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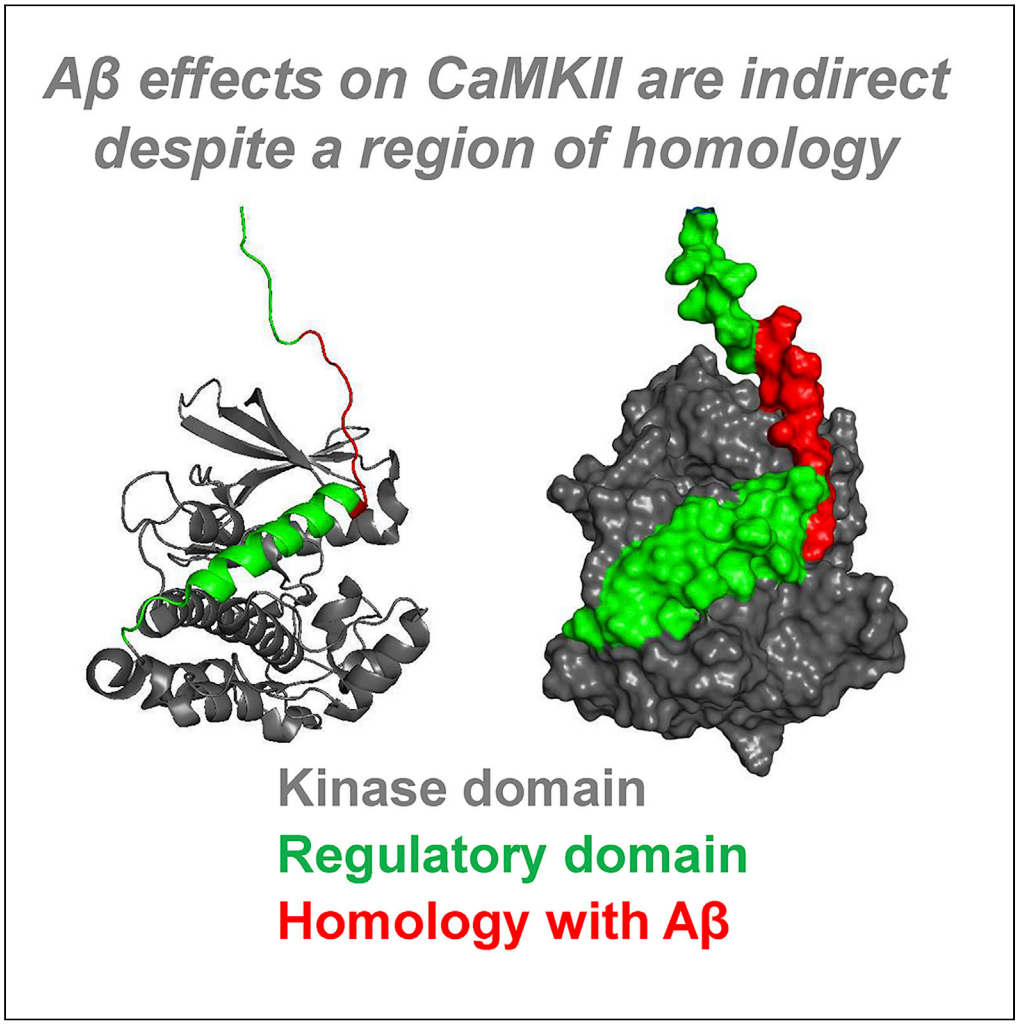

Carolyn Nicole Brown, Nicole L. Rumian, Jonathan E. Tullis, Steven J. Coultrap, K. Ulrich Bayer

steve.coultrap@cuanschutz.edu (S.J.C.)
ulli.bayer@cuanschutz.edu (K.U.B.)

Highlights

Aβ and the CaMKII regulatory domain share a region of homology

Suppression of CaMKII movement in neurons by Aβ requires CaMKII activity

Aβ does not directly affect CaMKII activity, T286 phosphorylation, or GluN2B binding

Thus, the Aβ effects on CaMKII in neurons require indirect signaling mechanisms

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Aβ-induced synaptic impairments require CaMKII activity that is stimulated by indirect signaling events

Carolyn Nicole Brown,[1,3] Nicole L. Rumian,[1,2,3] Jonathan E. Tullis,[1,3] Steven J. Coultrap,[1,*] and K. Ulrich Bayer[1,2,4,*]

SUMMARY

Aβ bears homology to the CaMKII regulatory domain, and peptides derived from this domain can bind and disrupt the CaMKII holoenzyme, suggesting that Aβ could have a similar effect. Notably, Aβ impairs the synaptic CaMKII accumulation that is mediated by GluN2B binding, which requires CaMKII assembly into holoenzymes. Furthermore, this Aβ-induced impairment is prevented by CaMKII inhibitors that should also inhibit the putative direct Aβ binding. However, our study did not find any evidence for direct effects of Aβ on CaMKII: Aβ did not directly disrupt CaMKII holoenzymes, GluN2B binding, T286 autophosphorylation, or kinase activity *in vitro*. Most importantly, in neurons, the Aβ-induced impairment of CaMKII synaptic accumulation was prevented by an ATP-competitive CaMKII inhibitor that would not interfere with the putative direct Aβ binding. Together, our results indicate that synaptic Aβ effects are not mediated by direct binding to CaMKII, but instead require CaMKII activation via indirect signaling events.

INTRODUCTION

The Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) is a central mediator for long-term potentiation (LTP) (reviewed in Bayer and Schulman, 2019), a form of synaptic plasticity that is thought to underlie learning and memory (Lynch, 2004; Martin et al., 2000; Nicoll, 2017) and that is critically impaired by soluble oligomeric amyloid β (oAβ), a central pathological agent in Alzheimer's disease (AD) (Lambert et al., 1998; Shankar et al., 2008; Walsh et al., 2002). Interestingly, CaMKII mediates not only normal LTP but also the LTP impairment that is induced by Aβ: Whereas CaMKII inhibition during an LTP stimulus prevents LTP induction (Malinow et al., 1989; Silva et al., 1992), CaMKII inhibition during preincubation of hippocampal slices with oAβ prevents the impairment of LTP (provided the CaMKII inhibitor is washed out prior to the LTP stimulus) (Cook et al., 2019; Opazo et al., 2018). At least one of the mechanisms by which oAβ impairs LTP is by preventing the accumulation of CaMKII at excitatory synapses (Cook et al., 2019). This accumulation is normally mediated by regulated binding to the NMDA receptor (NMDAR) subunit GluN2B (Bayer et al., 2001) and is required for normal LTP (Barria and Malinow, 2005; Halt et al., 2012). Like LTP, the Aβ-induced impairment of CaMKII movement can also be rescued by CaMKII inhibition (Cook et al., 2019), further supporting the notion that Aβ impairs LTP by impairing synaptic CaMKII accumulation.

