Cellular/Molecular

βCaMKII Plays a Nonenzymatic Role in Hippocampal Synaptic Plasticity and Learning by Targeting αCaMKII to Synapses

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The calcium/calmodulin-dependent kinase type II (CaMKII) holoenzyme of the forebrain predominantly consists of heteromeric complexes of the αCaMKII and βCaMKII isoforms. Yet, in contrast to αCaMKII, the role of βCaMKII in hippocampal synaptic plasticity and learning has not been investigated. Here, we compare two targeted *Camk2b* mouse mutants to study the role of βCaMKII in hippocampal function. Using a *Camk2b*$^{-/-}$ mutant, in which βCaMKII is absent, we show that both hippocampal-dependent learning and Schaffer collateral–CA1 long-term potentiation (LTP) are highly dependent upon the presence of βCaMKII. We further show that βCaMKII is required for proper targeting of αCaMKII to the synapse, indicating that βCaMKII regulates the distribution of αCaMKII between the synaptic pool and the adjacent dendritic shaft. In contrast, localization of αCaMKII, hippocampal synaptic plasticity and learning were unaffected in the *Camk2b*^{A303R} mutant, in which the calcium/calmodulin-dependent activation of βCaMKII is prevented, while the F-actin binding and bundling property is preserved. This indicates that the calcium/calmodulin-dependent kinase activity of βCaMKII is fully dispensable for hippocampal learning, LTP, and targeting of αCaMKII, but implies a critical role for the F-actin binding and bundling properties of βCaMKII in synaptic function. Together, our data provide compelling support for a model of CaMKII function in which αCaMKII and βCaMKII act in concert, but with distinct functions, to regulate hippocampal synaptic plasticity and learning.

Introduction

Calcium/calmodulin-dependent kinase type II (CaMKII) is one of the most abundant proteins of the hippocampus, and its role in hippocampal plasticity and learning has been thoroughly investigated by pharmacological and genetic approaches. However, in the hippocampus there are two major isoforms of CaMKII, α and β, which cannot be distinguished using pharmacological approaches. In addition, almost all genetic approaches have focused on the α isoform (Silva et al., 1992; Mayford et al., 1996; Giese et al., 1998; Elgersma et al., 2002; Miller et al., 2002; Wang et al., 2008; Yamagata et al., 2009) (for an overview of CaMKII mutants, see Elgersma et al., 2004). Collectively, these studies have demonstrated that activation of αCaMKII is necessary for normal synaptic plasticity and learning, and, not surprisingly, its deregulation can lead to severe cognitive impairments (van Woerden et al., 2007).

In the hippocampus, αCaMKII and βCaMKII form a holoenzyme consisting of ~12 subunits in a 2:1 ratio (Brocke et al., 1999). αCaMKII and βCaMKII are highly homologous, but they are encoded by two distinct genes (*Camk2a* and *Camk2b*, respectively) (Hudmon and Schulman, 2002a). The most noticeable difference between these isoforms is that βCaMKII is able to bind to F-actin in an activity-controlled manner, through its extra domain in the variable region (Shen et al., 1998; Shen and Meyer, 1999). Two βCaMKII subunits per holoenzyme are already sufficient to change the localization of the entire holoenzyme (Shen et al., 1998). Mainly due to these different actin-binding properties, αCaMKII and βCaMKII were shown to have opposing effects on synaptic strength in cultured neurons (Thiagarajan et al., 2002). Interestingly, βCaMKII not only binds to actin, but is also capable of bundling actin in a kinase-independent manner (O'Leary et al., 2006; Okamoto et al., 2007; Sanabria et al., 2009). This nonenzymatic bundling feature is likely achieved by single CaMKII oligomers binding to multiple actin filaments.

Studies addressing the role of βCaMKII in synaptic plasticity and learning have only recently been initiated. Inducible overexpression of βCaMKII in the dentate gyrus did not affect acquisition of hippocampal learning, but did affect the long-term consolidation of memories (Cho et al., 2007). Additionally, it was shown that the absence of βCaMKII reverses the polarity of plasticity at cerebellar parallel fiber–Purkinje cell synapses and causes significant cerebellar learning deficits (van Woerden et al., 2009).

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The reversal of plasticity is caused in part by a nonenzymatic property of βCaMKII, which prevents precocious activation of αCaMKII under low-calcium conditions.

Here we examined the role of βCaMKII in hippocampal synaptic plasticity and learning using two different βCaMKII mutants: (1) the *Camk2b* $^{-/-}$ mouse, which does not express βCaMKII; and (2) the *Camk2b*^{A303R} mouse, where a point mutation blocks calcium/calmodulin binding, selectively preventing its enzymatic activation, while preserving its ability to bind to actin (Shen and Meyer, 1999; Fink et al., 2003; Lin and Redmond, 2008). We found that the absence of βCaMKII causes mislocalization of αCaMKII, impaired hippocampal synaptic plasticity, and impaired hippocampus-dependent learning. In contrast, these phenotypes were not present in the *Camk2b*^{A303R} mutants, arguing that the actin binding and bundling function of βCaMKII governs a major aspect of its synaptic function. These results strongly suggest an essential, but nonenzymatic role for βCaMKII in hippocampal plasticity.

Materials and Methods

Generation of the Camk2b^{A303R} *mutants.* The *Camk2b*^{A303R} targeting construct was generated as follows. The *Camk2b* genomic sequence (ENSMUSG00000057897) was obtained from a public database (Ensembl) and used to design the primers for the targeting constructs. PCR fragments encompassing exon 6–11 using 5′ primer: 5′-GGTACCTGAGGAAGGTGCCAGCTCTGTCCC-3′ and 3′ primer: 5′-GTCGACCAGGGTAGTCACGGTTGTCC-3′ (5.3 kb; exon denotation according to ENSMUST00000019133) and exon 11–12 using 5′ primer: 5′-GCGGCCGCCTGTTAAAGGAATGGTTCTC-3′ and 3′ primer: 5′-ATGCATCTAAAAGGCAGGCAGGATGATCTGC-3′ (6 kb) were amplified using High Fidelity Taq Polymerase (Roche) on ES cell genomic DNA and cloned on either site of a PGK (phosphoglycerate kinase)–Neomycin selection cassette. All exons were sequenced to verify that no mutations were introduced accidentally. Site-directed mutagenesis was used to introduce the point mutation Ala303Arg. For counter selection, a gene encoding diphtheria toxin chain A (DTA) was inserted at the 5′ of the targeting construct. The targeting construct was linearized and electroporated into embryonic day 14 (E14) ES cells (derived from 129P2 mice). Cells were cultured in BRL cell-conditioned medium in the presence of leukemia inhibitory factor. After selection with G418 (200 μg/ml), targeted clones were identified by PCR (long-range PCR from neomycin resistance gene to the region flanking the targeted sequence). A clone with normal karyotype was injected into blastocysts of C57BL/6 mice. Male chimeras were crossed with female C57BL/6 mice (Harlan). The resulting F1 heterozygous mice (in the 129P2-C57BL/6 background) were used to generate F2 homozygous mutants and wild-type littermate controls. These mice, of either sex, were used for all the behavioral and electrophysiological experiments. The experimenter was blind for the genotype, but homozygous mice were easily recognizable by the ataxic gait. Therefore, a second person blind to the genotype also analyzed the data. Mice were housed on a 12 h light/dark cycle with food and water available *ad libitum*, and were used between 2 and 6 months of age for all experiments described (including electrophysiology). All animal procedures were approved by a Dutch Ethical Committee for animal experiments.

Western blot. Lysates were prepared by quick dissection of the brain and by homogenization of the brain tissue in lysis buffer (10 mM TRIS-HCl 6.8, 2.5% SDS, 2 mM EDTA, and protease and phosphatase inhibitor cocktails; Sigma). The concentration of the lysates was adjusted to 1 mg/ml. Ten micrograms was used for Western blot analysis. Western blots were probed with antibodies directed against αCaMKII (MAB3119, 1:10,000; Millipore Bioscience Research Reagents), βCaMKII (CB-β1, 1:10,000; Zymed), Ph-T286/T87 CaMKII antibody (1:5000; catalog #06–881, Millipore Cell Signaling Solutions), and actin (MAB1501R, 1:2000; Millipore Bioscience Research Reagents). Blots were stained using Enhanced ChemoLuminescence (catalog #32106, Pierce). Western blot quantification was performed using NIH-Image.

