



CaMKII Metaplasticity Drives A β Oligomer-Mediated Synaptotoxicity

Patricio Opazo, Silvia Viana da Silva, Mario Carta, Christelle Breillat, Steven Coultrap, Dolors Grillo-Bosch, Matthieu Sainlos, Françoise Coussen-Choquet, K. Ulrich Bayer, Christophe Mulle, et al.

► To cite this version:

Patricio Opazo, Silvia Viana da Silva, Mario Carta, Christelle Breillat, Steven Coultrap, et al.. CaMKII Metaplasticity Drives A β Oligomer-Mediated Synaptotoxicity. Cell Reports, 2018, 23 (11), pp.3137-3145. <10.1016/j.celrep.2018.05.036>. <hal-02325366>

HAL Id: hal-02325366

<https://hal.science/hal-02325366v1>

Submitted on 22 Oct 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

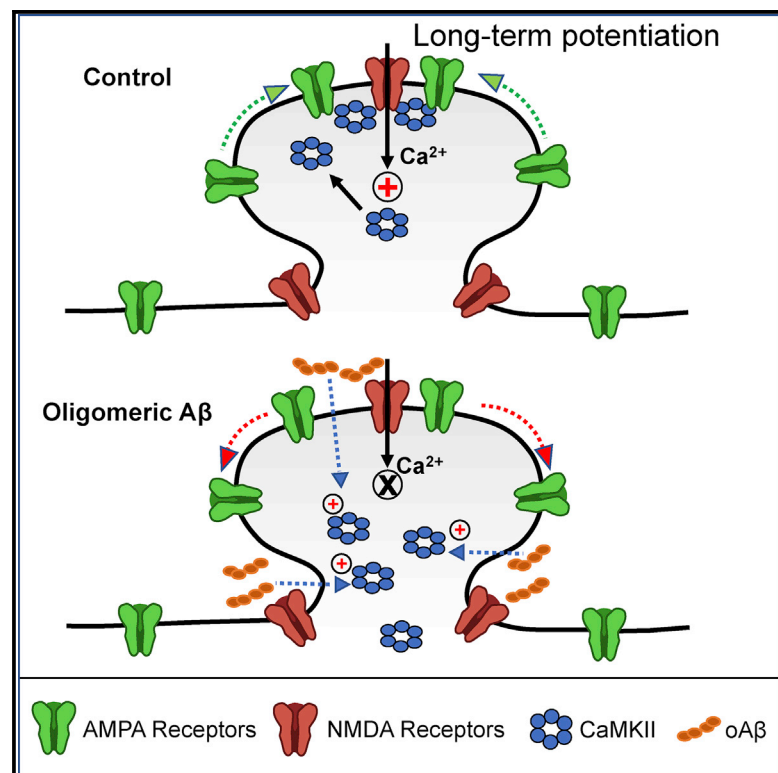
L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons CC BY-NC-ND 4.0 - Attribution - Non-commercial use - No Derivative Works - International License

CaMKII Metaplasticity Drives A β Oligomer-Mediated Synaptotoxicity

Graphical Abstract



Authors

Patricio Opazo, Silvia Viana da Silva, Mario Carta, ..., K. Ulrich Bayer, Christophe Mulle, Daniel Choquet

Correspondence

p.opazo@uq.edu.au (P.O.),
dchoquet@u-bordeaux.fr (D.C.)

In Brief

Opazo et al. show that oligomeric and synaptotoxic forms of the A β peptide trigger the rapid activation of CaMKII throughout the neuron. They find that aberrant CaMKII activation leads to deficits in long-term potentiation and ultimately synaptic loss via the destabilization of AMPA receptors.

Highlights

- Oligomeric A β triggers the non-autonomous activation of CaMKII
- Oligomeric A β prevents the activation of CaMKII by subsequent rounds of plasticity
- CaMKII activation leads to deficits in long-term potentiation and dendritic spine loss
- CaMKII drives synaptotoxicity via the destabilization of synaptic AMPA receptors



CaMKII Metaplasticity Drives A β Oligomer-Mediated Synaptotoxicity

Patricio Opazo,^{1,2,5,*} Silvia Viana da Silva,^{1,2} Mario Carta,^{1,2} Christelle Breillat,^{1,2} Steven J. Coultrap,³ Dolors Grillo-Bosch,^{1,2} Matthieu Sainlos,^{1,2} Françoise Coussen,^{1,2} K. Ulrich Bayer,³ Christophe Mulle,^{1,2} and Daniel Choquet^{1,2,4,6,*}

¹Université de Bordeaux, Interdisciplinary Institute for Neuroscience, UMR 5297, 33000 Bordeaux, France

²CNRS, Interdisciplinary Institute for Neuroscience, UMR 5297, 33000 Bordeaux, France

³Department of Pharmacology, Anschutz Medical Campus, University of Colorado School of Medicine, Aurora, CO 80045, USA

⁴Bordeaux Imaging Center, UMS 3420 CNRS, Université de Bordeaux, US4 INSERM, 33000 Bordeaux, France

⁵Present address: Clem Jones Centre for Ageing Dementia Research, Queensland Brain Institute, University of Queensland, St. Lucia, QLD, Australia

⁶Lead Contact

*Correspondence: p.opazo@uq.edu.au (P.O.), dchoquet@u-bordeaux.fr (D.C.)

<https://doi.org/10.1016/j.celrep.2018.05.036>

SUMMARY

Alzheimer's disease (AD) is emerging as a synaptopathology driven by metaplasticity. Indeed, reminiscent of metaplasticity, oligomeric forms of the amyloid- β peptide (oA β) prevent induction of long-term potentiation (LTP) via the prior activation of GluN2B-containing NMDA receptors (NMDARs). However, the downstream Ca²⁺-dependent signaling molecules that mediate aberrant metaplasticity are unknown. In this study, we show that oA β promotes the activation of Ca²⁺/calmodulin-dependent kinase II (CaMKII) via GluN2B-containing NMDARs. Importantly, we find that CaMKII inhibition rescues both the LTP impairment and the dendritic spine loss mediated by oA β . Mechanistically resembling metaplasticity, oA β prevents subsequent rounds of plasticity from inducing CaMKII T286 autophosphorylation, as well as the associated anchoring and accumulation of synaptic AMPA receptors (AMPA). Finally, prolonged oA β treatment-induced CaMKII misactivation leads to dendritic spine loss via the destabilization of surface AMPARs. Thus, our study demonstrates that oA β engages synaptic metaplasticity via aberrant CaMKII activation.

INTRODUCTION

Alzheimer's disease (AD) is emerging as a synaptopathology driven by metaplasticity (Hulme et al., 2013; Zorumski and Izumi, 2012). In a manner reminiscent of metaplasticity events, numerous studies have shown that oligomeric forms of the amyloid- β peptide (oA β) prevent the induction of long-term potentiation (LTP) via the prior activation of N-methyl-D-aspartate receptors (NMDARs) (Hulme et al., 2013; Malinow, 2012). The critical role of NMDARs in mediating the synaptotoxic effects

of oA β is highlighted by several studies showing that NMDAR antagonists can fully rescue the effects of oA β (Hsieh et al., 2006; Shankar et al., 2007). More recently, it was shown that oA β specifically targets NMDARs containing the N-methyl-D-aspartate receptor 2B (GluN2B) subunit, because the specific antagonist ifenprodil was able to completely reverse the oA β -mediated inhibition of LTP (Hu et al., 2009; Rammes et al., 2011; Röncke et al., 2011). Given the critical role of GluN2B-containing NMDARs in mediating the synaptotoxic effects of oA β , it has become critical to elucidate the downstream Ca²⁺-dependent signaling cascades triggering synaptotoxicity. In this study, we investigated the role of Ca²⁺/calmodulin-dependent kinase II (CaMKII) in oA β -mediated synaptotoxicity for a number of reasons. First, CaMKII is the most prominent protein associated with the GluN2B subunit, both at the structural and functional levels (Coultrap and Bayer, 2012; Hell, 2014). Second, it is well accepted that CaMKII is part of a core mechanism for LTP expression (Huganir and Nicoll, 2013). The fact that oA β consistently and robustly inhibits LTP suggests that it might be interfering with such a core LTP mechanism. Third, CaMKII has been recently shown to play a direct role in metaplasticity because prior activation of CaMKII prevents the subsequent induction of LTP (Yang et al., 2011). In addition, transgenic animals overexpressing active CaMKII present LTP deficits in a metaplasticity-like manner (Deisseroth et al., 1995; Mayford et al., 1995).