But how does Aβ impair CaMKII movement and why is the movement restored by CaMKII inhibitors? As Aβ is internalized by neurons (Hu et al., 2009; Riad et al., 2020), the effect could be mediated by direct binding of Aβ to CaMKII. Indeed, Aβ contains a sequence that is homologous to a region of the CaMKII regulatory domain, which binds to the CaMKII kinase domain in the inactive basal state (Figures 1A and 1B). Importantly, in the basal state, the regulatory domain blocks CaMKII binding to GluN2B (Bayer et al., 2001), and Aβ binding to the same site should thus also be expected to interfere with this GluN2B binding that mediates CaMKII movement to excitatory synapses. Furthermore, peptides derived from the CaMKII regulatory domain region that contains the Aβ homology have been recently shown to disrupt the 12meric CaMKII holoenzyme structure (Karandur et al., 2020) (see Figure 1A) that is also required for normal CaMKII binding to GluN2B and movement to synapses (Bayer et al., 2006; Strack et al., 2000). But why would inhibition of CaMKII activity prevent such a potential direct Aβ effect? This may not be related to CaMKII activity at all, but instead to the specific mechanisms of the inhibitors used: KN93 prevents the activation

[1]Department of Pharmacology, University of Colorado Anschutz Medical Campus, Aurora, CO 80045, USA

[2]Program in Neuroscience, University of Colorado Anschutz Medical Campus, Aurora, CO 80045, USA

[3]These authors contributed equally

[4]Lead contact

*Correspondence: steve.coultrap@cuanschutz.edu (S.J.C.), ulli.bayer@cuanschutz.edu (K.U.B.)

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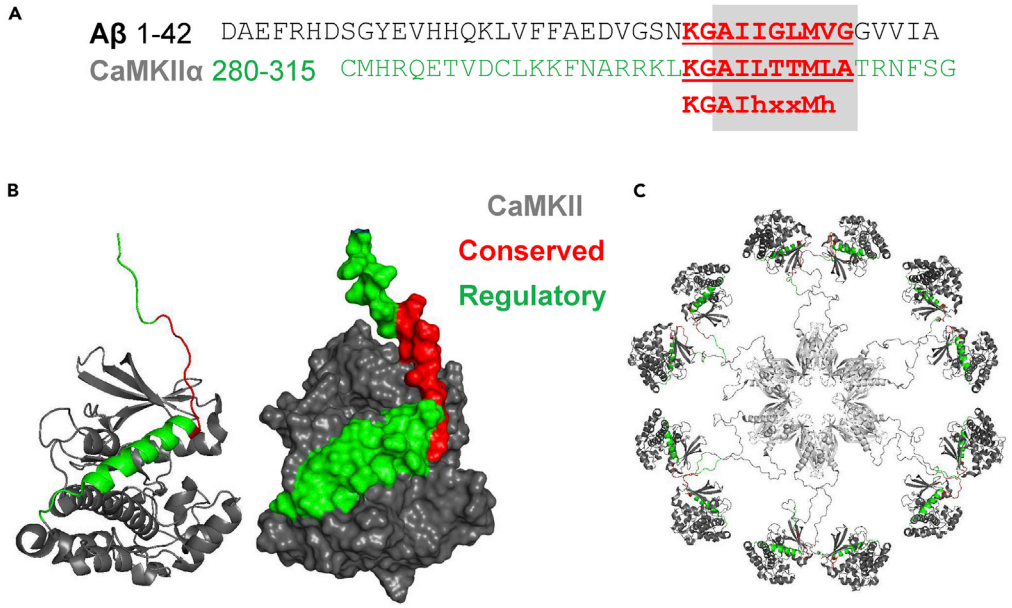

Figure 1. Aβ contains a region with homology to the CaMKII regulatory domain

(A) Sequence alignment of Aβ (black) and the CaMKII regulatory domain (green), with the homologous region indicated (red). The sequence of the CaMKII regulatory domain that has been reported to disrupt the CaMKII holoenzyme structure is underlaid in gray.

(B) The structure of the CaMKII kinase domain (gray) and regulatory domain (green), with the Aβ-homologous region indicated (red).

(C) The 12meric CaMKII holoenzyme in the same color code, with the linker region and C-terminal association domain added (in lighter gray). The color coding in the shown models of the CaMKII structure (PDB 5u6y) was generated in PyMOL.

of CaMKII by Ca^{2+}/CaM (Pellicena and Schulman, 2014; Sumi et al., 1991), which should also prevent the displacement of the regulatory domain and thereby prevent Aβ binding to the homologous site. By contrast, tatCN21 is an active kinase inhibitor that does not bind to CaMKII in the basal state, as it requires the displacement of the regulatory domain to bind to the kinase domain to block substrate access (Vest et al., 2007), which should also block any Aβ binding that occurs in a manner homologous to the binding of the CaMKII regulatory domain or GluN2B. Indeed, CaMKII binding to GluN2B is prevented by both KN93 and tatCN21 (Vest et al., 2007, 2010), but not by ATP-competitive inhibitors (Barcomb et al., 2013).

Here, we tested several potential direct effects of Aβ on CaMKII functions *in vitro*. Within neurons, we tested the effects of a CaMKII inhibitor that acts by an ATP-competitive mechanism that should not interfere with any direct Aβ binding. Our results indicate that synaptic Aβ effects are not mediated by direct Aβ effects on CaMKII holoenzyme integrity, GluN2B binding, or kinase activity, but instead require the stimulation of CaMKII kinase activity by indirect mechanisms.

