Calmodulin shuttling mediates cytonuclear signaling to trigger experience-dependent transcription and memory

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Learning and memory depend on neuronal plasticity originating at the synapse and requiring nuclear gene expression to persist. However, how synapse-to-nucleus communication supports long-term plasticity and behavior has remained elusive. Among cytonuclear signaling proteins, γCaMKII stands out in its ability to rapidly shuttle Ca2+/CaM to the nucleus and thus activate CREB-dependent transcription. Here we show that elimination of γCaMKII prevents activity-dependent expression of key genes (BDNF, c-fos, Arc), inhibits persistent synaptic strengthening, and impairs spatial memory in vivo. Deletion of γCaMKII in adult excitatory neurons exerts similar effects. A point mutation in γCaMKII, previously uncovered in a case of intellectual disability, selectively disrupts CaM sequestration and CaM shuttling. Remarkably, this mutation is sufficient to disrupt gene expression and spatial learning in vivo. Thus, this specific form of cytonuclear signaling plays a key role in learning and memory and contributes to neuropsychiatric disease.
long-term plasticity and learning requires activity-dependent nuclear transcription14, a form of regulated gene expression that contributes to a critical dialog between synapse and nucleus3–5. Excitation-induced recruitment of nuclear transcription factors is exemplified by the phosphorylation of calcium- and cAMP response element binding protein (CREB), a transcription factor particularly important for learning and memory6,7. Genetic manipulation of nuclear CREB by mutation of Ser133 to phosphomimetic or non-phosphorylatable forms dramatically alters synaptic plasticity and spatial memory8. This focuses attention on the location of communication initiated by the opening of post-synaptic ligand- and voltage-gated channels7 and culminates in the phosphorylation and resulting activation of CREB9,10 and subsequent expression of plasticity-related genes10. Multiple hypotheses concerning the molecular basis of this communication have been advanced11–14, some invoking nucleus-directed translocation of signaling molecules like NFκB, Rsk2, Jacob, and CRTCl14–19. However, manipulation of these molecules produces confounding changes in brain development and anatomy20–23, basal synaptic properties21, or excitability17. Thus, clear mechanistic links between neuronal activity, nuclear CREB phosphorylation, and memory consolidation have not been established, leading to consideration of the idea that no translocating protein is required at all11–14.

To address questions surrounding the relevance of nuclear signaling for synaptic plasticity, gene expression and behavior, while avoiding ambiguous effects from the elimination or sequestration of multifunctional signaling molecules, we sought to manipulate the transport of the signaling protein while sparing the protein itself. We applied this strategy to the nuclear translocation of Ca2+/calmodulin (CaM)18,19,25, which can switch on a calmodulin (CaM)18,19,24,25, that can switch on a CaMKII shuttle protein in excitatory neurons19,27. We show that genetic deletion of γCaMKII severely impairs activity-dependent expression of key plasticity genes (BDNF, c-Fos, Arc), persistent synaptic strengthening (late LTP or L-LTP), and memory, without detectable changes in anatomy. The necessity of Ca2+/CaM translocation per se was further established by introducing a γCaMKII point mutation discovered in an intellectually disabled boy28. The mutant γCaMKII (R292P) trapped Ca2+/CaM for only seconds rather than minutes, reached the nucleus without its Ca2+/CaM cargo, and failed to support gene expression, L-LTP or long-term memory. Thus, signaling to the nucleus by Ca2+/CaM translocation supports learning and memory and shows vulnerability in neuropsychiatric disease.