Using a combination of state-of-the-art microscopy and electrophysiology, we demonstrate that oA β triggers the activation of CaMKII in a GluN2B-dependent manner. More importantly, we unveil a critical role of CaMKII activation in oA β -induced LTP impairment and dendritic spine loss. In a manner resembling synaptic metaplasticity, the oA β -mediated activation of CaMKII prevents subsequent LTP-induced T286 autophosphorylation of CaMKII, as well as the associated synaptic translocation and anchoring of synaptic α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA). Our study substantiates the emerging notion that oA β engages synaptic metaplasticity to drive pathology at the early stages of AD.



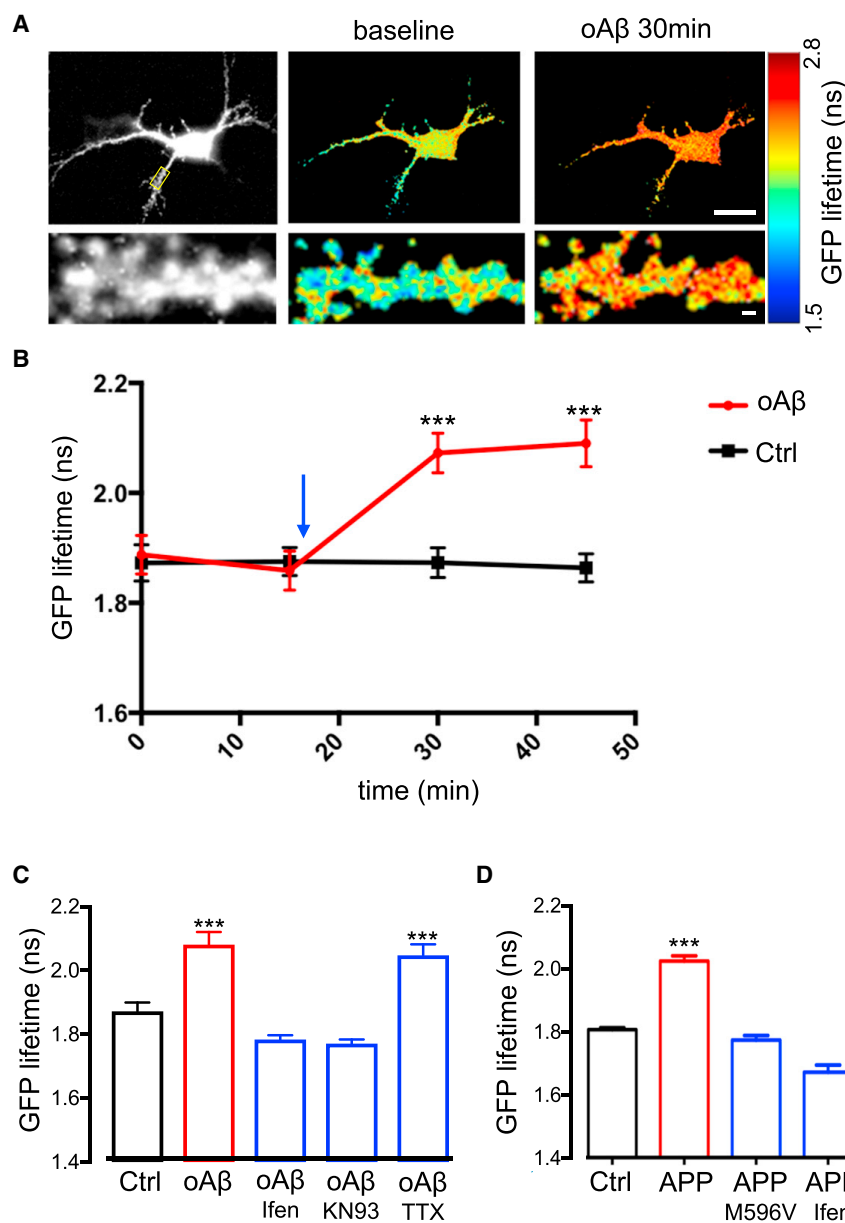


Figure 1. oAβ Activates CaMKII in a GluN2B-Dependent Manner

(A) Sample image of a cultured hippocampal neuron expressing the modified FRET-based CaMKII α sensor (left) and the lifetimes images of the same neuron before (middle) and after (right) incubation with oAβ (0.5 μ M for 30 min). Note that oAβ increased GFP lifetime (warm colors), reflecting a decrease in FRET because of the open-conformation of active CaMKII. Scale bars, 20 μ m (above); 1 μ m (below).

(B) Line graph displaying the time course of CaMKII activation in dendritic spines during oAβ incubation. For controls (black line), mean \pm SEM GFP lifetime: t0 = 1.872 ns, t15 = 1.875 ns, t30 = 1.873 ns, t45 = 1.864 ns. For oAβ (red line), mean \pm SEM GFP lifetime: t0 = 1.888 ns, t15 = 1.859 ns, t30 = 2.073 ns, t45 = 2.090 ns.

(C) Bar graph showing the mean \pm SEM GFP lifetime 15 min after oAβ incubation. Mean \pm SEM GFP lifetime: control (Ctrl) = 1.873 ns (n = 99), oAβ = 2.073 ns (n = 89), oAβ + ifenprodil = 1.784 ns (n = 69), oAβ + KN93 = 1.770 ns (n = 176), and oAβ + TTX = 2.048 ns (n = 124).

(D) Bar graph showing the mean \pm SEM GFP lifetime after APP overexpression. Mean \pm SEM GFP lifetime: Ctrl = 1.793 ns (n = 1079), APP = 2.003 ns (n = 348), APP MV = 1.761 ns (n = 348), and APP + ifenprodil = 1.663 ns (n = 157).

***p < 0.001.

RESULTS

oAβ Activates CaMKII in a GluN2B-Dependent Manner

To determine the impact of oAβ on the spatiotemporal dynamics of CaMKII activation, we used the modified fluorescence resonance energy transfer (FRET)-based CaMKII α sensor previously reported (Lee et al., 2009). Cultured hippocampal neurons expressing the CaMKII sensor were acutely exposed to an oligomeric preparation of the Aβ_{1–42} peptide (Figure S1A), and FRET was measured using fluorescence lifetime imaging microscopy (FRET-FLIM). We found that oAβ promoted a dose-dependent activation of CaMKII throughout the somatodendritic region including dendritic spines, as opposed to a vehicle-only control (Figures 1A and 1B; Figure S1B). Consistent with the proposed

role of GluN2B-containing NMDAR in the oAβ effects, we found that CaMKII activation was fully prevented by the GluN2B antagonist ifenprodil (Figure 1C) and also the CaMKII inhibitor KN93. On the other hand, the action potential blocker tetrodotoxin (TTX) (1 μ M) did not prevent CaMKII activation, ruling out an indirect effect via an oAβ-mediated hyperexcitability of neuronal circuits.

AD is triggered by the release of the Aβ peptide into the extracellular space after proteolysis of amyloid precursor protein (APP). To examine whether the endogenous cleavage of APP and the conse-

quent release of the Aβ peptide was sufficient to activate CaMKII, we coexpressed the CaMKII sensor along with APP. We found that APP overexpression caused a robust increase in CaMKII activity (Figure 1D). Importantly, overexpression of the non-cleavable APP mutant M596V had no effect on CaMKII activity, indicating that the release of the Aβ peptide (and/or other β -secretase products), and not APP itself, triggers CaMKII activation. In line with the role of GluN2B-containing NMDAR, we found that ifenprodil also fully prevented the APP-mediated activation of CaMKII (Figure 1D).