RESULTS

CaMKII activity is required for Aβ-induced suppression of CaMKII movement

CaMKII movement to excitatory synapses that is mediated by GluN2B binding is required for normal LTP (Barria and Malinow, 2005; Halt et al., 2012), and oAβ impairs LTP at least in part by inhibiting this CaMKII movement (Cook et al., 2019; Opazo et al., 2018). Notably, two different CaMKII inhibitors rescue both CaMKII movement and LTP (Cook et al., 2019; Opazo et al., 2018); however, these inhibitors should interfere not only with kinase activity but also with a potential direct binding of Aβ to CaMKII (see Figure 1). Thus, we here additionally tested the effect of a newer ATP-competitive CaMKII inhibitor, AS105 (Neef et al., 2018; Pellicena and Schulman, 2014), that should interfere neither with such binding of Aβ nor with binding to GluN2B (Barcomb et al., 2013). Movement of endogenous CaMKII induced by chemical LTP (cLTP) stimuli (1 min, 100 μM glutamate in presence of glycine) was monitored live in hippocampal neurons using a recently established intrabody labeling method (Cook et al., 2019, 2021; Gross et al., 2013). In this method,

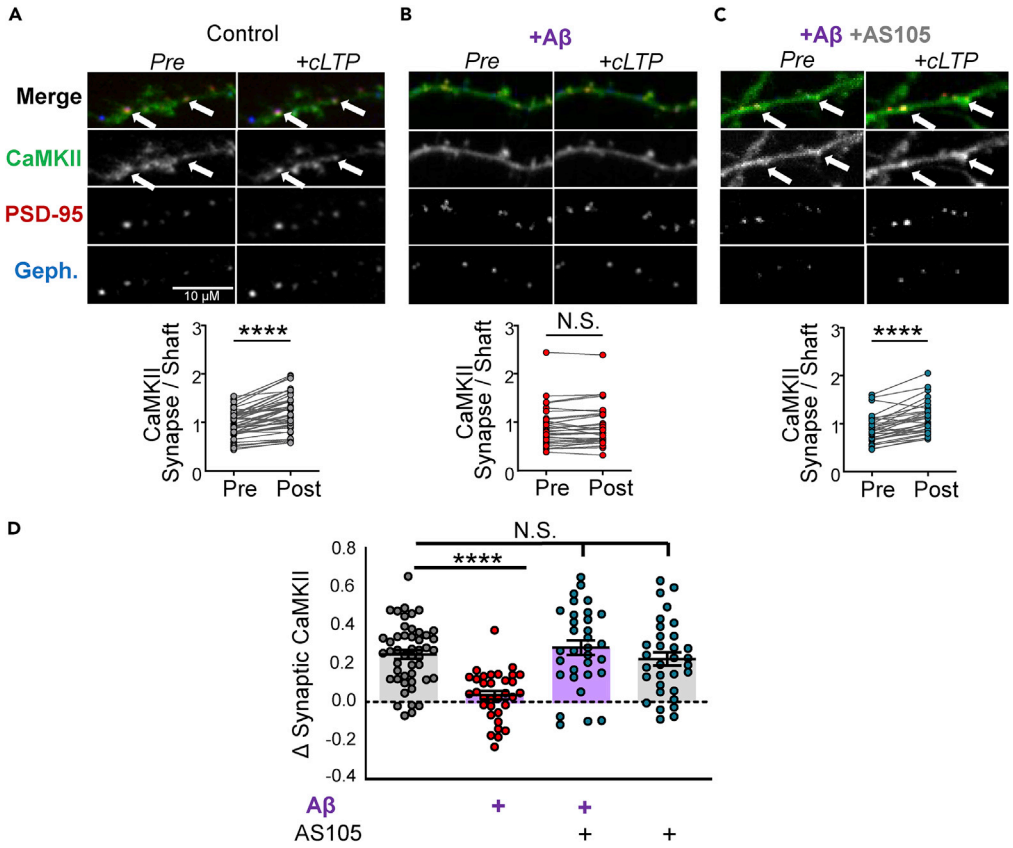

Figure 2. AS105 blocks the Aβ effect on CaMKII movement in hippocampal neurons

GFP-CaMKII intrabody was co-expressed with mCherry-labeled PSD-95 intrabody and iRFP-labelled Gephyrin intrabody in dissociated hippocampal neurons. CaMKII accumulation at excitatory synapses was induced by cLTP (100 μM glutamate, 10 μM glycine, 1 min).

(A) Example images and quantification of endogenous CaMKII at excitatory synapses before and 1 min after cLTP treatment, where CaMKII translocated to excitatory synapses (****$p < 0.0001$ in paired t-test, n = 49 neurons). Scale bar: 10 μm.

(B) Example images and quantification of endogenous CaMKII at excitatory synapses before and 1 min after cLTP treatment, with a 45 min oAβ pre-incubation (N.S. in paired t-test, n = 33). Before the cLTP stimulus, coverslips were washed in fresh ACSF.

(C) Example images and quantification of endogenous CaMKII at excitatory synapses before and 1 min after cLTP treatment, with a 45 min oAβ+AS105 pre-incubation, where CaMKII translocated to excitatory synapses (****$p < 0.0001$ in paired t-test, n = 32 neurons). The AS105 was added immediately after the oAβ, and the coverslips were washed in fresh ACSF prior to the cLTP stimulus.

(D) Column statistics of the cLTP-induced change in CaMKII synaptic enrichment at excitatory synapses (one-way ANOVA with Bonferroni post hoc test, ****$p < 0.0001$, N.S. $p > 0.05$, n = 49, 33, 32, 32 neurons). AS105 rescues from the Aβ-induced impairment in CaMKII enrichment; without Aβ, it did not affect CaMKII enrichment. Error bars indicate SEM.

tracking of movement to synapses is facilitated by simultaneous live imaging of endogenous marker proteins for excitatory and inhibitory synapses, i.e., PSD95 and gephyrin. As observed previously, 45 min pre-incubation with 0.5 μM oligomeric Aβ (oAβ) blocked the cLTP-induced movement of CaMKII to excitatory synapses (Figures 2A and 2B). However, co-incubation with 10 μM AS105 completely alleviated this block and allowed normal movement (Figures 2C and 2D). Thus, the Aβ-induced block of CaMKII movement requires CaMKII activity.