Immunocytochemistry. Immunocytochemistry was performed on free-floating 40-μm-thick frozen sections using a standard avidin-biotin-immunoperoxidase complex method (ABC, Vector Laboratories) with βCaMKII (CB-b1, 1:2000; Zymed) as the primary antibody and diaminobenzidine (0.05%) as the chromogen (Hansel, 2006). For gross brain morphology, sections were stained with thionin.

Dendritic arborization. Golgi–Cox staining on unfixed hippocampi of three *Camk2b* $^{-/-}$ mutants and three wild-type mice was performed using the FD Rapid GolgiStain Kit (FD NeuroTechnologies), according to the manufacturers' instructions. Sagittal sections, 100 μm thick, were cut on a microtome with cryostat adaptations. Pyramidal cell counting and selection for further detailed analysis was done by two independent observers who were both blind for genotype. A calibration grid was used to count the number of spines per 10 μm, using a 40× objective.

Primary hippocampal cultures and immunohistochemistry. βCaMKII or βCaMKII-A303R heterozygous mice were crossed, and wild-type, heterozygous, and knock-out hippocampal neuron cultures were prepared from brains of single E18 embryos out of mixed genotype litters. Mouse hippocampal neurons were isolated and prepared as described previously (Goslin and Banker, 1991). In short, the two hippocampi were removed from the embryonic brain, collected in 1 ml of DMEM on ice, washed two times with 1 ml of DMEM, and incubated in trypsin/EDTA solution (Invitrogen) at 37°C for 15 min. After washing with 1 ml of DMEM, the cells were resuspended in neurobasal medium (NB) supplemented with 2% B27, 1% penicillin/streptomycin, and 1% glutamax (Invitrogen), and dissociated using a gently flamed Pasteur pipette. Neurons were plated in a small drop on poly-L-lysine- (100 μg/ml, Sigma) and laminin-coated (50 μg/ml, Sigma) 15 mm glass coverslips at a density of 75,000 per coverslip in 12 well plates. After 2 h, 1 ml of NB supplemented with 2% B27, 1% penicillin/streptomycin, and 1% glutamax was added to the coverslips. Neuronal activity was suppressed by treating the neuronal cultures with TTX for 24 h (2 μM, Sigma).

For immunohistochemistry, two [guanylate kinase domain-associated protein (GKAP)] or three (bassoon) independent cultures of 14 d *in vitro* (DIV14) neurons were fixed for 10 min with 4% formaldehyde/4% sucrose in PBS at room temperature (Jaworski et al., 2009). After fixation, the cells were washed two times in PBS for 30 min at room temperature, and were incubated with primary antibodies in GDB buffer (0.2% BSA, 0.8 M NaCl, 0.5% Triton X-100, 30 mM phosphate buffer, pH 7.4) overnight at 4°C. The following primary antibodies were used: rabbit anti-bassoon (Synaptic Systems), rabbit anti-GKAP (Santa Cruz Biotechnology), and mouse anti-aCaMKII (Sigma). Phalloidin (Invitrogen) was used to stain filamentous actin (F-actin). Neurons were then washed three times in PBS for 30 min at room temperature and incubated with Alexa488- and Alexa568-conjugated secondary antibodies (Invitrogen) in GDB for 2 h at room temperature, and washed three times in PBS for 30 min. Slides were mounted using Vectashield mounting medium (Vector Laboratories). Confocal images were acquired using a LSM510 confocal microscope (Zeiss) with a 40× or 63× oil objective.

For quantification, confocal images of four to five neurons obtained from two to three independent experiments were obtained, with sequential acquisition settings at the maximal resolution of the microscope (1024 × 1024 pixels). Each image was a *z*-series of six to eight images each averaged two times was chosen to cover the entire region of interest from top to bottom. The resulting *z*-stack was "flattened" into a single image using maximum projection. Images were not further processed and were of similar high quality to the original single planes. The confocal settings were kept the same for all scans when fluorescence intensity was compared. Morphometric analysis, quantification, and colocalization were performed using MetaMorph software (Universal Imaging Corporation). The ratio of αCaMKII in the synapse over αCaMKII in the shaft was calculated by measuring the average intensity of the fluorescent αCaMKII signal in the synapses and the shaft.

Fear conditioning. Fear conditioning was performed in a conditioning chamber (Medical Associates) equipped with a grid floor via which the footshock could be administered. Each mouse was placed inside the conditioning chamber for 180 s. A footshock (2 s, 0.4 mA) was delivered 148 s after placement in the chamber. Twenty-four hours later, context-dependent freezing was measured during 3 min.

Electrophysiology. After the animals had been killed, sagittal slices (400 μm) were obtained and submerged in ice-cold artificial CSF (ACSF)

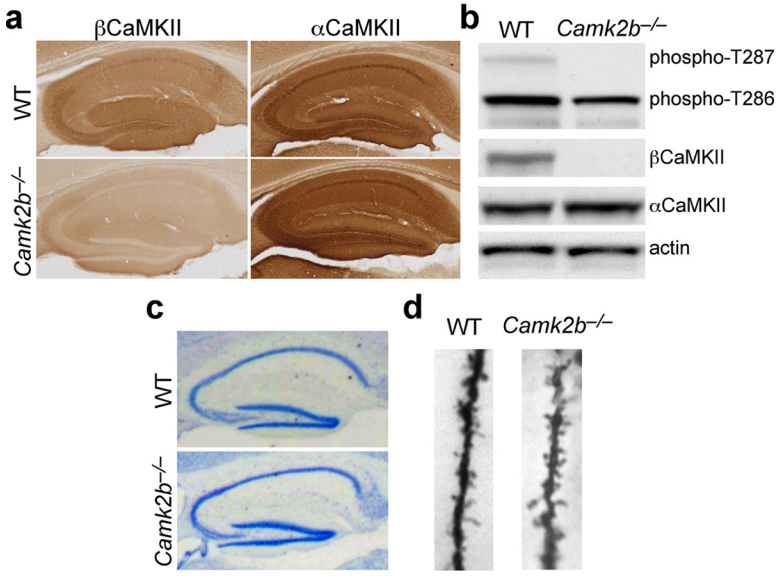

Figure 1. Morphological and molecular analysis of the *Camk2b* $^{-/-}$ mice. ***a***, Immunocytochemistry analysis using αCaMKII- and βCaMKII-specific antibodies shows complete absence of βCaMKII in the hippocampus, with no apparent change in αCaMKII expression. ***b***, Western blot analysis using αCaMKII- and βCaMKII-specific antibodies reveals no change in the levels of αCaMKII protein or in the levels of αCaMKII-T286 phosphorylation. In contrast, βCaMKII protein and βCaMKII-T287 phosphorylation are completely absent. ***c***, Thionin staining shows no apparent morphological change in the hippocampus of *Camk2b* $^{-/-}$ mice compared with wild-type mice. ***d***, Quantification of Golgi analysis of the hippocampal pyramidal cells does not reveal any difference in spine density.

using a vibratome, and hippocampi were dissected out. These sagittal hippocampal slices were maintained at room temperature for at least 1.5 h to recover before experiments were initiated. Then they were placed in a submerged recording chamber and perfused continuously at a rate of 2 ml/min with ACSF equilibrated with 95% O_2, 5% CO_2 at 31°C. ACSF contained the following (in mM): 120 NaCl, 3.5 KCl, 2.5 $CaCl_2$, 1.3 $MgSO_4$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, and 10 D-glucose. Extracellular recordings of field EPSPs (fEPSPs) were made in CA1 stratum radiatum with platinum (Pt)/iridium (Ir) electrodes (Frederick Haer Company). A bipolar Pt/Ir was used to stimulate Schaffer collateral/commissural afferents with a stimulus duration of 100 μs. Stimulus–response curves were obtained at the beginning of each experiment, 20 min after placing the electrodes. Long-term potentiation (LTP) was evoked using the following two different tetani: (1) 100 Hz (1 train of 1 s at 100 Hz); and (2) 200 Hz (4 trains of 0.5 s, spaced by 5 s). Both protocols were performed at one-third of the maximum fEPSP. fEPSP measurements were done once per minute. Potentiation was measured as the normalized increase of the mean fEPSP slope for the duration of the baseline. Only stable recordings were included, and this judgment was made blind to genotype. Average LTP was defined as the mean last 10 min of the normalized fEPSP slope.