**Results**

γCaMKII is critical for gene expression and L-LTP and learning. γCaMKII knockout (KO) mice were fully viable29 and displayed no detectable developmental, morphological or histological brain abnormalities (Supplementary Fig. 1a, b; see also Supplementary Fig. 3, below), minimizing concern about confounding effects on brain development. A custom antibody raised against γCaMKII confirmed the absence of γCaMKII in the γCaMKII KO brain (Supplementary Fig. 1c). Levels of α-, β-, and δCaMKII, as well as other critical neuronal activity-related proteins, were no different than in wild-type (WT) littermates (Supplementary Fig. 1d, e). Neuronal morphology, assessed by Golgi staining of pyramidal neuron dendrites, was not detectably altered (Supplementary Fig. 1f). To assess the role of γCaMKII in the brain, we subjected γCaMKII KO mice to an extensive battery of behavioral tests (Supplementary Fig. 2). γCaMKII KO mice displayed pronounced impairments in multiple spatial learning tasks designed to assess long-term memory, including the Morris Water Maze (MWM)3,8,24,30,31, the radial arm maze (RAM), and the inhibitory avoidance task, each of which invoke different motivations and motor systems. In MWM testing, γCaMKII KO mice were deficient in the learning of platform location compared to WT littermates; latency to escape improved more slowly in KO animals and remained longer than WT even after 3–4 days of training (Fig. 1a). In a probe trial (hidden platform removed) on day 5, γCaMKII KO mice took ~5-fold longer to reach the former platform zone and entered that zone half as often (Fig. 1a), suggesting shortcomings in prior memorization.

To look for biochemical underpinnings of the deficits in learning and memory3,9,32,33, we monitored the experience-driven expression of three major CREB-dependent target genes, BDNF, c-Fos, and Arc (Fig. 1b and Supplementary Fig. 1g). Before training, the expressed protein levels in vivo were similar in WT and γCaMKII KO hippocampus. One hour after MWM training, WT mice displayed a significant increase in hippocampal expression of these genes as compared to naïve animals, assessed by immunoblot (Fig. 1b, black bars), consistent with previous reports10. In contrast, deletion of γCaMKII prevented the training-induced elevation of expression of all three genes (p > 0.4, Fig. 1b, rightmost red bars). Thus, γCaMKII is critical for experience-dependent expression of CREB target genes over the course of spatial learning in vivo.

A similar comparison was made between behavior and gene expression for an inhibitory avoidance (IA) task, in which animals were mildly shocked following entry into the dark compartment of a 2-compartment chamber. WT and γCaMKII KO mice were indistinguishable in the latency to explore the dark compartment during trials prior to administration of a mild shock (dashed lines, Fig. 1c), and in test sessions if no shock was given in the training sessions (Supplementary Fig. 2a). Upon further testing 24 h later, WT animals reliably avoided the conditioned context whereas γCaMKII KO mice were deficient in this avoidance behavior (Fig. 1c, p < 0.05, Kolmogorov–Smirnov test). Correspondingly, the proportion of c-Fos-positive pyramidal neurons was elevated in the WT controls but not in the γCaMKII KO group. Thus, the inhibitory avoidance task provided another example of faulty learning associated with a lack of enduring gene expression. In both hippocampal-dependent tasks (MWM and IA), the absence of γCaMKII engendered clear deficiencies in behavioral performance, comparable to deficits produced by lesions to hippocampal area CA134.