We then examined the impact of oAβ on CaMKII autophosphorylation (T286), which is usually used as a proxy of CaMKII activation. Using western blots, we found that oAβ had no effect on CaMKII autophosphorylation at a concentration and

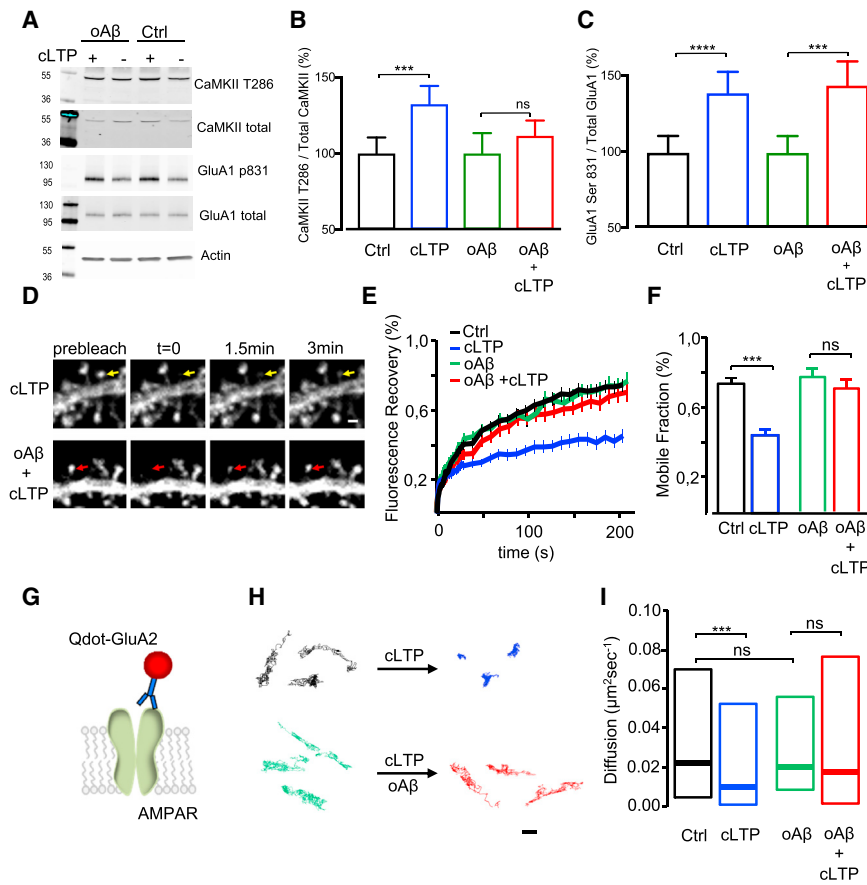


Figure 2. oAβ Prevents the Further cLTP-Mediated Activation of CaMKII

(A) Representative blots against autophosphorylated T286 CaMKII, total CaMKII, p831 GluA1, total GluA1, and actin.

(B) Bar graph of the mean \pm SEM pCaMKII:CaMKII ratio for the different conditions (Ctrl = 100%, cLTP = 132.3%, oAβ = 100%, and oAβ + cLTP = 111.6%).

(C) Bar graph of the mean \pm SEM p831GluA1:total GluA1 for the different conditions (Ctrl = 100%, cLTP = 139.5%, oAβ = 100%, and oAβ + cLTP = 144.9%).

(D) Sample images of dendritic region of a cultured hippocampal neuron expressing CaMKII::GFP before and after photobleaching. Scale bar, 1 μ m. The red and yellow arrows indicate the photobleached dendritic spine.

(E) Normalized fluorescence recovery after photobleaching of CaMKII::GFP under control conditions (black trace), after oAβ preincubation (green trace), after cLTP (blue trace), and after oAβ preincubation and cLTP together (red trace).

(F) Bar graph of the mean \pm SEM fluorescence recovery at the last time point recorded (3 min after photobleaching) for all the conditions (control 0.732, n = 51 spines; cLTP 0.444, n = 50 spines; oAβ preincubation, n = 50 spines; and oAβ and cLTP 0.699, n = 60 spines).

(G) Experimental scheme representing the labeling strategy. Endogenous GluA2-containing AMPARs were tracked using primary antibody against the GluA2 N-terminal domain and Qdots-coupled secondary antibody.

(H) Representative trajectories of the surface diffusion of endogenous GluA2-containing

AMPARs in mature cultured hippocampal neurons. Note that in control conditions (but not after exposure to oAβ) cLTP decreased the surface explored by endogenous AMPARs during the recording period (30 s). Scale bar, 0.5 μ m.

(I) Bar graph of the median diffusion coefficient of AMPARs ($\pm 20\%$ –75% interquartile range [IQR]) (in $\mu\text{m}^2/\text{sec}$: control 0.0236 [n = 1067]; cLTP 0.0098 [n = 459]; cLTP + oAβ 0.018 [n = 277]).

***p < 0.001.

incubation time similar to those used with the FRET assay (Figures 2A and 2B). Similarly, oAβ had no effect on the phosphorylation of S831 in the GluA1 subunit of the AMPAR, a known CaMKII substrate. As a positive control, we confirmed that activation of NMDARs via a standard chemical LTP (cLTP) protocol promoted strong CaMKII autophosphorylation and phosphorylation of S831 in GluA1 (Figures 2A and 2C).

In order to confirm that oAβ promoted CaMKII activation independently of T286 autophosphorylation, we applied oAβ to neurons expressing the CaMKII FRET sensor carrying a T286A mutation. In agreement with the western blot data, we found that oAβ still promoted a robust and significant activation of this autophosphorylation mutant (Figures S1C and S1D). In addition, we found that oAβ also effectively activates CaMKII FRET sensors carrying mutations at other post-translational modifications sites known to activate CaMKII such as oxidation (M281V), nitrosylation (C280/289V), and glycosylation (S279A) (Figures S1C and S1D).

Given that autophosphorylation, as well as other posttranslational modifications, normally results in autonomous Ca^{2+} -independent CaMKII activity, we performed *in vitro* experiments to

evaluate the effect of oAβ on autonomous kinase activity. As expected from the above experiments, we found that oAβ does not modify the levels of autonomous CaMKII activity (Figure S2). As a positive control, we confirmed that the autonomous CaMKII activity can be increased by prior *in vitro* autophosphorylation or phosphatase inhibitors (Figure S2).

Taken together, this set of experiments demonstrates that oAβ increases overall CaMKII activity but fails to promote CaMKII autophosphorylation and, consequently, an increase in autonomous activity.

oAβ Engages Metaplasticity via CaMKII

According to a metaplasticity mechanism of action, the oAβ-mediated activation of CaMKII may prevent CaMKII activation by subsequent plasticity-inducing stimuli. Using western blots, we found that prior incubation with oAβ (0.5 μ M for 30 min) prevented the subsequent autophosphorylation of CaMKII triggered by cLTP (Figures 2A and 2B). Similarly, using the FRET sensor, we found that both prior incubation with oAβ and APP overexpression decreased the dynamic range of cLTP-mediated CaMKII activation (Figure S3). We then examined whether oAβ