Aβ does not directly stimulate CaMKII activity *in vitro*

As CaMKII inhibition prevents the Aβ effect in neurons, we decided to test first if Aβ can directly stimulate CaMKII activity in *in vitro* assays with purified protein. As it is not entirely clear in which forms Aβ would exist after potential cellular uptake, we tested the effect of two different Aβ preparations, soluble oligomeric and

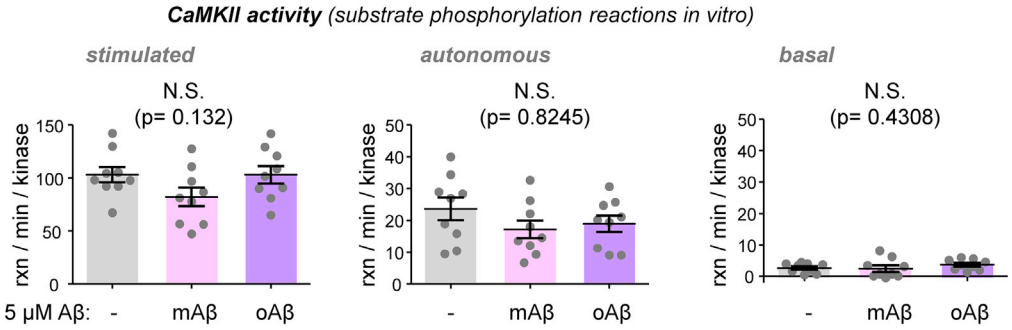

Figure 3. Aβ does not affect CaMKII activity *in vitro*

Tested were the effects of mAβ and oAβ (5 μM) on stimulated CaMKII activity generated by Ca^{2+}/CaM, autonomous CaMKII generated by T286 autophosphorylation, and basal CaMKII activity without autophosphorylation of T286 and in absence of any Ca^{2+}. N.S. indicates no significant differences by ANOVA. Error bars indicate SEM.

monomeric Aβ (oAβ and mAβ). The oAβ was prepared according to an established standard method (Klein, 2002), but with final peptide dilution in buffered saline instead of F12 media (Cook et al., 2019), as in the experiments in neurons; the mAβ was made by fresh reconstitution of lyophilized peptide. Neither of the Aβ preparations caused any increase in Ca^{2+}/CaM-stimulated CaMKII activity, in autonomous activity after T286 autophosphorylation, or in basal CaMKII activity (Figure 3). If any, mAβ appeared to slightly decrease stimulated CaMKII activity; however, this was not statistically significant even at the high Aβ concentrations used in the experiments (5 μM; i.e., at least 10–100-fold higher than the concentrations that show effects in neurons). Thus, Aβ neither directly increases nor decreases CaMKII activity, and the CaMKII activity responsible for the Aβ effects on CaMKII movement and LTP must be generated through indirect signaling steps.

Aβ does not disrupt the 12meric CaMKII holoenzyme structure

Peptides derived from the region of the CaMKII regulatory domain that has homology with the Aβ sequence (see Figure 1A) have been described to promote the disassembly of the 12meric CaMKII holoenzyme structure into monomers, dimers, tetramers, and other oligomeric states (Karandur et al., 2020). In turn, such holoenzyme disassembly would be expected to suppress T286 autophosphorylation, as this reaction occurs between subunits within a holoenzyme (Hanson et al., 1994). Indeed, such suppression of T286 autophosphorylation has been observed after treatment with Aβ (Opazo et al., 2018). Importantly, CaMKII holoenzyme disassembly would inhibit T286 autophosphorylation without inhibiting the CaMKII activity that mediates the observed Aβ-induced impairments of CaMKII movement and LTP (Cook et al., 2019; Opazo et al., 2018; see also Figure 2). Thus, we decided to test the effect of Aβ on the multimerization state of the CaMKII holoenzyme *in vitro*, which was monitored here by fluorescence correlation spectroscopy (FCS). Briefly, diffusion of fluorescently labeled in and out of a confocal microscope's focal volume creates fluctuations in the average fluorescence intensity of the sample (Figure 4A). The average fluorescence intensity is divided by the number of individual particles in the solution (N; determined by the autocorrelation value at G(0)) to determine each particle's relative brightness. The brightness of each particle is proportional to the number of fluorophores, and thus, the number of subunits, in that particle. Even an extremely high concentration of mAβ (50 μM) did not have any detectable effect on CaMKII holoenzyme oligomeric state neither in the presence or absence of additional Ca^{2+}/CaM stimuli (Figure 4B). These results suggest that mAβ does not disrupt the holoenzyme quaternary structure.

Aβ does not directly affect CaMKII T286 autophosphorylation *in vitro*

Next, we decided to test possible Aβ effects on two CaMKII functions that require the holoenzyme structure: T286 autophosphorylation (Hanson et al., 1994; Rich and Schulman, 1998) and GluN2B binding (Bayer et al., 2006; Strack et al., 2000). Thus, while Aβ did not affect the autonomous CaMKII activity that is generated by T286 autophosphorylation (see Figure 3), we additionally tested if Aβ may have a direct effect on the T286 autophosphorylation reaction itself (Figure 5). Although application of 0.5 μM oAβ to neurons blocks the LTP-induced increase in T286 autophosphorylation (Opazo et al., 2018), *in vitro*, even 5 μM of oAβ or mAβ caused only a slight apparent decrease that was not statistically significant (Figure 5). Thus, Aβ does not directly affect autonomous CaMKII activity or its generation by T286 autophosphorylation.

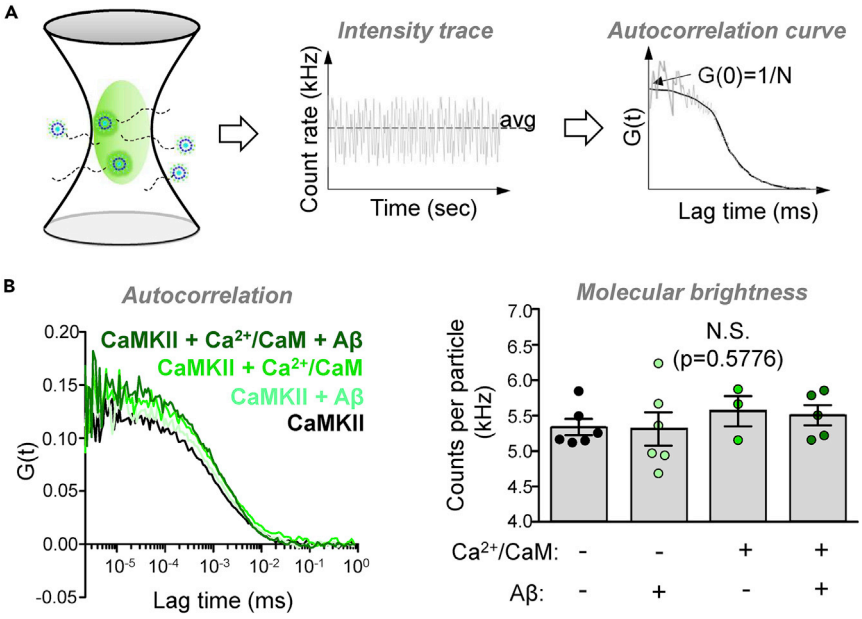

Figure 4. Aβ does not disrupt CaMKII holoenzyme structure

(A) Diffusion of fluorescent particles through a focal volume creates fluctuations in the average fluorescence intensity of the sample. Correlation analyses of the original intensity trace with the same trace offset by increasing lag times generate an autocorrelation curve. The y-intercept of the autocorrelation curve (G(0)) is equal to the inverse of the number of particles (N) in the focal volume. The average fluorescence intensity of the focal volume divided by the number of particles in the focal volume yields the brightness (counts per particle; cpp (kHz)) of each particle, which provides a readout for the oligomeric state of CaMKII.