Statistical analysis. All data are presented as means ± SEM and were tested for normality of distribution using the D'Agostino–Pearson test. If normality of distribution was violated or sample size was too small to determine normality, we used the Mann–Whitney *U* test. In all other cases, an appropriate *t* test was used to analyze differences between genotypes for spine density, freezing time, and LTP induction (based on the average of the last 10 min). A repeated-measures ANOVA was used to analyze differences between genotypes for fiber volley and fEPSP slope, even when in some cases the distribution was not normal because there is no nonparametric alternative for the repeated-measures ANOVA.

Results

Camk2b $^{-/-}$ mice show normal hippocampal morphology and no change in αCaMKII protein levels and autophosphorylation

Generation of the *Camk2b* $^{-/-}$ mouse has been described previously (van Woerden et al., 2009). Using immunohistochemistry and Western blot, we confirmed the absence of βCaMKII in the hippocampus of the *Camk2b* $^{-/-}$ mouse (Fig. 1*a*,*b*). Since *in vitro* experiments showed that upregulation of βCaMKII causes downregulation of αCaMKII (Thiagarajan et al., 2002), we tested whether the absence of βCaMKII caused upregulation of αCaMKII *in vivo*. However, we did not observe a change in αCaMKII protein levels (wild-type mice: 100 ± 5.4, $n = 6$; *Camk2b* $^{-/-}$ mice: 91.5 ± 6.3, $n = 7$; Mann–Whitney *U* test, $U = 13.00$, $p = 0.29$) (Fig. 1*b*), nor was there a significant change in basal levels of αCaMKII Thr^{286} phosphorylation (wild-type mice:100 ± 17.1 $n = 6$; *Camk2b* $^{-/-}$ mice: 92.7 ± 13.9 $n = 7$; Mann–Whitney *U* test, $U = 20.00$, $p = 0.95$) (Fig. 1*b*). These data show that in *Camk2b* $^{-/-}$ mice βCaMKII is absent and that protein expression and basal levels of autophosphorylation of αCaMKII are unaltered.

Previous studies showed that upregulation or downregulation of βCaMKII *in vitro* caused respectively an increased or decreased dendritic arborization, suggesting that βCaMKII might be critical for normal dendritic development *in vivo* (Fink et al., 2003). Therefore, we performed a detailed examination of the hippocampus using thionin staining. However, we found no evidence of significant changes in hippocampal structure at the light microscopy level (Fig. 1*c*). Furthermore, we investigated the morphology of the CA1 pyramidal cells using Golgi–Cox staining. We found no significant change in the density of spines (wild-type mice: 6.35 ± 0.36 $n =$ 15 cells from 3 mice; *Camk2b* $^{-/-}$ mice: 7.07 ± 0.61 $n = 15$ from 3 mice; unpaired two-tailed *t* test, $t_{(28)} = 1.8$, $p = 0.09$) (Fig. 1*d*), which is consistent with our previous findings for the cerebellum (van Woerden et al., 2009). Together, these data show that gross neuronal development is preserved in *Camk2b* $^{-/-}$ mice.

αCaMKII is mislocalized in *Camk2b* $^{-/-}$ neurons

Since βCaMKII is able to bind F-actin in an activity-controlled manner (Shen et al., 1998; Shen and Meyer, 1999) and pharmacologically induced changes in actin bundling have a large effect on CaMKII delivery in spines (Allison et al., 2000; Okamoto et al., 2004), it is possible that βCaMKII can change actin dynamics and the localization of the CaMKII holoenzyme. Therefore, we hypothesized that absence of βCaMKII might result in abnormalities in synaptic localization of αCaMKII. However, using brain sections for quantitative analysis of changes in localization of αCaMKII is challenging, because of its ubiquitous distribution, and its ability to self-aggregate and redistribute depending on multiple factors including fixation conditions (Tao-Cheng et al., 2002). Hence, we investigated the localization of endogenous αCaMKII by immunostaining of neuronal cultures obtained from E18 hippocampal neurons from *Camk2b* $^{-/-}$ and wild-type mice. Neurons were fixed at DIV14 and stained with an antibody against bassoon (a marker for the presynaptic active zone), GKAP (a marker for the postsynaptic density), phalloidin (which stains F-actin), and an antibody against αCaMKII (Fig. 2). Whereas αCaMKII showed a striking synaptic labeling in wild-type neurons, this was clearly less pronounced in the *Camk2b* $^{-/-}$-derived neurons. To quantify this, we measured the ratio of αCaMKII in

the synapse (colocalizing with bassoon or GKAP) to αCaMKII in the dendritic shaft, and found that this ratio was reduced by >40% in the *Camk2b*$^{-/-}$ neurons when measured over bassoon ($\alpha CaMKII_{synapse}/\alpha CaMKII_{shaft}$ ratio: wild-type neurons, 3.3 ± 0.2, $n = 15$; *Camk2b*$^{-/-}$ neurons, 2.1 ± 0.2, $n = 14$; Mann–Whitney U test, $U = 17.00$, $p = 0.0002$) (Fig. 2*a*), and by 60% when measured over GKAP ($\alpha CaMKII_{spine}/\alpha CaMKII_{shaft}$ ratio: wild-type neurons, 3.42 ± 0.16, $n = 10$; *Camk2b*$^{-/-}$ neurons, 1.50 ± 0.14 $n = 10$; Mann–Whitney U test, U = 8.00, $p = 0.001$) (Fig. 2*b*). This distinct difference was still observed after decreasing or increasing neuronal activity of *Camk2b*$^{-/-}$ neuronal cultures with tetrodotoxin or bicuculline for 24 h, as neither treatment changed the αCaMKII localization significantly ($\alpha CaMKII_{synapse}/\alpha CaMKII_{shaft}$ ratio: control medium, 1.66 ± 0.19, $n = 5$; tetrodotoxin, 1.74 ± 0.18, $n = 5$; bicuculline, 1.80 ± 0.13, $n = 6$; ANOVA $F_{(2,13)} = 0.05$; $p = 0.96$). This indicates that the changes were not due to differences in spontaneous activity between the cultures. In addition, even though the cultures were derived from E18 neurons, the ratio of αCaMKII/βCaMKII in wild-type DIV14 cultures was comparable to the ratio observed in the adult mouse hippocampus (Fig. 2*d*). Together, these findings highlight the importance of βCaMKII in regulating the distribution of endogenous αCaMKII between the synaptic pool and the adjacent dendritic shaft.