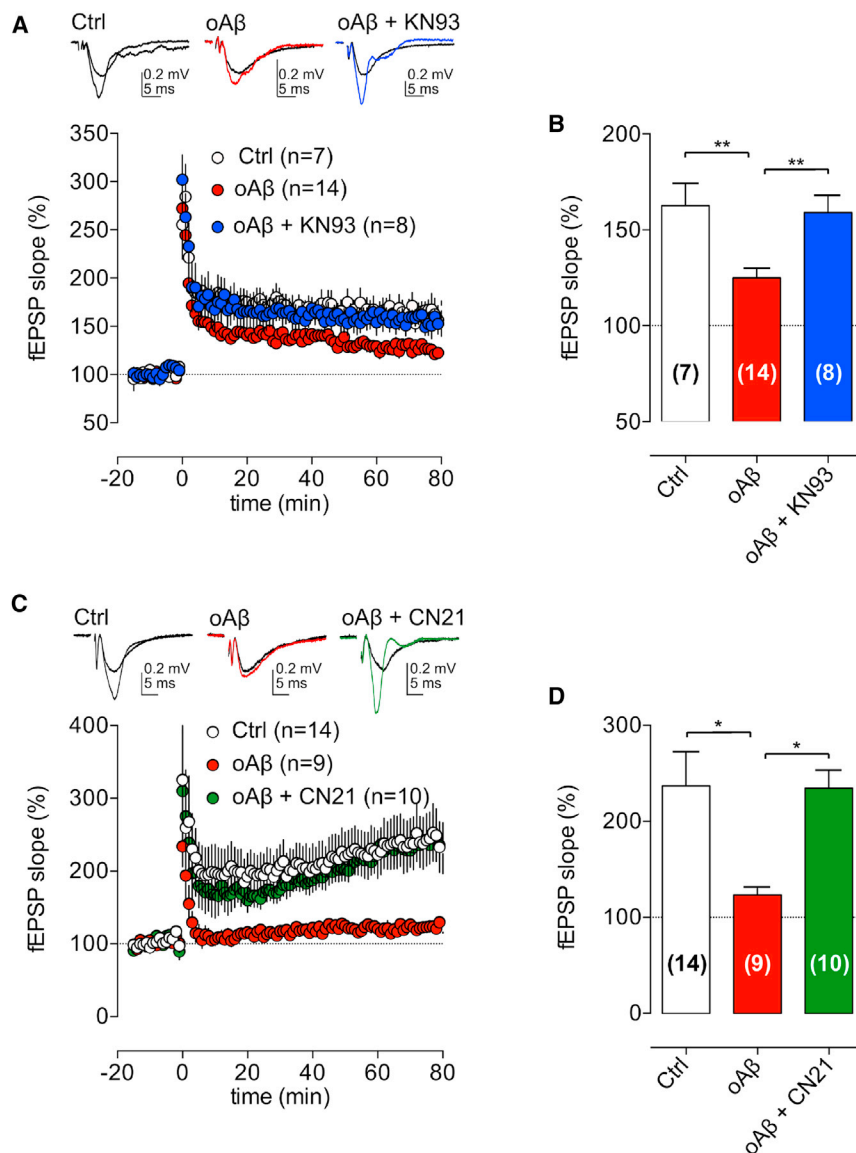


Figure 3. CaMKII Activation Drives the oAβ-Mediated Deficits in LTP

(A) The CaMKII inhibitor KN93 (0.5 μM; blue trace) completely rescued the oAβ-mediated impairment in LTP in acute hippocampal slices (red trace).

(B) Bar graph of the mean ± SEM fEPSP slope between 70 and 80 min after HFS used for statistical comparisons (fEPSP slope [%]: Ctrl = 162.7% [n = 7], oAβ = 125.1% [n = 14], and oAβ + KN93 = 159.0% [n = 8]).

(C) The CaMKII inhibitor tatCN21 (5 μM; green trace) completely rescued the oAβ-mediated impairment in LTP in acute hippocampal slices (red trace).

(D) Bar graph of the mean ± SEM fEPSP slope between 70 and 80 min after HFS used for statistical comparisons (fEPSP slope [%]: Ctrl = 237.2% [n = 14], oAβ = 123.5% [n = 9], and oAβ + tatCN21 = 234.7% [n = 10]). Note that Ctrl corresponds to pooled values from two control groups: vehicle (n = 6) and tatCN21-only (n = 8) pre-treated slices. *p < 0.05; **p < 0.01.

might also prevent other activity-dependent features of CaMKII that are known to be critical for the induction of LTP such as its synaptic translocation and the anchoring of synaptic AMPARs (Coultrap and Bayer, 2012; Hell, 2014; Lisman et al., 2012; Opazo et al., 2010). First, we found that oAβ pre-incubation prevented the cLTP-mediated immobilization of CaMKII at dendritic spines (Figures 2D–2F). Second, we found that oAβ pre-incubation also blocked the cLTP-mediated diffusional trapping of AMPARs (Figures 2G–2I). Together, these findings demonstrate that the oAβ-mediated activation of CaMKII prevents subsequent rounds of plasticity from engaging activity-dependent features of CaMKII that are critical for LTP.

CaMKII Activation Drives oAβ-Mediated Synaptotoxicity

Does CaMKII activation contribute to the detrimental effects of oAβ on LTP? Given that CaMKII is part of the core mechanism

for LTP induction (Huganir and Nicoll, 2013), we examined whether preventing the oAβ-mediated activation of CaMKII (and thus restoring CaMKII activity to basal levels) might be sufficient to rescue the LTP impairment. To that end, we pre-incubated acute hippocampal slices with oAβ (0.5 μM) alone or in the presence of CaMKII inhibitors, either KN93 (1 μM) or tatCN21 (5 μM). Importantly, to allow the proper activation of CaMKII during LTP, we washed out CaMKII inhibitors 40–45 min before the LTP-inducing protocol. As shown in Figure 3, we found that both KN93 and tatCN21 completely rescued the oAβ-mediated inhibition of LTP. Importantly, we found that pre-incubation with oAβ with or without KN93 or tatCN21 has no impact on input-output curves (fiber volley amplitude versus field excitatory postsynaptic potential [fEPSP] slope; Figure S4). Together with previous experiments showing that co-incubation with ifenprodil can also rescue LTP (Hu et al., 2009; Rammes et al., 2011; Röncke et al., 2011), our findings suggest that oAβ activates the canonical GluN2B-CaMKII pathway to prevent the subsequent induction of LTP in a manner reminiscent of the metaplasticity-mediated inhibition of LTP (Yang et al., 2011).

Although at first oAβ impacts the ability of synapses to undergo plasticity, a long-term exposure ultimately leads to the loss of dendritic spines (Um et al., 2012), the preferred site of excitatory synapses. We thus examined whether oAβ-mediated CaMKII activation might also contribute to dendritic spine loss. To this end, we used confocal microscopy to image the same neuron before and after a 5-hr-long exposure to oAβ, a time point known to be sufficient to induce significant spine loss (Um et al., 2012). Although dendritic spine density remained constant

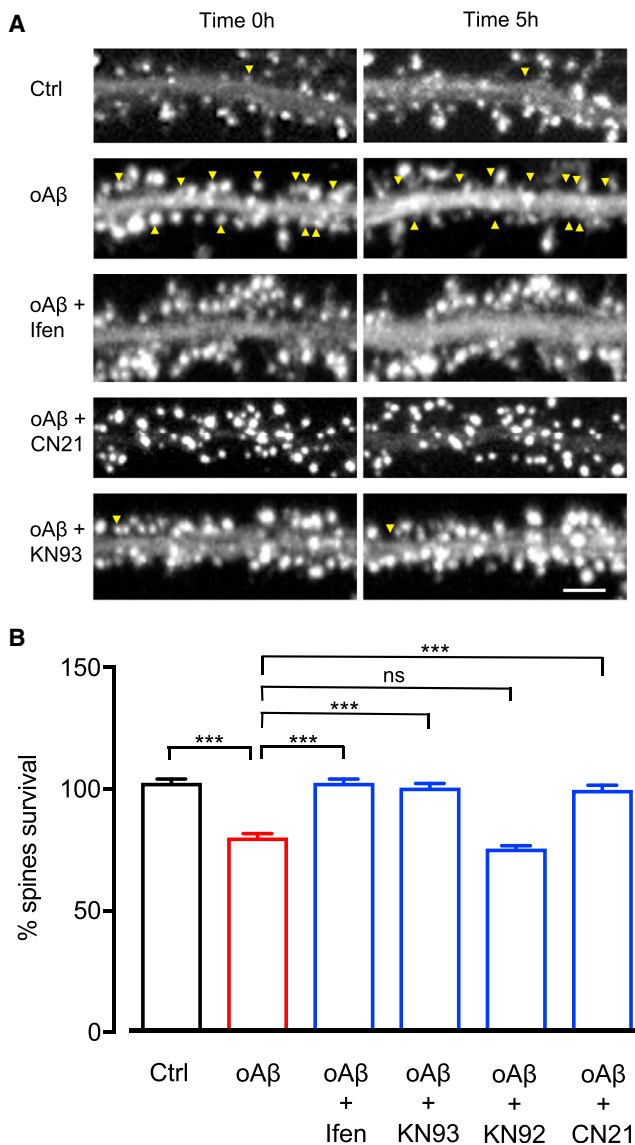


Figure 4. CaMKII Activation Drives the oAβ-Mediated Dendritic Spine Loss

(A) Sample images of dendritic regions from hippocampal neurons overexpressing the synaptic marker Homer1c::DsRed. Note that a prolonged incubation with oAβ (0.5 μM for 5 hr) promoted spine loss (yellow arrowheads) that was blocked by both the GluN2B-antagonist ifenprodil and the CaMKII inhibitors KN93 and tatCN21. Scale bar, 5 μm.