To clarify the mechanism underlying the deficit in learning, we next asked whether disruption of γCaMKII signaling affects hippocampal late-LTP (L-LTP). This enduring form of synaptic plasticity depends on transcription and translation and supports spatial memory formation3,6,24,35,36. We induced L-LTP at Schaffer collateral-CA1 synapses with a standard protocol, three trains of tetanic stimulation (100 Hz, 1 s) spaced by 5 min2,24,37, injected hippocampal slices derived from WT and γCaMKII KO mice. While no deficit was observed in early LTP (E-LTP) between genotypes, L-LTP was strongly affected (Fig. 1d): the initial potentiation in γCaMKII KO slices decayed much faster than that in WT, with a clear difference emerging at 2 h post tetanization (Fig. 1d) (WT: 140.7 ± 5.5%, KO: 121.5 ± 3.9%, p = 0.02), and growing up to the end of recording (WT: 134.9 ± 5.9%, KO: 105.9 ± 8.4%, n = 6 mice, p = 0.02). In contrast, no differences were found in basal properties of WT and γCaMKII KO slices (Fig. 1d): synaptic input–output relationship (ratios of fEPSP slope to fiber volley of 4.61 ± 0.81 for WT vs.
Fig. 1 Spatial memory, CREB-dependent gene expression, L-LTP are impaired in γCaMKII-deficient mice. a Spatial memory acquisition and retrieval were tested with the Morris Water Maze (MWM). Bottom left, mean escape latencies to reach a hidden platform were plotted against training day (4 trials per day) for WT mice (black, n = 21 for 1st 3 days, 12 for 4th day) and γCaMKII KO mice (red, n = 19 for 1st 3 days, 10 for 4th day). Top left, representative path used by mice to reach hidden platform (dotted circle) during the last trial of Day 4. Top right, activity histogram representing average proportion of total time spent by mice during probe trial; γCaMKII KO mice (n = 10). WT (n = 12); scale at far right, amount of time spent at different locations. Bottom middle, latency to reach platform zone during probe trial. Bottom right, number of zone entries during probe trial. b Western blot analysis of BDNF, c-Fos, and Arc expression in the hippocampus of WT (black, n = 9) and KO (red, n = 7) mice. Animals sacrificed 1 h after end of MWM training on 3rd day. c Inset, schematic depiction of inhibitory avoidance test. Left graph, cumulative distribution of latencies to enter the dark compartment before conditioning (dashed lines) and 24 h post conditioning (solid lines). Conditioning done by administration of a mild shock in the dark compartment. Right bar graph, percentage of c-Fos+ neurons in CA1 assessed 1 h after test trial. d Left, late-phase LTP (L-LTP) induced by three trains of tetanic stimulation (100 Hz, 1 s), spaced by 5 min intervals24,37, with timing denoted by the gap in the field EPSP trace (n = 6 mice for each group). Superimposed representative EPSPs show the basal EPSP (bold color) and the EPSP at 165 min (muted color) after L-LTP induction. Calibration bars, 1 mV and 10 ms. Top right, fluo-4 Ca2+ responses (∆F/F) to 5 s of 100 Hz field stimulation in acute hippocampal slices from wild-type and γCaMKII KO mice. Bottom right, paired-pulse ratio (slope of second EPSP/slope of first EPSP) plotted with interpulse intervals of 50, 100, and 200 ms (n ≥ 9 slices). e Radial arm maze behavior. Reference and working memory on day 2, reflecting various aspects of spatial memory 24 h after first exposure to maze, were assayed respectively by recording the number of entries into arms that were never baited (reference memory) or re-entries into arms that were initially baited (working memory) (n ≥ 9 for both genotypes). Statistical analysis was performed with one-way ANOVA followed by Holm–Sidak post hoc test unless otherwise noted. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001. Error bars represent SEM.

4.58 ± 0.57 for γCaMKII KO, p > 0.9), paired-pulse ratio, or somatic Ca2+ influx in response to 100 Hz stimulation38.

Our finding of reduced L-LTP with intact E-LTP suggested that γCaMKII KO mice might display deficits in long-term (reference) memory but not short-term (working) memory. In the radial arm maze, γCaMKII KO mice were no different than WT animals upon first presentation of arm locations (reference “errors”, day 1), but failed to show memory savings 24 h later (Fig. 1e). In contrast, KO and WT mice committed similar numbers of short-term (working memory) errors. Likewise, further tests in the MWM indicated that deficiency in γCaMKII KO performance specifically reflected a spatial learning deficit; our behavioral results could not be otherwise explained by reluctance to swim, swimming speed, wall-hugging behavior, muscle function...
γCaMKII downstream of CaV1/NMDARs regulates spatial learning. To narrow down the requirements for γCaMKII signaling, we selectively deleted γCaMKII in excitatory neurons late in development, using offspring of γCaMKIIloxP/LoxP mice crossed with aCaMKII-CRE mice (exc-KO, Fig. 2a). Hippocampal CA1 pyramidal neurons so deprived of γCaMKII were no different than WT with regard to passive membrane properties, excitability, dendritic branching (Supplementary Fig. 3c) or cell number (Supplementary Fig. 4a). Nonetheless, selective elimination of γCaMKII in excitatory neurons impaired spatial learning (Fig. 2a), recapitulating the deficiency in MWM performance observed with global γCaMKII deletion (p = 0.5, Fig. 1a) Befitting the role of γCaMKII as a surface-to-nucleus shuttle for Ca2+/CaM19, training-induced CaM translocation, seen as increased nuclear CaM immunostaining in WT mice, was eliminated in γCaMKII exc-KO animals (Fig. 2b, c). Likewise, elevation of nuclear c-Fos, reflective of training-induced gene expression in vivo18, was similarly inhibited (Fig. 2b, c). In contrast, basal levels of expression of c-Fos, Arc, BDNF, or nuclear CaM were no different in γCaMKII exc-KO mice and WT littermates (Supplementary Fig. 4), indicating that γCaMKII acted mainly under conditions of training.