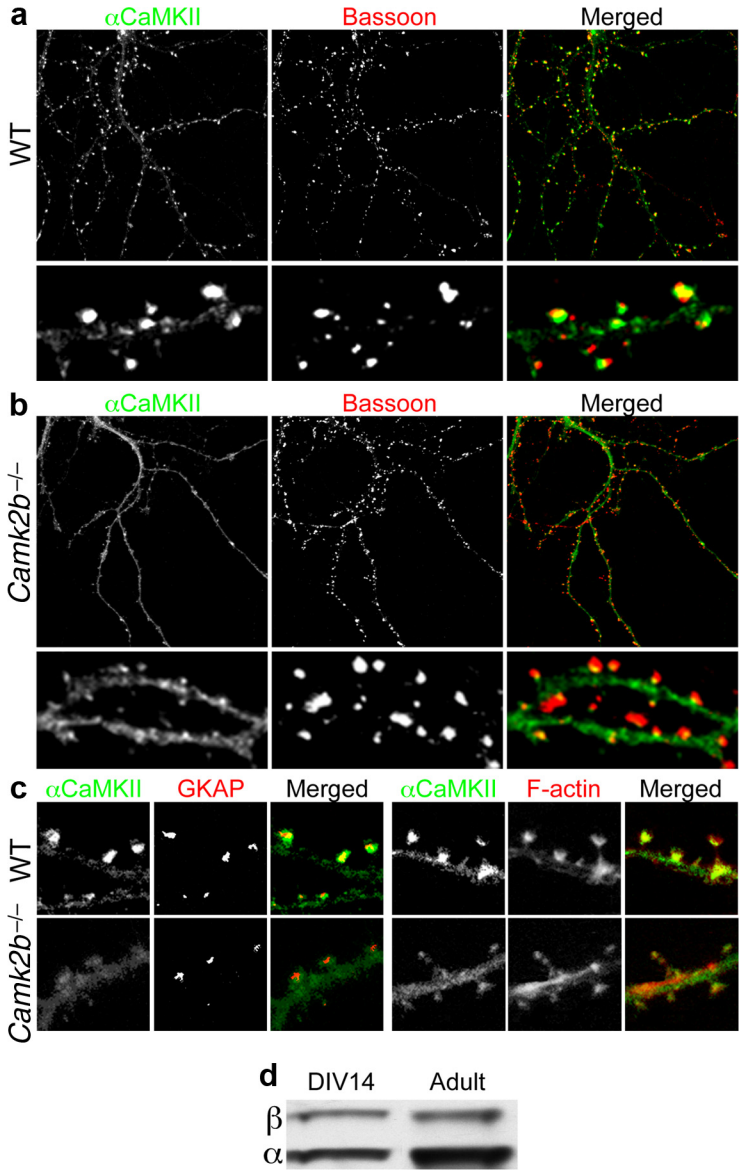

Figure 2. Impaired αCaMKII targeting to dendritic spines in *Camk2b*$^{-/-}$ mice. ***a***, Representative images of cultured wild-type hippocampal neurons (top), and an enlargement of a dendritic segment (bottom), labeled with αCaMKII (green) and bassoon (red), and their colocalization (merged). ***b***, Representative images of cultured *Camk2b*$^{-/-}$ hippocampal neurons (top), and an enlargement of a dendritic segment (bottom), labeled with αCaMKII (green) and bassoon (red), and their colocalization (merged), showing reduced targeting of αCaMKII to synapses in *Camk2b*$^{-/-}$ hippocampal neurons. ***c***, Representative images of wild-type (top row) and *Camk2b*$^{-/-}$ (bottom row) hippocampal dendritic segments, labeled with αCaMKII (green) and GKAP (red), and their colocalization (merged) (left) and labeled with αCaMKII (green) and F-actin (phalloidin staining) (red), and their colocalization (merged) (right), showing reduced targeting of αCaMKII to spines in *Camk2b*$^{-/-}$ hippocampal neurons. ***d***, Western blot shows comparable ratio of αCaMKII (α) to βCaMKII (β) in E18-derived wild-type cultured neurons on DIV14 compared with a lysate from an adult wild-type mouse.

Camk2b$^{-/-}$ mice show impaired LTP

Given that overall neuronal morphology was unaffected in *Camk2b*$^{-/-}$ mutants with an impaired localization of αCaMKII into dendritic spines, we examined the functional implications by investigating synaptic plasticity at the hippocampal CA1 synapse. Using extracellular recordings in acute hippocampal slices, we focused on the Schaffer–collateral pathway given the large literature implicating this synapse in many forms of hippocampus-dependent learning and memory. No significant impairment in basal synaptic transmission was observed in the *Camk2b*$^{-/-}$ mice (Fig. 3*a*), with significant changes in neither fiber volley (repeated-measures ANOVA, $F_{(1,135)} = 0.22$; $p = 0.64$) nor fEPSP slope (repeated-measures ANOVA, $F_{(1,135)} = 1.4$; $p = 0.23$). However, we found a significant deficit in LTP in *Camk2b*$^{-/-}$ mutants compared with wild-type littermates (wild-type mice, 152.6 ± 7.7, $n = 27$; *Camk2b*$^{-/-}$ mice, 119.3 ± 4.4, $n = 21$; Mann–Whitney U test, $U = 130.00$, $p = 0.0015$) (Fig. 3*b*), indicating that βCaMKII plays an essential role in synaptic plasticity at the hippocampal Schaffer–collateral pathway. Notably, the LTP deficit in the *Camk2b*$^{-/-}$ mice induced by a 100 Hz/1 s tetanus, is as severe as after the loss of the far more abundant αCaMKII (Elgersma et al., 2002). Moreover, it should be noted that hippocampal LTP is unaffected in the heterozygous *Camk2a* mutant (Frankland et al., 2001; Elgersma et al., 2002), which shows a larger decrease in total CaMKII level compared with the homozygous *Camk2b* mutant. This indicates that the LTP deficit of the *Camk2b*$^{-/-}$ mice cannot only be explained in terms of loss of CaMKII activity.

It has previously been shown that changes in the F-actin/G-actin equilibrium affect αCaMKII localization (Allison et al., 2000; Okamoto et al., 2004) and that stimulation of NMDA receptors affects CaMKII localization as well as the F-actin/G-actin equilibrium (Shen and Meyer, 1999; Okamoto et al., 2004). Together, these findings support a model in which CA1 LTP is

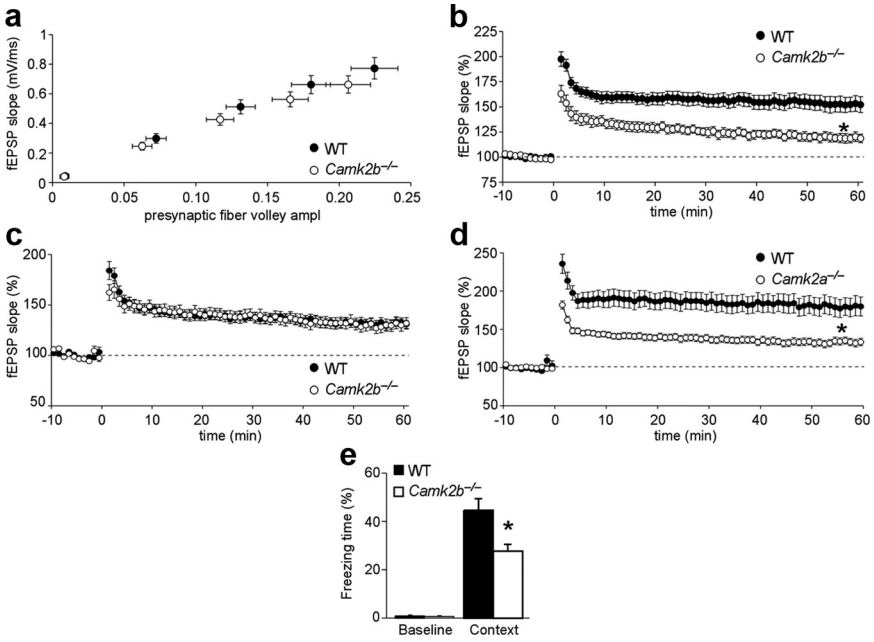

Figure 3. Hippocampal plasticity and learning in *Camk2b* $^{-/-}$ mice. ***a***, *Camk2b* $^{-/-}$ mice show normal synaptic transmission (wild-type, $n = 74$; *Camk2b* $^{-/-}$, $n = 63$). ***b***, *Camk2b* $^{-/-}$ mice show impaired 100 Hz LTP (wild-type, $n = 27$; *Camk2b* $^{-/-}$, $n = 21$). ***c***, *Camk2b* $^{-/-}$ mice show normal 200 Hz LTP (wild-type, $n = 14$; *Camk2b* $^{-/-}$, $n = 12$). ***d***, The 200 Hz LTP is dependent on αCaMKII, as *Camk2a* $^{-/-}$ mice show impaired 200 Hz LTP (wild-type, $n = 12$; *Camk2a* $^{-/-}$, $n = 18$). ***e***, Impaired contextual fear conditioning in *Camk2b* $^{-/-}$ mice. Percentage of time spent freezing during training before the footshock (Pre) and 24 h after conditioning (Post) indicates reduced postshock freezing, while preshock freezing is normal (wild-type, $n = 10$; *Camk2b* $^{-/-}$, $n = 10$). Error bars represent SEM; asterisks indicate a significant difference between mutant and control.