(B) Bar graph of the mean ± SEM survival fraction after oAβ incubation. Ctrl = 102.5% (n = 4 neurons), oAβ = 80% (n = 15 neurons), oAβ + ifenprodil = 102.5% (n = 4 neurons), oAβ + KN93 = 100.5% (n = 7 neurons), oAβ + KN92 = 75.6% (n = 8 neurons), and oAβ + tatCN21 = 99.7% (n = 9 neurons).

***p < 0.001.

during control conditions (vehicle, 5 hr), a prolonged incubation with oAβ leads to a significant loss in dendritic spines in a GluN2B-dependent manner (Figure 4). To test the role of oAβ-mediated CaMKII activation in spine loss, we co-incubated oAβ and either KN93 or tatCN21, and found that both CaMKII inhibitors (but not the inactive analog KN92) completely prevented

dendritic spine loss (Figure 4). Taken together, these findings demonstrate that CaMKII activation contributes to oAβ-mediated synaptotoxicity at the functional and structural levels.

CaMKII Metaplasticity Leads to AMPAR Destabilization and Spine Loss

How does CaMKII metaplasticity lead to dendritic spine loss? Given that oAβ interferes with the normal functioning of CaMKII, it is likely that oAβ precludes CaMKII from anchoring and stabilizing AMPARs, one of its most critical synaptic functions (Coultrap and Bayer, 2012; Hell, 2014; Lisman et al., 2012; Opazo et al., 2010). Because AMPAR content is strongly correlated to dendritic spine structure (Matsuzaki et al., 2004), we hypothesized that long-term exposure to oAβ leads to the destabilization and escape of synaptic AMPARs and, consequently, to the collapse of dendritic spines. In order to start testing this hypothesis, we first overexpressed APP and tracked the surface mobility of synaptic AMPARs using single-particle QDot tracking. As shown in Figures 5A–5C, we found that APP triggered a dramatic destabilization of synaptic AMPARs as evidenced by their enhanced diffusion coefficient and decreased immobile fraction. Importantly, we found that the non-cleavable APP (M596V) had no effect on AMPAR diffusion (Figure 5C), suggesting that the release of the Aβ peptide, rather than APP itself, triggered AMPAR destabilization. In agreement with the role of released Aβ, we found that non-transfected neurons within the same field of view (80 × 80 μm) of APP-expressing neurons also presented an increased destabilization of AMPARs (Figure 5D). Consistent with the metaplasticity model, we found that both the general NMDAR antagonist 2-amino-5-phosphopentanoic acid (AP5) and ifenprodil prevented AMPAR destabilization (Figure 5E). To investigate the causal role of CaMKII misactivation in AMPAR destabilization, we co-expressed APP along with the kinase-dead CaMKII mutant K42R. As shown in Figure 5F, we found that CaMKII K42R rescued the effects of APP expression in AMPAR surface diffusion. Similarly, we found that the CaMKII inhibitor tatCN21 also prevented AMPAR destabilization. Together, these findings underlie the causal link between prolonged CaMKII misactivation and AMPAR destabilization.

It is widely accepted that AMPARs synaptic content and dendritic spine size go hand in hand (Matsuzaki et al., 2004). Because a 2-hr oAβ incubation time was sufficient to destabilize AMPARs (Figure S5), which preceded dendritic spine loss (5 hr), we explored the possibility that these events might be causally related. First, we reasoned that if AMPAR destabilization is driving spine loss, then artificially destabilizing AMPARs might worsen the effects of oAβ on spines loss. We tested this hypothesis by pre-incubating neurons with a recently described peptide-based ligand that destabilizes endogenous AMPARs by disrupting the interaction between the AMPARs auxiliary subunit Stargazin and PSD95 (STG^{pep}) (Sainlos et al., 2011). As shown in Figures 5G and 5H, we found that indeed pre-incubation with STG^{pep} exacerbated oAβ-mediated spine loss. Lastly, in order to establish a causal relationship between AMPAR destabilization and spine loss, we prevented AMPAR destabilization by specifically crosslinking GluA2-containing AMPARs via antibodies against their extracellular N-terminal domains

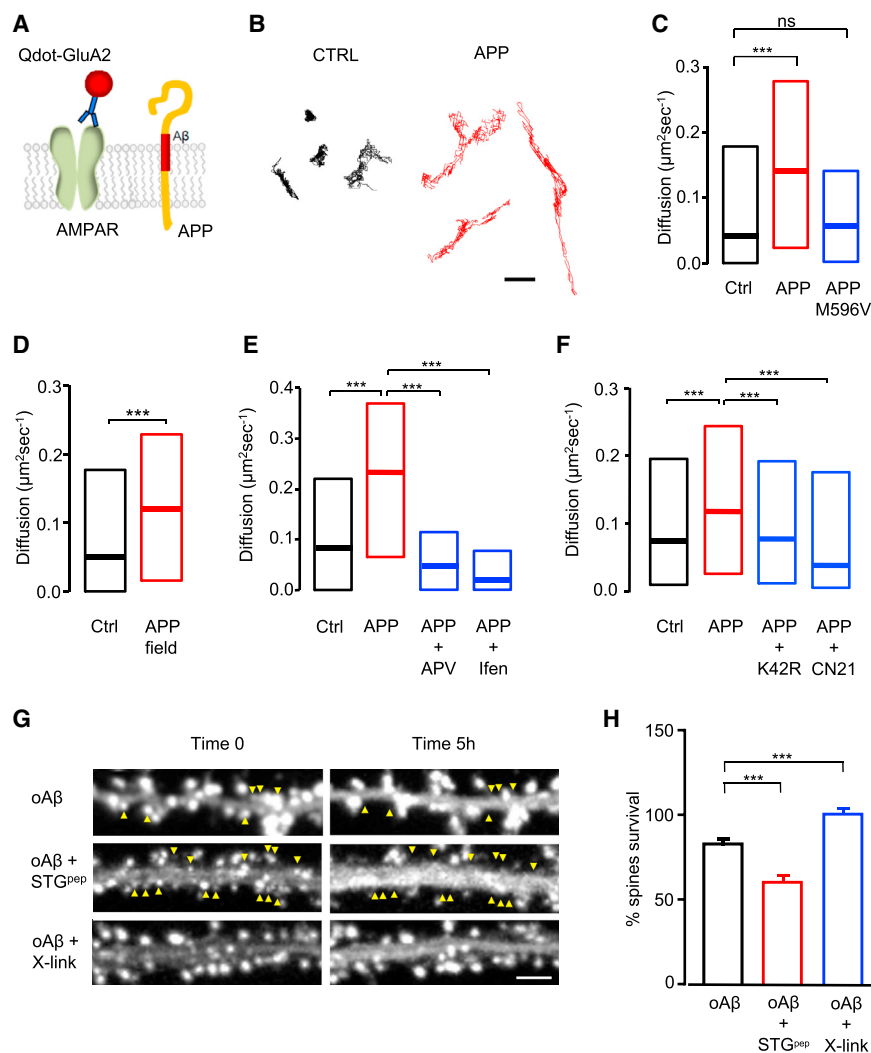


Figure 5. oA β Leads to AMPAR Destabilization and Dendritic Spine Loss

(A) Schematic representation of the experimental manipulation. Cultured hippocampal neurons were transfected with the amyloid precursor protein (APP), and the lateral diffusion of endogenous AMPARs was evaluated after 1–2 days using a primary antibody against the GluA2 N-terminal domain and Qdots-coupled secondary antibody.

(B) Representative trajectories of the surface diffusion of endogenous GluA2-containing AMPARs. Note that APP overexpression dramatically increased the surface explored by AMPARs during the recording period (30 s). Scale bar, 1 μ m.