These results raised questions about signaling by NMDA receptors, whose importance for induction of L-LTP and learning is well known31,32 but whose linkage to nuclear shuttling of Ca2+/CaM by γCaMKII remains mysterious. To address this,
we exposed cultured hippocampal or cortical neurons to 25 μM NMDA and 5 μM glycine to activate NMDARs, as confirmed with NMDAR-selective blocker APV (Fig. 2d–k and Supplementary Fig. 5a, b). NMDA stimulation elevated nuclear CaM (reflected by the nucleus/cytoplasm ratio, nuc/cyt) and increased pCaMKIV. Involvement of γCaMKII in NMDA-dependent cytonuclear signaling was tested by infecting neurons with viruses encoding a specific γCaMKII shRNA or with a nonsilencing control19. Increases in nuclear CaM, pCaMKIV, pCREB, and c-Fos evoked by NMDA stimulation were prevented or largely reduced by the γCaMKII shRNA (Fig. 2d–g). Thus, the γCaMKII pathway supported NMDA receptors in signaling to the nucleus (Fig. 2l).

Further experiments clarified how signaling by γCaMKII relates to the classical MAP kinase pathway. NMDA-driven elevation of nuclear CaM and CaMKIV activity were prevented by nimodipine (inhibits CaV1) or KN93 (inhibits CaMKs), but not PD98059 (inhibits MAP2K1/MEK1, enzymes upstream of
ERK) (Fig. 2h, i). NMDA stimulation also triggered an increase in pCREB that was prevented by nimodipine or KN93 (Fig. 2j), and also reduced by PD98059, consistent with involvement of both CaMK and MAPK pathways (ref. 39 for earlier references). NMDA-driven elevation of c-Fos protein, assayed 40 min following stimulation to allow time for transcription and translation, was prevented by nimodipine, KN93, or PD98059 (Fig. 2k).

These results indicate that NMDAR activation drives divergent γCaMKII- and MAPK-mediated pathways that reconverge to control gene expression. We verified that deletion of γCaMKII spared critical markers of the MAPK signaling branch (elevation of nuclear levels of pMAPK, Rsk2, CRTC1; Supplementary Fig. 5). Thus, the dramatic behavioral consequences of γCaMKII KO (Figs. 1a, c, e and 2a) are specifically indicative of the role of γCaMKII-mediated branch of the signaling to the nucleus.

ID-associated γCaMKII R292P mutation impairs CaM shuttling. To gain further insight into the functional significance of cytonuclear communication by protein translocation, we focused on genetic variation in γCaMKII that alters human intellectual performance. Extending an early study of heritability of intellectual disability (ID) that implicated γCaMKII40, de Ligt et al.48 performed exome sequencing of a boy with severe ID and his unaffected parents and discovered a de novo coding mutation in γCaMKII (R292P, Fig. 3a). The positively charged residue at position 292 is highly conserved across CaMKII isoforms (Supplementary Fig. 6a and b) and neighbors a largely α-helical region required for CaM binding41 (Fig. 3a); mutation to proline could disrupt that region and its function. Accordingly, we reasoned that this ID-linked mutation might illuminate the mechanism by which γCaMKII supports learning and memory.