highly dependent upon proper targeting of αCaMKII into spines, for which βCaMKII is required. If the LTP deficit is due to mislocalization of αCaMKII, it is conceivable that the LTP deficit can be rescued by using a stronger LTP-inducing protocol (4 trains of 200 Hz for 0.5 s, spaced 5 s apart) (Grover and Teyler, 1990). Indeed, using this stimulation protocol, LTP is normal in *Camk2b* $^{-/-}$ mice, confirming the functional implications of the altered CaMKII localization (wild-type mice, 131.6 ± 4.4, $n = 14$; *Camk2b* $^{-/-}$ mice, 129.7 ± 5.7, $n = 12$; unpaired two-tailed *t* test, $t_{(24)} = 0.36$, $p = 0.72$) (Fig. 3*c*). To confirm that this 200 Hz LTP protocol requires αCaMKII, we also performed this experiment in *Camk2a* $^{-/-}$ mice. Indeed, *Camk2a* $^{-/-}$ mice show a significant impairment (wild-type mice, 179.6 ± 13.3, $n = 12$; *Camk2a* $^{-/-}$, 133.3 ± 4.8, $n = 18$; Mann–Whitney *U* test, $U = 36.00$, $p = 0.0025$) (Fig. 3*d*), indicating that this LTP protocol is indeed dependent on CaMKII activity. Together, these results show that the LTP deficit in the *Camk2b* $^{-/-}$ mice can be overcome by a strong LTP-inducing protocol. These findings are consistent with, but not a proof of, the hypothesis that the deficit in hippocampal synaptic plasticity is a result of a failure to properly target αCaMKII into dendritic spines of the *Camk2b* $^{-/-}$ mouse.

Camk2b $^{-/-}$ mice show impaired hippocampus-dependent learning

To test whether the mislocalization of CaMKII in the *Camk2b* $^{-/-}$ mouse also affected hippocampal learning, we made use of contextual fear conditioning. In this task, mice are conditioned to associate a certain context with a mild, aversive footshock. Learning is assessed by measuring freezing behavior (i.e., the cessation of all movement except respiration), which is a natural expression of fear in mice. *Camk2b* $^{-/-}$ mutants did not differ from their wild-type littermates in preshock baseline freezing behavior (wild-type mice, 0.9 ± 0.2%, $n = 11$; *Camk2b* $^{-/-}$ mice, 0.7 ± 0.2%, $n = 9$; two-tailed *t* test, $t_{(18)} = 0.72$, $p = 0.48$) (Fig. 3*e*), but showed significantly less freezing in the 24 h long-term memory test, demonstrating an impairment of hippocampus-dependent memory (wild-type mice, 44.8 ± 4.6%, $n = 11$; *Camk2b* $^{-/-}$ mice, 27.8 ± 2.7%, $n = 9$; two-tailed *t* test, $t_{(18)} = 3.0$, $p = 0.0079$) (Fig. 3*e*).

Generation and characterization of the *Camk2b* ^{A303R} mouse

The results above suggest that βCaMKII strongly influences hippocampal plasticity by regulating αCaMKII localization into dendritic spines. However, the affinity of βCaMKII for calcium/calmodulin is nearly 10-fold higher than αCaMKII, and the sensitivity range of the heteromeric holoenzyme is dependent on the ratio of α to β subunits (De Koninck and Schulman, 1998; Brocke et al., 1999). Hence, we cannot rule out that the deficits in CA1 LTP and hippocampus-dependent learning are caused by the loss of the enzymatic activity of βCaMKII, rather than the abnormal localization of αCaMKII. Therefore, we sought to distinguish between these possibilities using a well described mutation of βCaMKII (A303R), which prevents kinase activation (by interfering with calcium/calmodulin binding) while preserving F-actin binding and bundling (Shen and Meyer, 1999; Fink et al., 2003; O'Leary et al., 2006). In addition, the βCaMKII-A303R protein does not show a dominant-negative effect on dendritic arborization *in vitro*, in contrast to cells expressing the catalytically dead βCaMKII-K42R protein, which cannot bind ATP (Fink et al., 2003). Accordingly, this mutation elegantly permits us to dissect the requirement of βCaMKII kinase activity and F-actin bundling on LTP and learning.

We created a knock-in mutant of the *Camk2b* gene, substituting alanine ^{303} for arginine (A303R) (Fig. 4*a*,*b*). Immunostaining and Western blot analysis of brains of homozygous point mutants (designated as *Camk2b* ^{A303R} mice) revealed no change in expression of βCaMKII and αCaMKII in the *Camk2b* ^{A303R} mouse (Fig. 4*c*,*d*). Since binding of calcium/calmodulin is a prerequisite for autophosphorylation of CaMKII at Thr286/287 (for review, see Hudmon and Schulman, 2002b; Lisman et al., 2002; Colbran, 2004), we used the phospho-Thr286/287 antibody to confirm that this mutation renders the βCaMKII-A303R protein insensitive to calcium/calmodulin activation. Indeed, Western blot analysis showed that βCaMKII Thr ^{287} phosphorylation was entirely absent in the *Camk2b* ^{A303R} mouse (Fig. 4*d*), confirming *in vitro* studies that the A303R mutation blocks activation of βCaMKII. We also observed a significant reduction of αCaMKII Thr ^{286} autophosphorylation (wild-type mice, 100 ± 7.2, $n = 7$; *Camk2b* ^{A303R} mice, 69.9 ± 8.3, $n = 5$; Mann–Whitney *U* test, $U = 4.0$, $p = 0.03$) (Fig. 4*d*), which is not unexpected given that most of the αCaMKII subunits are associated with βCaMKII subunits, and that it takes two adjacent activated CaMKII subunits to get inter-subunit autophosphorylation at Thr286/Thr287 (Hudmon and Schulman, 2002b; Lisman et al., 2002; Colbran, 2004). Thionin staining did not reveal any gross morphological changes in

the brain (Fig. 4*e*), indicating that development of the brain is normal despite the presence of an inactive form of βCaMKII.

αCaMKII shows normal subcellular distribution in *Camk2b*^{A303R} neurons

To directly examine the influence of βCaMKII on the localization of αCaMKII, we tested whether synaptic targeting of αCaMKII was altered in neurons of the *Camk2b*^{A303R} mice. Remarkably, and in strong contrast to the findings in *Camk2b*$^{-/-}$ neurons, *Camk2b*^{A303R} neurons showed normal αCaMKII synaptic localization ($\alpha CaMKII_{synapse}/\alpha CaMKII_{shaft}$: wild-type neurons, 4.2 ± 0.7, $n = 6$; *Camk2b*^{A303R} neurons, 3.9 ± 0.6, $n = 7$; Mann–Whitney U test, $U = 14.00$, $p = 0.37$) (Fig. 5). These results demonstrate that βCaMKII protein but not its calcium/calmodulin-dependent activation is required for targeting αCaMKII to synapses.

The *Camk2b*^{A303R} mice show normal LTP

Camk2b^{A303R} mice retain normal synaptic localization of αCaMKII, despite a complete abrogation of calcium/calmodulin-dependent kinase activity of βCaMKII. This provides a unique opportunity to dissect the mechanism by which βCaMKII influences synaptic plasticity and learning.