(C) APP overexpression strongly increases the mobility of endogenous AMPARs. Bar graph of the median diffusion coefficient of endogenous AMPARs ($\pm 20\%$ – 75% interquartile range [IQR]) (median diffusion in $\mu\text{m}^2/\text{sec}$: control 0.0411 [n = 1840]; APP 0.140 [n = 1470]; APP M596V 0.056 [n = 929]).

(D) APP overexpression increases the mobility of neighboring non-transfected neurons within the same field of view of APP-overexpressing neurons (median diffusion in $\mu\text{m}^2/\text{sec}$: control 0.0411 [n = 1,840]; neighboring non-transfected neurons [field APP] 0.1195 [n = 555]).

(E) AMPAR destabilization requires NMDAR activity (median diffusion in $\mu\text{m}^2/\text{sec}$: control 0.074 [n = 380]; APP 0.2334 [n = 633]; APP + AP5 0.0483 [n = 448]; APP + ifenprodil 0.042 [n = 750]).

(F) APP overexpression increases the mobility of AMPAR in a CaMKII-dependent manner (median diffusion in $\mu\text{m}^2/\text{sec}$: control 0.074 [n = 4976]; APP 0.1141 [n = 6359]; APP + CaMKII K42R 0.0744 [n = 7,945]; APP + tatCN21 0.0352 [n = 638]).

(G) Sample images of dendritic regions from hippocampal neurons overexpressing the synaptic marker Homer1c::DsRed. Note that a prolonged incubation with oA β (0.5 μ M for 5 hr) promoted spine loss (yellow arrowheads) that was fully rescued by the prior immobilization of AMPARs and exacerbated by prior destabilization of AMPARs. Scale bar, 5 μ m.

spine loss (yellow arrowheads) that was fully rescued by the prior immobilization of AMPARs and exacerbated by prior destabilization of AMPARs. Scale bar, 5 μ m.

(H) Bar graph of the mean \pm SEM survival fraction 5 hr after oA β incubation for all conditions. oA β = 82% (n = 7 neurons), oA β + STG^{pep} = 60.2% (n = 5 neurons), and oA β + crosslinking = 100.4% (n = 9 neurons).

***p < 0.001.

prior to oA β incubation (Heine et al., 2008). As shown in Figures 5G and 5H, AMPAR crosslinking was sufficient to completely prevent the spine loss mediated by oA β . Together, these findings support the hypothesis that oA β prevents CaMKII from serving its critical role on AMPAR anchoring, leading to their synaptic escape and ultimately to dendritic spine loss.

DISCUSSION

In this study, we provide mechanistic insight for a metaplasticity model of oA β action. In the same way that a subthreshold activation of GluN2B-containing NMDAR and CaMKII prevents the subsequent induction of LTP during metaplasticity (Yang et al., 2011), we found that oA β engaged the same pathway to drive impairment of LTP and spine loss. In accordance with a meta-

plasticity mechanism of action, oA β prevented the further T286 autophosphorylation of CaMKII, its synaptic translocation, and the functional anchoring of AMPARs, all critical steps in the strengthening of synaptic transmission (Coultrap and Bayer, 2012; Hell, 2014; Lisman et al., 2012). In line with a metaplasticity mechanism of action, we found that preventing the oA β -mediated activation of CaMKII was sufficient to rescue LTP.

Although our findings are consistent with the well-documented coupling between GluN2B and CaMKII, they are at odds with previous studies suggesting that oA β inhibits rather than activates CaMKII (Gu et al., 2009; Townsend et al., 2007; Zhao et al., 2004). We found that the reason behind these discrepancies resides on the different approaches used to measure CaMKII activation. Although in this study we used a highly sensitive FRET approach to measure “total” CaMKII activity,

all previous studies used western blots with an antibody directed against CaMKII autophosphorylation as a proxy of CaMKII activity. Because CaMKII autophosphorylation contributes very little to the overall maximal levels of CaMKII activity (Coultrap et al., 2012; Otmakhov et al., 2015), the $\alpha\beta$ -mediated activation of “total” CaMKII activation reported here was not detected. As before, we were unable to detect induction of CaMKII autophosphorylation using western blots or autonomous Ca^{2+} -independent activity using *in vitro* kinase assays at concentrations and incubation times similar to those used with the FRET assay. Besides this discrepancy, this and previous studies converge on the notion that $\alpha\beta$ prevents the activation of CaMKII by further rounds of plasticity.

While acute exposure to $\alpha\beta$ impacts the ability of synapses to undergo plasticity, the long-term exposure ultimately leads to the loss of dendritic spines. Our current work and previous studies suggest that $\alpha\beta$ also promotes dendritic spine loss via a metaplasticity mechanism. First, it has been shown that NMDAR antagonists are sufficient to rescue the dendritic spine loss triggered by $\alpha\beta$ (Shankar et al., 2007; Um et al., 2012). Second, in the current study we show that two mechanistically distinct CaMKII inhibitors fully rescue dendritic spine loss. How does CaMKII activation trigger dendritic spine loss? In accordance with a metaplasticity mechanism, it is likely that the $\alpha\beta$ -mediated activation of CaMKII precludes the physiological activation of CaMKII occurring under resting conditions. As we previously showed, interfering with the normal activation of CaMKII for prolonged periods of time leads to the destabilization and escape of synaptic AMPARs (Opazo et al., 2010). Consistent with this hypothesis, we found that APP overexpression leads to the surface destabilization of AMPARs in an NMDAR- and CaMKII-dependent manner. More importantly, we found that preventing AMPAR destabilization using a crosslinking approach was sufficient to rescue dendritic spine loss. Although these results identify a causal relationship between AMPAR destabilization and dendritic spine loss, the underlying mechanism remains to be identified. In addition, these findings are in agreement with previous studies showing that $\alpha\beta$ drives synaptotoxicity by promoting AMPAR endocytosis (Hsieh et al., 2006). Because the endocytic machinery is located at extrasynaptic sites (Rácz et al., 2004), it is likely that destabilized AMPARs escape the synapse prior to undergoing endocytosis.

Although our results suggest that the $\alpha\beta$ -mediated activation of CaMKII triggers synaptotoxicity simply by preventing further CaMKII activation, we cannot rule out the possibility that $\alpha\beta$ -activated CaMKII might phosphorylate off-target substrates such as the microtubule-associated protein Tau (Amar et al., 2017; Steiner et al., 1990; Yoshimura et al., 2003), which in turn leads to synaptotoxicity (Roberson et al., 2007). Regardless of whether the $\alpha\beta$ -mediated activation of CaMKII drives synaptotoxicity directly (via off-target phosphorylation) or indirectly (by preventing further CaMKII activation), our finding showing that AMPAR crosslinking fully rescued dendritic spine loss suggests that either mechanism should eventually lead to the surface destabilization of AMPARs.

Taken together, our study provides mechanistic insights into the emerging view of $\alpha\beta$ synaptotoxicity as an NMDAR-depen-

dent metaplasticity phenomenon. Our current study demonstrates that CaMKII is downstream of NMDAR-dependent synaptotoxicity and suggests that CaMKII inhibition might also prove beneficial as a therapeutic agent for AD (Ghosh and Giese, 2015). However, as in the case of NMDAR, it will be first necessary to develop inhibitors that selectively prevent the $\alpha\beta$ -mediated activation of CaMKII and not the physiological CaMKII activation critical for learning and memory.

In general, the emerging view of $\alpha\beta$ synaptotoxicity as a metaplasticity phenomenon raises the possibility that our current understanding of synaptic metaplasticity might shed light into the mechanisms underlying AD (Hulme et al., 2013).

EXPERIMENTAL PROCEDURES

Cultures of Sprague-Dawley rat hippocampal neurons were prepared from embryonic day 18 (E18) Sprague-Dawley rat embryos of either sex. Animals were used according to the guidelines of the University of Bordeaux/ Centre national de la recherche scientifique (CNRS) Animal Care and Use Committee.

Primary Neuronal Cultures and Transfection

Banker cultures of hippocampal neurons from E18 Sprague-Dawley rat embryos of either sex were prepared as described previously (Penn et al., 2017). Neurons (10–12 days *in vitro* [DIV]) were transfected using Effectene as per the manufacturer's instructions.