Lentiviral expression of human γCaMKII R292P in hippocampal slices from KO mice failed to rescue L-LTP (red trace, Fig. 3b); early potentiation and late-phase decay were indistinguishable from KO slices (black). In contrast, slices expressing human WT γCaMKII (blue) showed strong early potentiation that was maintained throughout the recording, and was significantly greater than that with γCaMKII R292P. The disabling effect of the R292P mutation on synaptic plasticity prompted us to analyze its impact on biochemical signaling in vitro (Fig. 3c–f) and in neurons (Fig. 3g–j). Both purified WT and R292P γCaMKII readily bound CaM as seen with CaM overlays (Fig. 3c), underwent phosphorylation at T287 as detected by γCaMKII-specific immunoblotting (Fig. 3d), and displayed catalytic activity as a protein kinase (Fig. 3e). Beyond these similarities between WT and mutant molecules, we noted a potentially critical contribution of γCaMKII in which its kinase activity is dispensable but its ability to trap and translocate CaM to the nucleus is crucial. Knowing that phosphorylation of αCaMKII at T286 increased its affinity for Ca2+/CaM >1000-fold, largely because of slowed CaM dissociation42,43, we characterized such “CaM trapping” by WT and R292P mutant forms of γCaMKII. The dissociation of fluorescently labeled CaM (CaM(C75)) from T287-phosphorylated γCaMKII was monitored as a decrease in fluorescence upon sudden addition of non-fluorescent CaM41. Strikingly, dissociation from the R292P mutant was ~1500-fold faster than from WT γCaMKII, occurring within seconds rather than minutes, even in the presence of Ca2+/CaM (Fig. 3f). Thus, by the time the mutant γCaMKII began to accumulate in the nucleus (~30 s), CaM would no longer be bound and would therefore not translocate effectively.

To test this prediction, we introduced WT or R292P forms of γCaMKII into cultured hippocampal and cortical neurons from γCaMKII KO mice. Neurons were depolarized with 40 mM K+ and protein levels in isolated nuclei were analyzed. Stimulation-induced increases in c-Fos, nuclear CaM and nuclear γCaMKII were absent in neurons from γCaMKII KO mice (Fig. 3g–j and Supplementary Fig. 6b), as previously found in cultured rat cortical neurons.19 Each of these events was rescued by re-introduction of WT human γCaMKII. In contrast, γCaMKII R292P was able to translocate to the nucleus, but unable to support nuclear CaM translocation or c-Fos expression (Fig. 3g–j).

Having demonstrated the necessity of intact γCaMKII and CaM shuttling for gene expression, we proceeded to test for sufficiency of nuclear CaM delivery by directly uncaging CaM in the nucleus. The vehicle was neurogranin, an endogenous buffer for apoCaM that releases CaM once it becomes Ca2+/CaM >1000-fold, that we HA-tagged and targeted to the nucleus (NLS-Nrgn)19. Immunocytochemistry verified that introduction of γCaMKII R292P in cultured γCaMKII KO neurons failed to rescue c-Fos expression, consistent with results from isolated cultured hippocampal and cortical neurons from WT or γCaMKII KO mice stimulated with 40 mM KCl for 1 h. Both γCaMKII (WT) and γCaMKII R292P translocated to the nucleus (g, h); however, only re-introduction of transgenic γCaMKII (WT), not γCaMKII R292P, into γCaMKII KO neurons rescued CaM translocation (g, i) and c-Fos expression (g, j). k c-Fos response in cultured hippocampal and cortical neurons from γCaMKII KO mice stimulated with 40 mM KCl for 1 h. Both γCaMKII (WT) but not γCaMKII R292P (no HA tag) could restore c-Fos response. Co-expressing γCaMKII R292P with NLS-Nrgn but not NLS-Nrgn S36D was able to rescue the c-Fos response, consistent respectively with an ability or inability to harbor CaM for release upon nuclear Ca2+ elevation45. Scale bar, 10 μm. Statistical analysis performed with one-way ANOVA followed by Holm–Sidak post hoc test unless otherwise noted.

*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001. Error bars represent SEM.
nuclei. In contrast, introduction of NLS-Nrgn along with γCaMKII R292P fully restored stimulation-induced c-Fos expression (Fig. 3k and Supplementary Fig. 6c, d). The rescue of c-Fos expression failed when the NLS-Nrgn was modified (S36D) to render it incompetent to bind apoCaM and thus release CaM upon Ca\(^{2+}\) influx (Fig. 3k and Supplementary Fig. 6c, d). Thus, the failure of γCaMKII R292P could be bypassed by exogenous replacement of nuclear Ca\(^{2+}\)/CaM elevation to circumvent the defective shuttle. Given that kinase activity of γCaMKII is dispensable for signaling to the nucleus\(^{19}\), the critical events for gene expression, operative in WT but lost in the ID-linked mutant, are CaM trapping and nuclear CaM delivery.