As observed in *Camk2b*$^{-/-}$ mice (Fig. 3*a*), extracellular recordings in acute hippocampal slices of *Camk2b*^{A303R} mice showed a slight, but not significant, reduction in basal synaptic transmission (basal synaptic transmission: repeated-measures ANOVA fiber volley, $F_{(1,46)} = 0.44$, $p = 0.51$; fEPSP slope, $F_{(1,46)} = 0.93$; $p = 0.34$; $n = 25$ and 23, respectively, for wild-type and *Camk2b*^{A303R} mice) (Fig. 6*a*). However, in contrast to the *Camk2b*$^{-/-}$ mice, which have a severe deficit in 100 Hz LTP, *Camk2b*^{A303R} mice showed normal 100 Hz LTP (wild-type mice, 149.7 ± 9.2, $n = 15$; *Camk2b*^{A303R} mice, 133.4 ± 7.5, $n = 10$; two-tailed t test, $t_{(23)} = 1.40$, $p = 0.17$) (Fig. 6*b*). These results indicate that the kinase activity of βCaMKII is dispensable for hippocampal synaptic plasticity and suggest that βCaMKII functions principally to regulate the targeting of αCaMKII into dendritic spines.

Contextual fear learning of the *Camk2b*^{A303R} mice

Given that *Camk2b*$^{-/-}$ mice showed a significant deficit in contextual fear conditioning (Fig. 3*e*), the results from *Camk2b*^{A303R} mice provide an opportunity to determine the mechanism of βCaMKII function in hippocampus-dependent learning. *Camk2b*^{A303R} mice showed indistinguishable freezing behavior from their wild-type littermates (wild-type mice, 62.6 ± 4.1, $n = 13$; *Camk2b*^{A303R} mice, 53.5 ± 5.0 $n = 11$; two-tailed t test, $t_{(22)} = 1.42$, $p = 0.17$) (Fig. 6*c*) without any change in baseline freezing behavior (wild-type mice, 3.5 ± 0.8, $n = 13$; *Camk2b*^{A303R} mice, 4.6 ± 1.6, $n = 11$; Mann–Whitney U test, $U = 65.00$, $p = 0.73$) (Fig. 6*c*). Hence, together with the results obtained using *Camk2b*$^{-/-}$ mice, these results suggest that βCaMKII-dependent localization of αCaMKII is required for normal hippocampus-dependent learning.

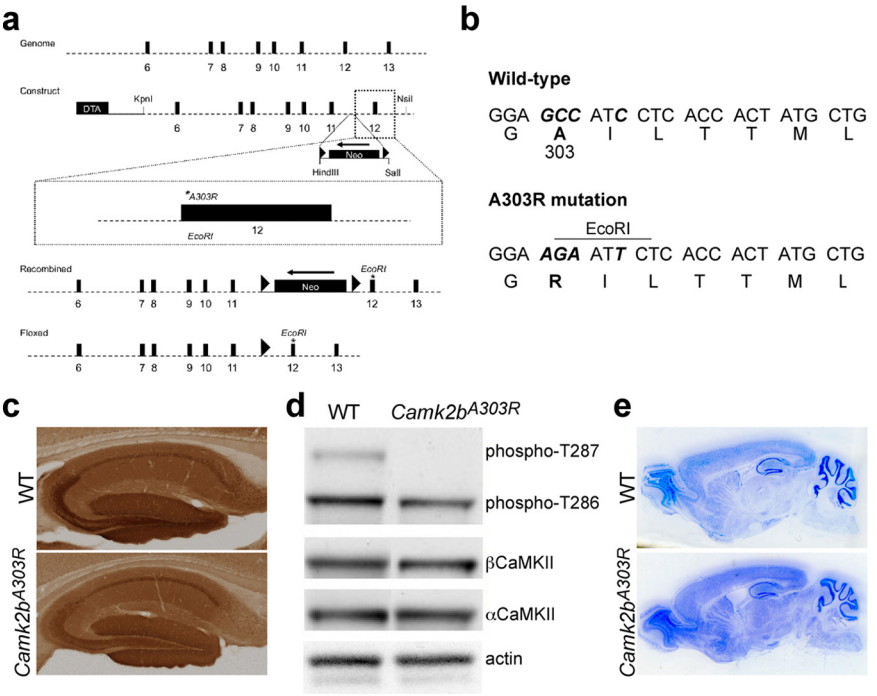

Figure 4. Generation of *Camk2b*^{A303R} mice. ***a***, Schematic diagram for the generation of the *Camk2b*^{A303R} mutants. Genome, Wild-type *Camk2b* locus with the exons around Ala303 depicted as black boxes; Construct, targeting construct used for introducing the mutation in Ala303. The asterisk in exon 12 indicates the mutation in Ala303. The LoxP sites flanking the neomycin gene are depicted as triangles. The DTA was cloned in the construct for positive selection. Recombined, Mutant *Camk2b*^{A303R} locus after homologous recombination in ES cells; Floxed, mutant *Camk2b*^{A303R} locus after Cre recombination. ***b***, Sequence of Ala303 in exon 12 showing the specific mutation made to induce Ala303Arg. ***c***, Immunocytochemistry staining using an antibody specific for βCaMKII shows no difference in βCaMKII staining in the hippocampus of *Camk2b*^{A303R} mice (bottom) compared with wild-type mice (top). ***d***, Western blot analysis using antibodies specific for αCaMKII and βCaMKII shows no difference in the levels of αCaMKII and βCaMKII in the hippocampus of the *Camk2b*^{A303R} mice. Western blot analysis using an antibody specific for detecting the phosphorylation levels of αCaMKII-T286 and βCaMKII-T287 reveals a slight reduction of Thr286 phosphorylation, whereas the Thr287 phosphorylation is completely absent in the hippocampus of the *Camk2b*^{A303R} mice. ***e***, Thionin staining showed no gross morphological difference in the brains of *Camk2b*^{A303R} mice.

Discussion

Through a genetic dissection in mice, we have identified that βCaMKII functions at the Schaffer collateral–CA1 synapse principally to target αCaMKII into dendritic spines. We found that the complete loss of βCaMKII protein leads to mislocalization of αCaMKII in cultured neurons. Furthermore, we found that loss of βCaMKII severely impairs hippocampal learning and synaptic plasticity. In contrast, the βCaMKII-A303R mutant provided an elegant dissection of βCaMKII function, since it fully retains F-actin binding despite a loss of calcium/calmodulin-dependent kinase activity. Indeed, *Camk2b*^{A303R} mice showed proper αCaMKII localization, robust LTP, and normal learning. Since we found that the subcellular localization of αCaMKII is changed only in the absence of βCaMKII, and that the LTP deficit in the absence of βCaMKII can be overcome by using a strong LTP-inducing protocol, our data support a role of βCaMKII, in which it regulates the targeting of αCaMKII into spines. Given the extensive literature showing that βCaMKII has F-actin binding and bundling properties, which are fully conserved in the βCaMKII-A303R mutant, we have concluded that the synaptic plasticity and learning deficits observed in the *Camk2b*$^{-/-}$ mutants result from the impaired βCaMKII-dependent targeting of αCaMKII into spines.