(B) Representative autocorrelation curves and molecular brightness quantitation of GFP-CaMKII with or without mAβ (50 μM) and Ca^{2+}/CaM (2 mM/1 μM) as indicated. N.S. indicates no significant differences by Kruskal-Wallis test. Error bars indicate SEM.

Additionally, this result further supports the finding that Aβ does not disrupt the CaMKII holoenzyme structure.

Aβ does not prevent CaMKII binding to GluN2B CaMKII *in vitro*

Potential direct binding of Aβ near the GluN2B binding site on the CaMKII kinase domain is suggested by the Aβ homology to the CaMKII regulatory domain that binds to this site (see Figure 1) and such binding could potentially disrupt CaMKII binding to GluN2B (Bayer et al., 2006) even if it does not disrupt the CaMKII holoenzyme. Thus, we determined the effect of Aβ on CaMKII binding to GluN2B in our established *in vitro* assay that utilizes the cytoplasmic C-terminus of GluN2B (GluN2Bc; amino acids 1120–1482) immobilized as GST-fusion protein to anti-GST-coated microtiter plates (Bayer et al., 2001, 2006; Tullis et al., 2020). Again, even 5 μM of oAβ or mAβ had no direct effect on the Ca^{2+}/CaM-induced binding of CaMKII to GluN2B *in vitro* (Figure 6). This indicates that the Aβ-induced suppression of CaMKII binding to GluN2B in neurons is triggered by indirect signaling events rather than by direct binding of Aβ to CaMKII. It also further validates that Aβ does not directly disrupt the CaMKII holoenzyme structure that is required for the GluN2B binding.

Overall, while it cannot be formally ruled out that there is a direct binding of oAβ or mAβ to CaMKII holoenzymes, we did not detect any direct biochemical effect on holoenzyme structure, T286 autophosphorylation, GluN2B binding, or kinase activity.

DISCUSSION

Our results demonstrate that kinase activity of CaMKII is required for the Aβ-induced suppression of CaMKII movement to excitatory synapses in hippocampal neurons, an effect that is responsible for the LTP impairment by Aβ. On its own, this did not rule out a potential co-requirement also for the mechanism

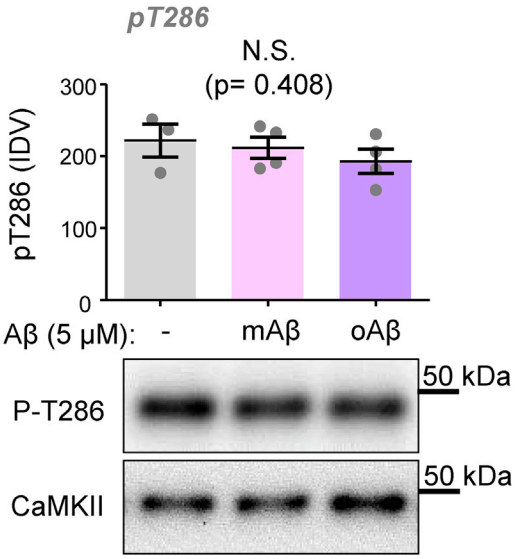

Figure 5. Aβ does not significantly affect CaMKII T286 autophosphorylation *in vitro*

Addition of 5 μM mAβ or oAβ does not reduce CaMKII T286 autophosphorylation. Western blot and quantification by arbitrary immune-detection value (IDV). N.S. indicates no significant differences by Kruskal-Wallis test. Error bars indicate SEM.

that involves the direct binding of Aβ to CaMKII, as we had initially hypothesized based on the homology of Aβ with a region of the CaMKII regulatory domain that interacts with the CaMKII kinase domain (see Figure 1). However, we did not detect any direct biochemical effects of Aβ on CaMKII holoenzyme structure, T286 autophosphorylation, GluN2B binding, or kinase activity *in vitro*. Thus, even in the case that there is a direct binding of Aβ to CaMKII, such a binding alone would not be associated with any impairment of the CaMKII functions tested here. Together, these findings show that CaMKII activity is required for the disruption of LTP by Aβ and suggest that this involves indirect signaling mechanisms rather than direct Aβ binding to CaMKII.

Our findings highlight that CaMKII activity is required not only for mediating LTP but also for mediating the LTP impairments triggered by Aβ. Such a dual role also in the Aβ-induced impairment of LTP has been suggested by our previous studies (Cook et al., 2019; Opazo et al., 2018); however, this was based on two CaMKII inhibitors that could have acted by preventing direct Aβ binding to CaMKII rather than by the inhibition of CaMKII activity. By contrast, the ATP-competitive CaMKII inhibitor AS105 (Neef et al., 2018; Pellicena and Schulman, 2014) that was used here would not interfere with the predicted putative Aβ binding. Furthermore, no indication for such a direct effect of Aβ on CaMKII was found in our biochemical studies.

A dual function of CaMKII activity in both LTP and LTP impairment may appear counterintuitive; however, a similar dual role of CaMKII has been established in both LTP and LTD, two forms of synaptic plasticity in opposing directions (Bayer and Schulman, 2019). In the case of the LTP versus LTD decision, the two opposing CaMKII functions are enabled by differential substrate selection (Coultrap et al., 2014) and a complex cross-regulation involving GluN2B binding, DAPK1, and inhibitory autophosphorylation at T305/306 (Barcomb et al., 2014; Cook et al., 2021; Goodell et al., 2017). In the case of LTP versus Aβ-induced LTP impairment, the underlying mechanisms remain to be elucidated. However, based on our recent results, it is clear that (i) the mechanisms of LTP impairment involve suppression of CaMKII binding to GluN2B and that (ii) this suppression requires CaMKII activity. One way how CaMKII activity could suppress GluN2B binding would be the phosphorylation of GluN2B at S1303 (O'Leary et al., 2011; Strack et al., 2000); however, our previous studies indicated that such phosphorylation is not increased by treatment with Aβ (Cook et al., 2019).

