $P < 0.0001$; t test between APP and APP + L685,458 and between EGFP and EGFP + Aβ (1-42), $*P < 0.0004$). **(c)** AP5 rescued Aβ-mediated impairment of cLTP spine enlargement. Data from untreated APP-expressing neurons in **b** is also shown (APP + AP5: $n = 254$ spines, 8 slices; dsRed + AP5: $n = 185$ spines, 5 slices; ANOVA, $F = 1.34$, $P < 0.0001$; t test between APP and APP + AP5, $*P < 0.001$). **(d)** α-bungarotoxin rescued Aβ-mediated impairment of spine enlargement after cLTP induction. Data from untreated APP-expressing neurons in **b** is also shown (APP + α-bungarotoxin: $n = 153$ spines, 5 slices; EGFP + α-bungarotoxin: $n = 119$ spines, 4 slices; ANOVA, $F = 7.62$, $P < 0.0001$; t test between APP and APP + α-bungarotoxin, $*P < 0.01$). Error bars represent s.e.m.

Figure 6.

Axonal secretion of A β reduces local spine structural plasticity. **(a)** Example traces of cell-attached recordings from a pair of uninfected CA1 and CA3 pyramidal neurons (top) or a pair of EGFP-infected CA1 cells and APP-infected CA3 cells (bottom) before, during and after cLTP induction. **(b)** Left, examples of EGFP-labeled spines more than 50 μ m away from APP-labeled axons before and after cLTP. Right, EGFP-labeled spines within 3 μ m of an APP-labeled axon before and after cLTP (arrows). Scale bar represents 3 μ m. **(c)** The volume change of spines >50 μ m from APP axons or <3 μ m from APP axons before, during and after cLTP induction (>50 μ m from APP axon: $n = 139$ spines, 25 slices; <3 μ m from APP axon: $n = 143$ spines, 25 slices; ANOVA, $F = 6.13$, $P = 0.0001$; t test between >50 μ m from APP axon and <3 μ m from APP axon, $*P < 0.0005$). Error bars represent s.e.m.

Figure 7.

Dendritic secretion of A β reduces local spine structural plasticity. **(a)** EGFP-labeled spines before and after cLTP induction. Left, spines more than 50 μ m away from APP-labeled structures. Right, EGFP-labeled spines within 3 μ m of an APP-labeled dendrite. Scale bar represents 1 μ m. **(b)** The volume change of spines >50 μ m from APP dendrites and of those <3 μ m from APP dendrites before, during and after cLTP induction (>50 μ m from APP dendrite: $n = 194$ spines, 42 slices; <3 μ m from APP dendrite: $n = 243$ spines, 42 slices; ANOVA, $F = 33.1$, $P < 0.0001$; t test between >50 μ m from APP dendrite and <3 μ m from APP dendrite, $*P < 0.01$). **(c)** Bar graph of spine volume change after cLTP (log transformed) in groups of spines 0–3 μ m, 3–6 μ m, 6–20 μ m or >50 μ m from APP dendrites (0–3 μ m: 0.079 ± 0.011 , 358 spines; 3–6 μ m: 0.101 ± 0.013 , 235 spines; 6–20 μ m: 0.134 ± 0.016 , 180 spines; >50 μ m: 0.143 ± 0.009 , 580 spines, from 68 slices; ANOVA, $F = 7.77$, $P < 0.0001$; t test, $*P = 0.008$, $**P < 0.001$). Error bars represent s.e.m.