βCaMKII is not required for neuronal development

Considering the developmentally earlier expression of βCaMKII compared with αCaMKII (Sahyoun et al., 1985; Bayer et al.,

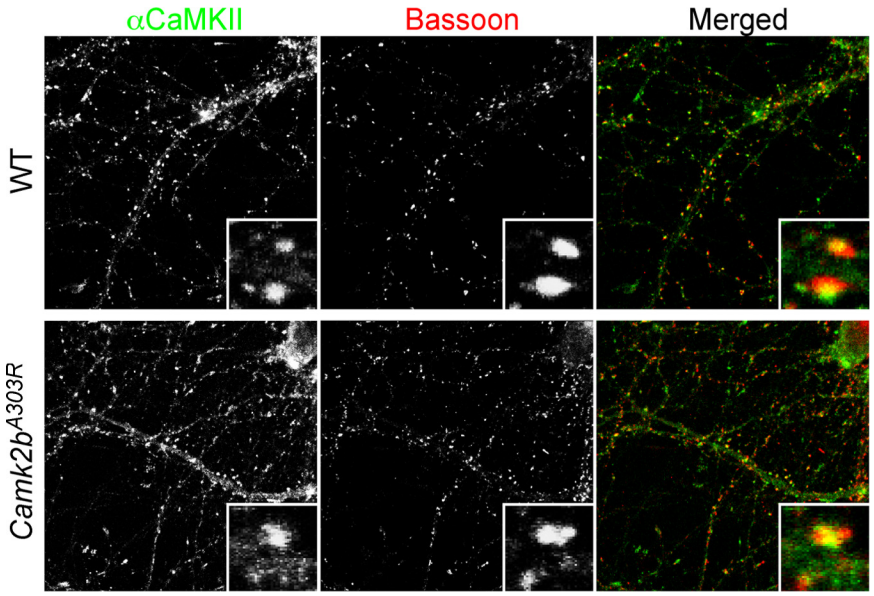

Figure 5. Normal distribution of αCaMKII in cultured neurons of *Camk2b*^{A303R} mice. Representative images of cultured wild-type and *Camk2b*^{A303R} hippocampal neurons labeled with αCaMKII (green) and bassoon (red), and their colocalization (merged) showing normal distribution of αCaMKII in *Camk2b*^{A303R} hippocampal neurons. Inserts show an enlargement of a dendritic segment with two spines.

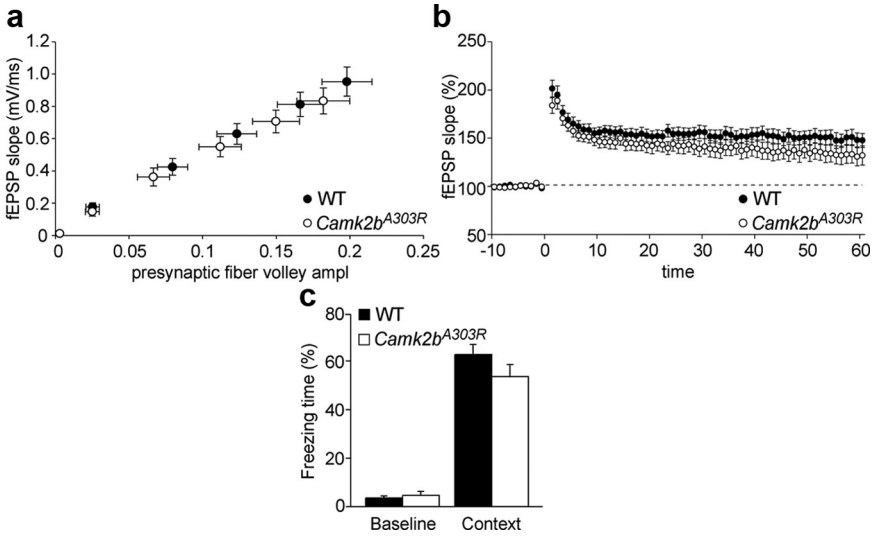

Figure 6. Normal hippocampal synaptic plasticity and learning of *Camk2b*^{A303R} mice. ***a***, *Camk2b*^{A303R} mice show normal synaptic transmission (wild-type, $n = 68$; *Camk2b*^{A303R}, $n = 58$). ***b***, *Camk2b*^{A303R} mice show normal 100 Hz LTP (wild-type, $n = 28$; *Camk2b*^{A303R}, $n = 19$). ***c***, *Camk2b*^{A303R} mice show normal contextual fear conditioning. Percentage of time spent freezing during training before the footshock (Pre) and 24 h after conditioning (Post), showing normal learning in *Camk2b*^{A303R} mice (wild-type, $n = 14$; *Camk2b*^{A303R}, $n = 6$). Error bars represent SEM.

1999) and the observation that knockdown or overexpression of βCaMKII affects synapse number and neurite outgrowth in cultured hippocampal neurons or neuroblastoma cells (Nomura et al., 1997; Fink et al., 2003), a large effect on hippocampal development could be expected. However, we found that neither the absence nor the inactivation of βCaMKII affects brain development at the light microscopic level. Hence, we conclude that βCaMKII is not required for normal brain development and for normal spine number of CA1 pyramidal neurons. This is similar to previous findings in the cerebellum (van Woerden et al., 2009). However, although our data show that βCaMKII is dispensable for normal brain development, we cannot rule out that germ line mutations in βCaMKII result in homeostatic compensatory mechanisms to prevent the changes seen in neuronal cultures shortly after the expression of βCaMKII was changed.

βCaMKII-dependent hippocampal synaptic plasticity

We showed that βCaMKII is required for normal hippocampal NMDA receptor-dependent plasticity as well as learning. Notably, despite the fact that βCaMKII has a 10-fold higher affinity for calcium/calmodulin compared with αCaMKII, the impairment in NMDA receptor-dependent LTP in *Camk2b*$^{-/-}$ mice cannot be explained by a reduction in calcium/calmodulin sensitivity, since LTP deficits were not observed in the *Camk2b*^{A303R} mice, in which βCaMKII has a negligible affinity for calcium/calmodulin. Rather, the deficit in NMDA-dependent LTP is most likely caused by the reduced synaptic localization of αCaMKII, as our data show that the subcellular localization of αCaMKII is strongly influenced by βCaMKII. Specifically, there is approximately a 50% reduction in the αCaMKII spine/shaft ratio in *Camk2b*$^{-/-}$ neurons, whereas the localization of αCaMKII in *Camk2b*^{A303R} neurons is indistinguishable from wild-type mice. Hence, βCaMKII functions independently of its calcium-dependent kinase activity to regulate the synaptic localization of αCaMKII.

We realize that our study has some limitations. First, to be able to quantify the subcellular localization of αCaMKII we had to revert to (*ex vivo*) dissociated neuronal cultures obtained from the mutant mice, which is the commonly used technique to study CaMKII localization. Whether the observed changes also hold true *in vivo* remains to be shown. In addition, our localization studies cannot distinguish between presynaptic and postsynaptic αCaMKII. However, given the relative abundance of postsynaptic αCaMKII, and the important role of postsynaptic αCaMKII in LTP, we believe that our findings strongly suggest that βCaMKII is particularly important for targeting postsynaptic CaMKII. Last, although the observed reduction of synaptic αCaMKII is likely underlying the NMDA-receptor-dependent LTP deficit, we cannot rule out that the loss of βCaMKII also affects other synaptic processes associated with the βCaMKII-dependent actin binding and bundling properties. But regardless of the precise nature of the deficits, it is interesting to note that the plasticity deficits can be overcome by a very strong LTP-inducing protocol consisting of four trains of 200 Hz (Grover and Teyler, 1990). Possibly, this protocol is able to activate the pool of αCaMKII, which is present in the shaft. In this respect, it is interesting to note that a recent study showed that voltage-gated calcium channels (VGCCs) are able to activate a different pool of αCaMKII than NMDA receptors, with a stronger activation of αCaMKII in

dendritic shafts compared with αCaMKII in spines (Lee et al., 2009). However, whether activation of VGCCs is responsible for the LTP rescue remains to be investigated.

Together, our data show that βCaMKII is essential for hippocampus-dependent learning and for normal plasticity at the Schaffer collateral–CA1 synapse. Despite the fact that βCaMKII has a higher affinity for calcium/calmodulin compared with αCaMKII, we found that the calcium/calmodulin-dependent activation of βCaMKII was fully dispensable for hippocampal LTP and learning. Rather, our data show that βCaMKII in hippocampal pyramidal neurons plays a structural role, which serves to target αCaMKII to synapses.

References

Allison DW, Chervin AS, Gelfand VI, Craig AM (2000) Postsynaptic scaffolds of excitatory and inhibitory synapses in hippocampal neurons: maintenance of core components independent of actin filaments and microtubules. J Neurosci 20:4545–4554.