Our here disproven hypothesis that Aβ effects may be mediated by direct binding to CaMKII was particularly appealing based on recent findings that homologous peptides could disrupt the 12meric CaMKII holoenzyme structure (Karandur et al., 2020) (see also Figure 1). In this case, the interaction of Aβ-homologous CaMKII regulatory domain sequences with the CaMKII association domain may have a physiological function in aiding subunit exchange in the holoenzyme (Bhattacharyya et al., 2016; Sloutsky et al., 2020; Stratton et al., 2014). A similar pathological Aβ function in disrupting the CaMKII holoenzyme would be expected to suppress both CaMKII binding to GluN2B (Bayer et al., 2006; Strack et al., 2000) and T286

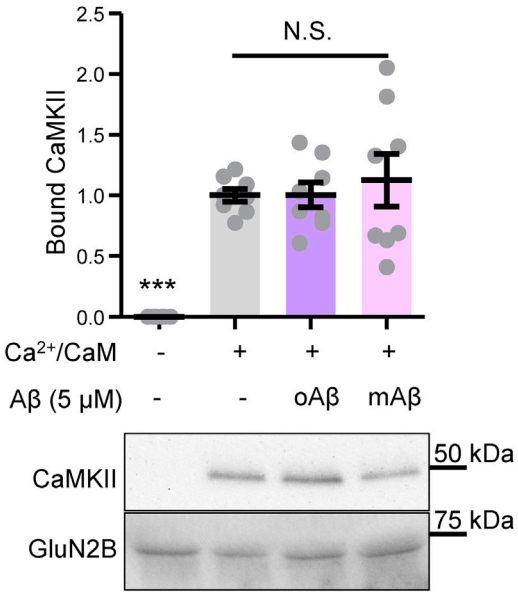

Figure 6. Aβ does not affect CaMKII binding to GluN2B *in vitro*

Binding of purified CaMKIIα to immobilized GST-GluN2B-c (a GST-fusion with the cytoplasmic C-terminus of GluN2B) was induced by Ca^{2+}/CaM (Ca^{2+}). This Ca^{2+}/CaM-induced CaMKII binding to GluN2B was not affected by 5 μM oAβ or mAβ (N.S.; $p = 0.9998$ or 0.7632, respectively, in ANOVA with Bonferroni post hoc test). Without Ca^{2+}/CaM, binding was significantly less (***; $p < 0.001$); in fact, binding without Ca^{2+}/CaM was not detectable at all. Error bars indicate SEM.

autophosphorylation (Hanson et al., 1994), two effects, indeed, caused by Aβ in neurons (Cook et al., 2019; Opazo et al., 2018). However, our biochemical experiments *in vitro* clearly show that Aβ does not cause these disruptions directly.

Our results indicate that CaMKII inhibition can prevent at least some of the adverse Aβ effects on excitatory synapses. However, it is not likely that these findings can be exploited for the treatment of Alzheimer's disease: While chronic CaMKII inhibition may prevent the mechanisms of LTP-impairments caused by Aβ, it would instead directly cause LTP-impairments by other mechanisms, as CaMKII mediates not only the Aβ-induced impairment of LTP but also LTP itself. However, a better understanding of the specific CaMKII signaling that mediates the Aβ-induced impairment may lead to the identification of druggable targets for the protection of LTP in Alzheimer's disease in future studies.

Limitations of the study

Our study demonstrates that (i) CaMKII activity is required to mediate the Aβ-induced impairment of synaptic CaMKII accumulation and that (ii) Aβ does not directly affect CaMKII activity, T286 autophosphorylation, GluN2B binding, or holoenzyme assembly. However, this does not rule that Aβ may still directly bind to CaMKII and that such binding could have other effects on CaMKII that were not tested here. Nonetheless, the impairment of the synaptic CaMKII accumulation that is required for normal LTP does not appear to be among such potential direct Aβ effects.

STAR★METHODS

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AUTHOR CONTRIBUTIONS

C.N.B., N.L.R., J.E.T, and S.J.C. performed experiments and analyses; S.J.C. and K.U.B. conceived the initial study design, with input from all authors. K.U.B. wrote the initial draft and all authors contributed to the final article.

DECLARATION OF INTERESTS

The University of Colorado holds the patent rights for a CaMKII inhibitor mentioned in this study, tatCN21, its derivatives, and their uses (PCT/US08/077934 ''Compositions and methods for improved CaMKII inhibitors and uses thereof''). K.U.B. is co-founder and board member of Neurexis Therapeutics.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
CaMKIIα	Made in House	CBα2
CaMKIIα pT286	Phospho-Solutions	p1005-286; RRID: AB_2492051
GST	Millipore	AB3282; RRID: AB_91439
Goat anti-Rabbit	GE Healthcare	NA934V; RRID: AB_2722659
Goat anti-Mouse	GE Healthcare	NA931V; RRID: AB_772210
Chemicals, peptides, and recombinant proteins		
Papain	Worthington	LS 03126
Lipofectamine 2000	Invitrogen	11668027
B-27 supplement	Gibco	17504044
Glutamate	Sigma	6106-04-3
Glycine	Sigma	56-40-6
cOmplete protease inhibitor cocktail (EDTA-free)	Roche	1187380001
Fluorescein	Sigma	F2456-2.5G
Microcystin-LR	Calbiochem	475815
Anti GST-coated plates	Pierce	15144
Calmodulin	Made in house	CaM
Ca^{2+}/CaM-dependent kinase II α	Made in house	CaMKII
GST-GluN2B C-tail	Made in house	GST-GluN2Bc
AS105	Howard Schulman	N/A (gift)
Unlabelled amyloid beta (1–42) peptide	Anaspec	AS-20276
Critical commercial assays		
Pierce BCA protein assay	Thermo-Fisher	23225
SuperSignal West Femto	Thermo Fisher	34095
Deposited data		
Raw and analyzed data	This paper, Mendeley Data	https://data.mendeley.com/datasets/y5g4czwt3w/1
Experimental models: Cell lines		
Primary hippocampal cultures	Laboratory of K. Ulrich Bayer	N/A
Experimental models: Organisms/strains		
Rat: Sprague-Dawley	Charles River Labs	N/A
Recombinant DNA		
CaMKIIα-FingR-GFP	Don Arnold	Addgene #46295
PSD-95-FingR-GFP	Don Arnold	Addgene #46296
Gephyrin-FingR-GFP	Don Arnold	Addgene #15214
Software and algorithms		
Slidebook 6.0	Intelligent Imaging Innovations (3i)	RRID:SCR_014300
Prism 7.0	Graphpad	RRID: SCR_002798
AlphaEase FC 4.0	Alpha Innotech	N/A
ImageJ	NIH	RRID:SCR_003070
PyCorrFit 1.1.7	Lab of T. Weidemann	PMID 24825612
PyMOL	Schrödinger	RRID:SCR_000305

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, K. Ulrich Bayer (ulli.bayer@ucdenver.edu).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- The datasets generated during this study are available through Mendeley Data: https://data.mendeley.com/datasets/y5g4czwt3w/1.
- No original code was generated during this study.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Experimental animals

All animal treatment was approved by the University of Colorado Institutional Animal Care and Use Committee, in accordance with NIH guidelines, and was done as described previously (Buonarati et al., 2020). Animals are housed at the Animal Resource Center at the University of Colorado Anschutz Medical Campus (Aurora, CO) and are regularly monitored with respect to general health, cage changes, and overcrowding. Pregnant Sprague-Dawley rats were supplied by Charles River Labs.