Oligomeric Preparation of the $\text{A}\beta_{1-42}$ Peptide

$\alpha\beta$ was generated as in Rönnicke et al. (2011). The lyophilized $\text{A}\beta_{1-42}$ peptide from Biochem (catalog number [Cat. #] H-1368) was dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP; Sigma, St. Louis, MO, USA) and aliquoted before removing HFIP. $\alpha\beta$ was obtained by incubating at 4°C for 24 hr in F12 medium. The quality of the oligomer preparation was controlled with western blots against the amyloid- β peptide (6E10; Covance, CA, USA).

Western Blots

Western blots and cLTP experiments were prepared as described previously (Penn et al., 2017). Cultured hippocampal neurons at DIV 14 were treated either with vehicle or 1 μM $\alpha\beta$ diluted in Tyrode's buffer during 30 min. Neurons were then treated with the cLTP protocol (200 μM glycine, 30 μM bicuculline in Tyrode's buffer 0 Magnesium) for 5 min. Neuronal homogenates were then western blotted against GluA1 (clone N355/1; NeuroMab), GluA1 Phospho Ser 831 (Cat. #04-823; Millipore), CamKII G-1 (Cat. #sc-5306; Santa Cruz), and Phospho CamKII T286 (Cat. #ab5683; Abcam).

Frequency Domain-Based FRET-FLIM

Experiments were performed on an inverted Leica DMI6000B (Leica Microsystems) spinning disk microscope and using the LIFA frequency domain lifetime attachment (Lambert Instruments; Roden, the Netherlands) and the LI-FLIM software. Cells were excited using a sinusoidally modulated 1-W 478-nm light-emitting diode (LED) under wide-field illumination. Lifetimes were calibrated using a 1 mM saline solution of fluorescein (pH 10; 4.00 ns).

Fluorescence Recovery after Photobleaching

The fluorescence recovery after photobleaching (FRAP) experiments were performed in a spinning disk microscope Leica DMI6000 (Leica Microsystems) equipped with a confocal Scanner Unit CSU-X1 (Yokogawa Electric Corporation, Tokyo, Japan). Diffraction limited regions on neurons expressing CaMKII-GFP were photobleached for 5 ms with the 488-nm laser. Recovery from photobleaching was monitored by 45 consecutive acquisitions at three different rates (each 0.5 s during 10 s, each 2 s during 20 s, and each 10 s during 180 s in time-lapse mode). Acquisitions and image corrections for background noise and continuous photobleaching were done using the MetaMorph software (Molecular Devices, Sunnyvale, CA, USA).

AMPA Labeling and Synaptic Live Staining

GluA2 labeling was performed in two steps. First, neurons were incubated for 10 min with a GluA2 antibody (1:200, MAB387; Chemicon). Second, neurons were incubated for 5 min with Quantum dots (QDs) 655 Goat F(ab')₂ anti-mouse IgG Conjugate (H+L) from Invitrogen Corporation. Synapses were labeled by transfection with Homer1C::GFP.

Single-Molecule Optical Microscopy

QDs and Homer1C-GFP signals were detected by using a mercury lamp (for QDs: excitation filter 560RDF55 or 460BP40 and emission filters 655WB20 or 655WB40). Fluorescent images from QDs were obtained with an integration time of 50 ms with up to 1,200 consecutive frames. Signals were recorded with a back-illuminated thinned CCD97 camera (Photometrics Cascade 512B; Roper Scientific).

AMPA Tracking and Analysis

The tracking of single QDs was performed with homemade software based on MATLAB (MathWorks, Natick, MA, USA). Owing to the random blinking events of the QDs, sub-trajectories of the same receptor were reconnected based on maximal position changes between consecutive frames (2–3 pixels; 0.32–0.48 μ m) and blinking rates (maximal dark periods of 25 frames; 1.25 s). Diffusion coefficients were calculated by a linear fit of the first 4–8 points of the mean-square displacement (MSD) plots versus time. The QDs were considered synaptic if colocalized with Homer1C dendritic clusters for at least five frames.

GluR2-AMPA Crosslinking

For crosslinking with GluR2-containing AMPARs, neurons were pre-incubated with the commercial monoclonal antibody against GluA2 (MAB387; Chemicon) for 10 min followed by an incubation with the secondary AB for 10 min.

CaMKII Activity Assays in Neuronal Extracts

Primary neuronal cultures (800,000 cells/60-mm plate) were pre-treated with either oA β (0.5 μ M for 30 min) or vehicle. Neuronal extracts were then assessed for CaMKII activity via phosphate incorporation into the AC3 peptide substrates as previously described (Coultrap et al., 2012).

Slice Preparation and Electrophysiology

Standard techniques were used to prepare slices (350 μ m thick) from the hippocampus of ketamine/xylazine anesthetized C57BL/6 mice (male, 2–3 months old). Slices were incubated with either vehicle, oA β (0.5 μ M), oA β (0.5 μ M) + KN93 (1 μ M), oA β (0.5 μ M) + tatCN21 (5 μ M), or tatCN21 alone (5 μ M) for 2.5–3 hr in an “incubation chamber” before transferring the slice to the electrophysiology setup. After transfer, slices were allowed to wash out for around 45 min before LTP induction (1 high frequency stimulation [HFS] train; 100 pulses at 100-Hz stimulation). Incubation and recording were done at room temperature (RT).

Statistics

Unless stated otherwise, data values are given as mean \pm SEM. Comparison of the means of two independent samples were made with either two-tailed Welch's t test (parametric) or Mann-Whitney test (non-parametric) using Prism (GraphPad, San Diego, CA, USA). To compare the means of more than two independent samples, we used one-way ANOVA test. When used in reporting statistical results, asterisks denote the following significance levels: *p < 0.05; **p < 0.01; ***p < 0.001.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and five figures and can be found with this article online at <https://doi.org/10.1016/j.celrep.2018.05.036>.

ACKNOWLEDGMENTS

We thank the IINS cell biology core facility for cell culture and plasmid production, Ryohei Yasuda for the CaMKII FRET sensor (Addgene plasmid #26933),

and Prof. Stefan Leutgeb for support with the revision experiments. We thank the Bordeaux Imaging Center, part of the national infrastructure France Biolmaging (ANR-10INBS-04-0), the Biochemistry and Biophysics Platform of Bordeaux Neurocampus (LABEX BRAIN ANR-10-LABX-43), and the help of J.M. Blanc. We thank funding from the Ministère de l'Enseignement Supérieur et de la Recherche, Centre National de la Recherche Scientifique, Conseil Régional d'Aquitaine, and Agence Nationale pour la Recherche Grant Nano-dom (ANR-12-BSV4-0009) and the ERC grants nano-dyn-syn (232942) and ADOS (339541) to D.C., Marie Curie Postdoctoral Fellowship and Fondation pour la Recherche Médicale Postdoctoral Fellowship to P.O., and NIH R01 NS081248 grant to K.U.B.

AUTHOR CONTRIBUTIONS

P.O. and D.C. conceived the study and formulated the models. P.O. performed the imaging experiments, analyzed the data, and prepared the figures. S.V.d.S. and M.C. performed electrophysiology experiments and data analysis. C.B. and F.C. performed the immunoblots experiments and data analysis. D.G.-B. and M.S. designed and synthesized the divalent stargazin peptides. S.J.C. performed the *in vitro* biochemical assays to measure CaMKII enzymatic activity and analyzed the data. All authors discussed the results and contributed to the preparation of the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