Bayer KU, Löhler J, Schulman H, Harbers K (1999) Developmental expression of the CaM kinase II isoforms: ubiquitous gamma- and delta-CaM kinase II are the early isoforms and most abundant in the developing nervous system. Brain Res Mol Brain Res 70:147–154.

Brocke L, Chiang LW, Wagner PD, Schulman H (1999) Functional implications of the subunit composition of neuronal CaM kinase II. J Biol Chem 274:22713–22722.

Cho MH, Cao X, Wang D, Tsien JZ (2007) Dentate gyrus-specific manipulation of beta-Ca2+/calmodulin-dependent kinase II disrupts memory consolidation. Proc Natl Acad Sci U S A 104:16317–16322.

Colbran RJ (2004) Targeting of calcium/calmodulin-dependent protein kinase II. Biochem J 378:1–16.

De Koninck P, Schulman H (1998) Sensitivity of CaM kinase II to the frequency of Ca2+ oscillations. Science 279:227–230.

Elgersma Y, Fedorov NB, Ikonen S, Choi ES, Elgersma M, Carvalho OM, Giese KP, Silva AJ (2002) Inhibitory autophosphorylation of CaMKII controls PSD association, plasticity, and learning. Neuron 36:493–505.

Elgersma Y, Sweatt JD, Giese KP (2004) Mouse genetic approaches to investigating calcium/calmodulin-dependent protein kinase II function in plasticity and cognition. J Neurosci 24:8410–8415.

Fink CC, Bayer KU, Myers JW, Ferrell JE Jr, Schulman H, Meyer T (2003) Selective regulation of neurite extension and synapse formation by the beta but not the alpha isoform of CaMKII. Neuron 39:283–297.

Frankland PW, O'Brien C, Ohno M, Kirkwood A, Silva AJ (2001) Alpha-CaMKII-dependent plasticity in the cortex is required for permanent memory. Nature 411:309–313.

Giese KP, Fedorov NB, Filipkowski RK, Silva AJ (1998) Autophosphorylation at Thr286 of the alpha calcium-calmodulin kinase II in LTP and learning. Science 279:870–873.

Goslin K, Banker G, eds (1991) Culturing nerve cells. Cambridge, MA: MIT.

Grover LM, Teyler TJ (1990) Two components of long-term potentiation induced by different patterns of afferent activation. Nature 347:477–479.

Hansel C, de Jeu M, Belmeguenai A, Houtman SH, Buitendijk GH, Andreev D, De Zeeuw CI, Elgersma Y (2006) αCaMKII is essential for cerebellar LTD and motor learning. Neuron 51:835–843.

Hudmon A, Schulman H (2002a) Structure-function of the multifunctional Ca2+/calmodulin-dependent protein kinase II. Biochem J 364:593–611.

Hudmon A, Schulman H (2002b) Neuronal CA2+/calmodulin-dependent protein kinase II: the role of structure and autoregulation in cellular function. Annu Rev Biochem 71:473–510.

Jaworski J, Kapitein LC, Gouveia SM, Dortland BR, Wulf PS, Grigoriev I, Camera P, Spangler SA, Di Stefano P, Demmers J, Krugers H, Defilippi P, Akhmanova A, Hoogenraad CC (2009) Dynamic microtubules regulate dendritic spine morphology and synaptic plasticity. Neuron 61:85–100.

Lee SJ, Escobedo-Lozoya Y, Szatmari EM, Yasuda R (2009) Activation of CaMKII in single dendritic spines during long-term potentiation. Nature 458:299–304.

Lin YC, Redmond L (2008) CaMKIIbeta binding to stable F-actin in vivo regulates F-actin filament stability. Proc Natl Acad Sci U S A 105:15791–15796.

Lisman J, Schulman H, Cline H (2002) The molecular basis of CaMKII function in synaptic and behavioural memory. Nat Rev Neurosci 3:175–190.

Mayford M, Bach ME, Huang YY, Wang L, Hawkins RD, Kandel ER (1996) Control of memory formation through regulated expression of a CaMKII transgene. Science 274:1678–1683.

Miller S, Yasuda M, Coats JK, Jones Y, Martone ME, Mayford M (2002) Disruption of dendritic translation of CaMKIIalpha impairs stabilization of synaptic plasticity and memory consolidation. Neuron 36:507–519.

Nomura T, Kumatoriya K, Yoshimura Y, Yamauchi T (1997) Overexpression of alpha and beta isoforms of Ca2+/calmodulin-dependent protein kinase II in neuroblastoma cells—H-7 promotes neurite outgrowth. Brain Res 766:129–141.

Okamoto K, Nagai T, Miyawaki A, Hayashi Y (2004) Rapid and persistent modulation of actin dynamics regulates postsynaptic reorganization underlying bidirectional plasticity. Nat Neurosci 7:1104–1112.

Okamoto K, Narayanan R, Lee SH, Murata K, Hayashi Y (2007) The role of CaMKII as an F-actin-bundling protein crucial for maintenance of dendritic spine structure. Proc Natl Acad Sci U S A 104:6418–6423.

O'Leary H, Lasda E, Bayer KU (2006) CaMKIIbeta association with the actin cytoskeleton is regulated by alternative splicing. Mol Biol Cell 17:4656–4665.

Sahyoun N, LeVine H 3rd, Burgess SK, Blanchard S, Chang KJ, Cuatrecasas P (1985) Early postnatal development of calmodulin-dependent protein kinase II in rat brain. Biochem Biophys Res Commun 132:878–884.

Sanabria H, Swulius MT, Kolodziej SJ, Liu J, Waxham MN (2009) βCaMKII regulates actin assembly and structure. J Biol Chem 284:9770–9780.

Shen K, Meyer T (1999) Dynamic control of CaMKII translocation and localization in hippocampal neurons by NMDA receptor stimulation. Science 284:162–166.

Shen K, Teruel MN, Subramanian K, Meyer T (1998) CaMKIIbeta functions as an F-actin targeting module that localizes CaMKIIalpha/beta heterooligomers to dendritic spines. Neuron 21:593–606.

Silva AJ, Stevens CF, Tonegawa S, Wang Y (1992) Deficient hippocampal long-term potentiation in alpha-calcium-calmodulin kinase II mutant mice. Science 257:201–206.

Tao-Cheng JH, Vinade L, Pozzo-Miller LD, Reese TS, Dosemeci A (2002) Calcium/calmodulin-dependent protein kinase II clusters in adult rat hippocampal slices. Neuroscience 115:435–440.

Thiagarajan TC, Piedras-Renteria ES, Tsien RW (2002) alpha- and beta-CaMKII. Inverse regulation by neuronal activity and opposing effects on synaptic strength. Neuron 36:1103–1114.

van Woerden GM, Harris KD, Hojjati MR, Gustin RM, Qiu S, de Avila Freire R, Jiang YH, Elgersma Y, Weeber EJ (2007) Rescue of neurological deficits in a mouse model for Angelman syndrome by reduction of alphaCaMKII inhibitory phosphorylation. Nat Neurosci 10:280–282.

van Woerden GM, Hoebeek FE, Gao Z, Nagaraja RY, Hoogenraad CC, Kushner SA, Hansel C, De Zeeuw CI, Elgersma Y (2009) betaCaMKII controls the direction of plasticity at parallel fiber-Purkinje cell synapses. Nat Neurosci 12:823–825.

Wang H, Feng R, Phillip Wang L, Li F, Cao X, Tsien JZ (2008) CaMKII activation state underlies synaptic labile phase of LTP and short-term memory formation. Curr Biol 18:1546–1554.

Yamagata Y, Kobayashi S, Umeda T, Inoue A, Sakagami H, Fukaya M, Watanabe M, Hatanaka N, Totsuka M, Yagi T, Obata K, Imoto K, Yanagawa Y, Manabe T, Okabe S (2009) Kinase-dead knock-in mouse reveals an essential role of kinase activity of Ca^{2+}/calmodulin-dependent protein kinase IIα in dendritic spine enlargement, long-term potentiation, and learning. J Neurosci 29:7607–7618.