Primary hippocampal cultures

Primary hippocampal neurons were prepared similar as previously described (Buonarati et al., 2021; Cook et al., 2021). Hippocampi were dissected from mixed sex rat pups (P0), incubated in dissociation solution (7 mL HBSS buffered saline, 150 μL 100mM $CaCl_2$, 10 μL 1M NaOH, 10 μL 500mM EDTA, 200 units Papain [Worthington]) for 1 h, washed 5x with plating media (MEM, FBS, 50 units/ml Penn/strep, 2mM Glutamax, filter sterilized), then manually dissociated. Cells were plated at 100,000 cells/mL for imaging (on poly-D-lysine (0.1 mg/mL in 1M Borate Buffer: 3.1g Boric Acid, 4.75 g borax, in 1 L deionized H20, filter sterilized) and laminin (0.01 mg/mL in PBS)-coated 18 mm glass coverslips in 12 well plates) and 500,000 cells/mL for biochemistry (on poly-D-lysine-coated 6 well plates). After day *in vitro* (DIV) 1, media was switched to feeding media (Neurobasal-A, B27 supplements, and 2 mM Glutamax, filter sterilized). At DIV 7, to suppress glial growth, cells were treated with anti-mitotic FDU (70 μM 5-fluoro-2′-deoxyuridine/140 μM uridine) and half of the media was replaced with fresh feeding media. At DIV 12–14, neurons were transfected at a 1:1:1 ratio with 1 μg total cDNA per well using Lipofectamine 2000 (Invitrogen), then imaged 2–3 days later.

METHOD DETAILS

Material and DNA constructs

Material was obtained from Sigma, unless noted otherwise. Amyloid beta (1–42) peptide was purchased from Anaspec. AS105 was a generous gift from Dr. Howard Schulman.

Protein preparations

Expression and purification of CaMKIIα, CaM, and GST-GluN2Bc was conducted according to established protocol described in detail previously (Bayer et al., 2001; Cook et al., 2021; Coultrap and Bayer, 2012; Singla et al., 2001). CaMKIIα was purified from a baculovirus/Sf9 cell expression system. CaM and GST-GluN2Bc were purified after expression in BL21 bacteria. GFP-CaMKIIα for FCS analysis was from HEK cell extracts, prepared as described previously (Coultrap et al., 2012). Briefly, HEK293 cells were transfected by the calcium phosphate method at approximately 70% confluency. The transfection media was replaced with fresh media one day later. Two days following transfection, the media was replaced with cold PBS, the cells were gently scraped, and the cell/PBS mixture was transferred to an Eppendorf tube for centrifugation (1000 rpm, 5 min, 4°C). The PBS supernatant was removed and replaced with homogenization buffer (50mM PIPES pH 7.12, 1mM EGTA, 10% glycerol, and complete protease inhibitor cocktail). A pestle homogenizer

was used to lyse cells on ice for 30s each. The homogenized cells were centrifuged at 14,000RPM for 20 min at 4C and the supernatant was stored at −20°C until analysis.

Aβ preparations

Amyloid beta (1–42) peptide was purchased from Anaspec. Upon arrival, the peptide was resuspended in 1,1,1,3,3,3-hexafluoro-2-propanol (HFP) and aliquoted in separate tubes. The solubilized peptide was left overnight at room temperature and then stored at −80°C. To reconstitute Aβ for experiments, 6 μL DMSO is added to the lyophilized Aβ tube and vortexed vigorously. An additional 54 μL of PBS was added to the tube and vortexed again to achieve a concentration of 100 μM. For mAβ, the solution was centrifuged at 14,000RPM for 10 min and the supernatant was diluted as needed in PBS and used immediately. For oAβ, the 60 μL of DMSO/PBS/Aβ was left at 4°C overnight or for two nights and then centrifuged at 14,000 rpm for 10 min, similarly as previously described (Cook et al., 2019). The supernatant was diluted as needed in PBS and used for experiments.

Fluorescence correlation spectroscopy (FCS)

Fluorescence correlation spectroscopy (FCS) experiments were performed with a Zeiss LSM780 spectral microscope equipped with an FCS/RICS package, similarly as described previously (Brown et al., 2021). The microscope pinhole was adjusted prior to experimentation using dilute (~50nM) fluorescein. GFP-CaMKII extracts from HEK293 cells were prepared to achieve a baseline count rate of 20,000–40,000 counts per second (CPS; kHz) to achieve a particle number (N) between 5 and 10 (autocorrelation curve y-intercept between 0.1 and 0.2 when curve decays to 0). Ten 10 s acquisitions were collected and averaged together for each experimental replicate. PyCorrFit 1.1.7 software was used to fit autocorrelation curves with a 3D Gaussian distribution model. Molecular brightness is reported as counts per particle (cpp; kHz). For groups without Aβ, an Aβ vehicle solution (10% DMSO in PBS) was used to account for the DMSO in our Aβ preparation.

CaMKII binding to GluN2B *in vitro*

CaMKII binding to the GluN2B C-tail was assessed similarly as previously described (Bayer et al., 2001). GST- coated well plates were washed 3 times in wash buffer (PST) containing (in mM) 50 PIPES pH 7.12, 150 NaCl, and 0.1% Tween-20. GST-GluN2Bc (diluted in PST containing 0.05% BSA) was added in saturating amounts to wells for 1 h under gentle agitation at room temperature and washed three times with PST. Wells were then blocked in 5% BSA in PST for 1 h under gentle agitation at room temperature. Purified CaMKII (50 nM) was bound for 1 h in kinase binding buffer containing (final concentration, in mM) 50 PIPES pH 7.12, 150 NaCl, 9.5 $MgCl_2$, 0.1 ADP, 0.05% BSA, and 0.05% Tween-20 and either EGTA (5 mM), Ca^{2+}/CaM (2 mM/1 μM), or Ca^{2+}/CaM with oligomeric or monomeric Aβ (oAβ or mAβ, 5 μM. Supernatant was discarded and wells were washed 4 times in PST containing EGTA (5 mM). Wells were then incubated in gel loading buffer containing 1% SDS at 90°C for 10 min to dissociate bound proteins.