Received: April 17, 2017

Revised: March 26, 2018

Accepted: May 12, 2018

Published: June 12, 2018

REFERENCES

- Amar, F., Sherman, M.A., Rush, T., Larson, M., Boyle, G., Chang, L., Götz, J., Buisson, A., and Lesné, S.E. (2017). The amyloid- β oligomer A β *56 induces specific alterations in neuronal signaling that lead to tau phosphorylation and aggregation. *Sci. Signal.* 10, eaal2021.
- Coultrap, S.J., and Bayer, K.U. (2012). CaMKII regulation in information processing and storage. *Trends Neurosci.* 35, 607–618.
- Coultrap, S.J., Barcomb, K., and Bayer, K.U. (2012). A significant but rather mild contribution of T286 autophosphorylation to Ca²⁺/CaM-stimulated CaMKII activity. *PLoS ONE* 7, e37176.
- Deisseroth, K., Bito, H., Schulman, H., and Tsien, R.W. (1995). Synaptic plasticity: a molecular mechanism for metaplasticity. *Curr. Biol.* 5, 1334–1338.
- Ghosh, A., and Giese, K.P. (2015). Calcium/calmodulin-dependent kinase II and Alzheimer's disease. *Mol. Brain* 8, 78.
- Gu, Z., Liu, W., and Yan, Z. (2009). beta-Amyloid impairs AMPA receptor trafficking and function by reducing Ca²⁺/calmodulin-dependent protein kinase II synaptic distribution. *J. Biol. Chem.* 284, 10639–10649.
- Heine, M., Groc, L., Frischknecht, R., Béique, J.C., Lounis, B., Rumbaugh, G., Huguinir, R.L., Cognet, L., and Choquet, D. (2008). Surface mobility of postsynaptic AMPARs tunes synaptic transmission. *Science* 320, 201–205.
- Hell, J.W. (2014). CaMKII: claiming center stage in postsynaptic function and organization. *Neuron* 81, 249–265.
- Hsieh, H., Boehm, J., Sato, C., Iwatsubo, T., Tomita, T., Sisodia, S., and Malenka, R. (2006). AMPAR removal underlies Abeta-induced synaptic depression and dendritic spine loss. *Neuron* 52, 831–843.
- Hu, N.W., Klyubin, I., Anwyl, R., and Rowan, M.J. (2009). GluN2B subunit-containing NMDA receptor antagonists prevent Abeta-mediated synaptic plasticity disruption in vivo. *Proc. Natl. Acad. Sci. USA* 106, 20504–20509.
- Huguinir, R.L., and Nicoll, R.A. (2013). AMPARs and synaptic plasticity: the last 25 years. *Neuron* 80, 704–717.

- Hulme, S.R., Jones, O.D., and Abraham, W.C. (2013). Emerging roles of metaplasticity in behaviour and disease. *Trends Neurosci.* 36, 353–362.
- Lee, S.J., Escobedo-Lozoya, Y., Szatmari, E.M., and Yasuda, R. (2009). Activation of CaMKII in single dendritic spines during long-term potentiation. *Nature* 458, 299–304.
- Lisman, J., Yasuda, R., and Raghavachari, S. (2012). Mechanisms of CaMKII action in long-term potentiation. *Nat. Rev. Neurosci.* 13, 169–182.
- Malinow, R. (2012). New developments on the role of NMDA receptors in Alzheimer's disease. *Curr. Opin. Neurobiol.* 22, 559–563.
- Matsuzaki, M., Honkura, N., Ellis-Davies, G.C., and Kasai, H. (2004). Structural basis of long-term potentiation in single dendritic spines. *Nature* 429, 761–766.
- Mayford, M., Wang, J., Kandel, E.R., and O'Dell, T.J. (1995). CaMKII regulates the frequency-response function of hippocampal synapses for the production of both LTD and LTP. *Cell* 81, 891–904.
- Opazo, P., Labrecque, S., Tigaret, C.M., Frouin, A., Wiseman, P.W., De Koninck, P., and Choquet, D. (2010). CaMKII triggers the diffusional trapping of surface AMPARs through phosphorylation of stargazin. *Neuron* 67, 239–252.
- Otmakhov, N., Gorbacheva, E.V., Regmi, S., Yasuda, R., Hudmon, A., and Lisman, J. (2015). Excitotoxic insult results in a long-lasting activation of CaMKII α and mitochondrial damage in living hippocampal neurons. *PLoS ONE* 10, e0120881.
- Penn, A.C., Zhang, C.L., Georges, F., Royer, L., Breillat, C., Hosy, E., Petersen, J.D., Humeau, Y., and Choquet, D. (2017). Hippocampal LTP and contextual learning require surface diffusion of AMPA receptors. *Nature* 549, 384–388.
- Rácz, B., Blanpied, T.A., Ehlers, M.D., and Weinberg, R.J. (2004). Lateral organization of endocytic machinery in dendritic spines. *Nat. Neurosci.* 7, 917–918.
- Rammes, G., Hasenjaeger, A., Sroka-Saidi, K., Deussing, J.M., and Parsons, C.G. (2011). Therapeutic significance of NR2B-containing NMDA receptors and mGluR5 metabotropic glutamate receptors in mediating the synaptotoxic effects of β -amyloid oligomers on long-term potentiation (LTP) in murine hippocampal slices. *Neuropharmacology* 60, 982–990.
- Roberson, E.D., Scarce-Levie, K., Palop, J.J., Yan, F., Cheng, I.H., Wu, T., Gerstein, H., Yu, G.Q., and Mucke, L. (2007). Reducing endogenous tau ameliorates amyloid β -induced deficits in an Alzheimer's disease mouse model. *Science* 316, 750–754.
- Röncke, R., Mikhaylova, M., Röncke, S., Meinhardt, J., Schröder, U.H., Fändrich, M., Reiser, G., Kreutz, M.R., and Reymann, K.G. (2011). Early neuronal dysfunction by amyloid β oligomers depends on activation of NR2B-containing NMDA receptors. *Neurobiol. Aging* 32, 2219–2228.
- Sainlos, M., Tigaret, C., Poujol, C., Olivier, N.B., Bard, L., Breillat, C., Thiolon, K., Choquet, D., and Imperiali, B. (2011). Biomimetic divalent ligands for the acute disruption of synaptic AMPAR stabilization. *Nat. Chem. Biol.* 7, 81–91.
- Shankar, G.M., Bloodgood, B.L., Townsend, M., Walsh, D.M., Selkoe, D.J., and Sabatini, B.L. (2007). Natural oligomers of the Alzheimer amyloid- β protein induce reversible synapse loss by modulating an NMDA-type glutamate receptor-dependent signaling pathway. *J. Neurosci.* 27, 2866–2875.
- Steiner, B., Mandelkow, E.M., Biernat, J., Gustke, N., Meyer, H.E., Schmidt, B., Mieskes, G., Söling, H.D., Drechsel, D., Kirschner, M.W., et al. (1990). Phosphorylation of microtubule-associated protein tau: identification of the site for Ca²⁺-calmodulin dependent kinase and relationship with tau phosphorylation in Alzheimer tangles. *EMBO J.* 9, 3539–3544.
- Townsend, M., Mehta, T., and Selkoe, D.J. (2007). Soluble A β inhibits specific signal transduction cascades common to the insulin receptor pathway. *J. Biol. Chem.* 282, 33305–33312.
- Um, J.W., Nygaard, H.B., Heiss, J.K., Kostylev, M.A., Stagi, M., Vortmeyer, A., Wisniewski, T., Gunther, E.C., and Strittmatter, S.M. (2012). Alzheimer amyloid- β oligomer bound to postsynaptic prion protein activates Fyn to impair neurons. *Nat. Neurosci.* 15, 1227–1235.
- Yang, Q., Liao, Z.H., Xiao, Y.X., Lin, Q.S., Zhu, Y.S., and Li, S.T. (2011). Hippocampal synaptic metaplasticity requires the activation of NR2B-containing NMDA receptors. *Brain Res. Bull.* 84, 137–143.
- Yoshimura, Y., Ichinose, T., and Yamauchi, T. (2003). Phosphorylation of tau protein to sites found in Alzheimer's disease brain is catalyzed by Ca²⁺/calmodulin-dependent protein kinase II as demonstrated tandem mass spectrometry. *Neurosci. Lett.* 353, 185–188.
- Zhao, D., Watson, J.B., and Xie, C.W. (2004). Amyloid β prevents activation of calcium/calmodulin-dependent protein kinase II and AMPA receptor phosphorylation during hippocampal long-term potentiation. *J. Neurophysiol.* 92, 2853–2858.
- Zorumski, C.F., and Izumi, Y. (2012). NMDA receptors and metaplasticity: mechanisms and possible roles in neuropsychiatric disorders. *Neurosci. Biobehav. Rev.* 36, 989–1000.