Western analysis

Western analysis was performed similar as described previously (Cook et al., 2021; Tullis et al., 2020). Protein concentration was determined using the Pierce BCA protein assay (Thermo-Fisher). Before undergoing SDS-PAGE, samples were boiled in Laemmli sample buffer for 5 min at 95°C. Proteins were separated in a resolving phase polymerized from 7.5% acrylamide, then transferred to a polyvinylidene difluoride membrane at 24 V for 1–2 h at 4°C. Membranes were blocked in 5% milk or BSA and incubated with anti-CaMKIIα (1:4000, CBα2, available at Invitrogen but made in house), pT286-CaMKII (1:2500, Phospho-Solutions), anti-GST (1:1000, Millipore), followed by either Amersham ECL goat anti-mouse HRP-linked secondary 1:10000 (GE Healthcare) or goat anti-rabbit horseradish peroxidase conjugate 1:10000 (Bio-Rad). Blots were developed using chemiluminescence (Super Signal West Femto, Thermo-Fisher), imaged using the Chemi-Imager 4400 system (Alpha-Innotech), and analyzed by densitometry (ImageJ). Phospho-signal was corrected to total protein. Relative band intensity was normalized as a percent of control conditions on the same blot, which was set at a value of one to allow for comparison between multiple experiments.

Live imaging and image analysis

Neurons from early postnatal (P0) rat pups were cultured on glass coverslips for imaging experiments. Preparation of dissociated cultures was conducted as described above in detail and similar as previously described (Buonarati et al., 2021; Cook et al., 2021). At DIV14-17, chemical LTP (cLTP) was induced with

100 μM glutamate, 10 μM glycine in ACSF for 1 min, followed by 5x volume washout in fresh ACSF. Coverslips were pre-incubated with 0.5 μM oAβ or 0.5 μM oAβ and 10 μM AS105 in culture medium for 45 min before; then coverslips were washed in ACSF prior to the cLTP stimuli. Cells were imaged using a 100 × 1.4NA objective on a Zeiss Axiovert 200 M (Carl Zeiss, Thornwood, NY) controlled by SlideBook software (Intelligent Imaging Innovations, Denver, CO). All imaging analysis was completed using SlideBook software. During image acquisition, neurons were maintained at 34°C in ACSF solution containing (in mM): 130 NaCl, 5 KCl, 10 HEPES pH 7.4, 20 glucose, 2 $CaCl_2$, and 1 $MgCl_2$, adjusted to proper osmolarity with sucrose. Pre and 1 min post cLTP washout images were collected. 2D maximum intensity projection images were then generated and the mean GFP intensity (CaMKII) at excititory (PSD-95) or inhibitory (gephyrin) synapses was quantified, as well as the mean intensity of a line drawn in the dendritic shaft. Changes in CaMKII synaptic accumulation were determined by dividing the net change in CaMKII at PSD-95 or gephyrin puncta-to shaft ratio by the pre-stimulation puncta-to-shaft ratio. All representative images were prepared using Fiji software (Imagej, NIH).

Kinase assays

Standard CaMKII reactions were done for 1 min at 30°C and started by adding CaMKIIα (2.5 nM subunits, unless stated otherwise) to a mix of 50 mM PIPES pH 7.1, 0.1% BSA, 1 μM CaM, 1 mM $CaCl_2$, 10 mM $MgCl_2$, 100 μM [γ^{32}P] ATP (1 Ci/mmole), 75 μM syntide-2 substrate peptide, similarly as previously described (Coultrap and Bayer, 2012; Coultrap et al., 2010). Basal CaMKII activity was measures as above with the CaM or $CaCl_2$ replaced with 1.5 mM EGTA in the reaction mix. To measure autonomous CaMKII activity T286 was first phosphorylated by reacting the kinase (100 nM) on ice for 5 min with a buffer similar to above without the syntide substrate and only non-radioactive ATP. The reaction was stopped by addition of 5 mM EDTA and dilution to 12.5 nM kinase on ice for 5 min. This kinase was diluted 5 fold into the final reaction. Autonomous kinase activity was then measured using the same reaction mix as the basal reactions. Monomeric or oligomeric A beta was preincubated with the naïve or p-T286 kinase (12.5 nM) for 5 min at RT before the final reaction. For all kinase activity assays, reactions were stopped by spotting onto P81 cation exchange chromatography paper (Whatman) squares. After extensive washes in water, phosphorylation of the substrate peptide bound to the P81 paper was measured by submersing in Bio-safe N/A scintillation fluid and counting in a liquid scintillation counter.

T286 autophosphorylation

To determine the effects of Aβ on T286 phosphorylation CaMKII (80 nM subunits) was diluted in a mix of 50 mM PIPES pH 7.1, 0.1% BSA, 1 μM CaM, 1 mM $CaCl_2$, 10 mM $MgCl_2$ then preincubated for 5 min at RT with or without 5 μM mAβ or oAβ. The kinase was then chilled on ice for 5 min before addition of 100 μM ATP. After 15 or 60 s, reactions were stopped by addition of Laemmli sample buffer with 5 mM EDTA added, then boiled for 5 min. Phosphorylation was determined by western blot.

QUANTIFICATION AND STATISTICAL ANALYSIS

All data are shown as mean ± SEM. Statistical significance is indicated in the figure legends. Statistics were performed using Prism (GraphPad) software. Imaging experiments were obtained and analyzed using SlideBook 6.0 software. Western blots were analyzed using ImageJ (NIH). All data were tested for their ability to meet parametric conditions, as evaluated by a Shapiro-Wilk test for normal distribution and a Brown-Forsythe test (3 or more groups) or an F-test (2 groups) to determine equal variance. All comparisons between two groups met parametric criteria, and independent samples were analyzed using unpaired, two-tailed Student's t-tests. Comparisons between three or more groups meeting parametric criteria were done by one-way ANOVA with specific post-hoc analysis indicated in figure legends. Non-parametric data comparisons between 3 or more groups were done by Kruskal-Wallis test.