**R292P mutation impairs gene expression and learning in vivo.** Returning full circle to spatial learning, we tested whether the behavioral deficit in excitatory neuron-specific γCaMKII KO mice could be repaired by acute expression of γCaMKII in the mature hippocampus (Fig. 4a). Hippocampi of adult WT or γCaMKII exc-KO mice were targeted with bilateral injections of saline or lentiviruses containing WT or mutant human γCaMKII, resulting in levels of expression of γCaMKII three-fold greater than endogenous (Supplementary Fig. 6e). Strikingly, the deficit in MWM spatial learning seen in γCaMKII exc-KO mice was fully rescued by introduction of γCaMKII. To verify that the behavioral repair was associated with rescue of nuclear signaling, we stained CA1 pyramidal cells\(^{31}\) for CaM and c-Fos 1 h after...
MWM training. Both CaM translocation and c-Fos expression were fully rescued by the re-introduction of γCaMKII (Fig. 4b, c). Hence, behavioral performance depends critically on the availability of γCaMKII during the task, not its presence during brain development.  

Unlike WT γCaMKII, γCaMKII R292P failed to rescue the spatial learning deficit when introduced into the mature hippocampus. Mice expressing the mutant performed no differently than exc-KO animals (Fig. 4a). Likewise, they displayed levels of nuclear c-Fos and CaM in CA1 that were no higher than in γCaMKII exc-KO (Fig. 4c; p > 0.8) when examined after MWM training. The strong correlation between post-training nuclear CaM and c-Fos in WT mice (Fig. 4d) was rescued in exc-KO mice by expression of human WT γCaMKII (Fig. 4e); likewise, nuclear c-Fos was correlated with γCaMKII (Fig. 4f). In contrast, following introduction of γCaMKII R292P, nuclear c-Fos was not correlated with nuclear CaM (Fig. 4e), nor with nuclear γCaMKII (Fig. 4f), even though basal levels of expressed γCaMKII R292P were not different than expressed γCaMKII (Supplementary Fig. 6e, f). These new cells reflect inefficient CaM shuttling by the mutant protein. In all respects, our observations with mutant γCaMKII R292P sharply contrasted with those obtained with γCaMKII.

**γCaMKII R292P demonstrates dominant negative effect.** We next tested for a dominant-negative effect of the R292P mutant, of possible relevance to the pathogenesis of ID in a human heterozygote. Interestingly, overexpressed γCaMKII R292P in cultured WT hippocampal neurons prevented nuclear CaM translocation, suggesting a strong dominant negative effect of γCaMKII R292P in regulating nuclear CaM signaling (Supplementary Fig. 6g). Moreover, following expression of γCaMKII R292P in the hippocampus, escape latencies in the MWM were no faster in WT than in exc-KO animals (Fig. 4g). Experience-dependent c-Fos expression was compared in WT and exc-KO hippocampus following behavioral testing. Unlike the striking difference in experience-dependent c-Fos staining between WT and exc-KO (Fig. 2b), in γCaMKII R292P-infected pyramidal neurons, distinguished by their HA-tag (Fig. 4h), such post-training differences were no longer observable (Fig. 4i). Evidently, exogenous expression of mutant γCaMKII can nullify the impact of normal protein with regard to gene expression as well as behavior, consistent with some kind of dominant negative effect.

**Discussion**

Here we show the importance of Ca\(^{2+}\)/CaM translocation to the nucleus for learning and memory, and how this translocation is disrupted by a human mutation linked to intellectual disability. Mediated by γCaMKII in excitatory neurons, the Ca\(^{2+}\)/CaM shuttle links activation of NMDARs\(^{44}\) and Ca\(_{v}1\) channels\(^{19}\) to nuclear transcription, thus supporting L-LTP and long-term memory. Our in vivo experiments indicate that this mechanism is necessary for classic hippocampal CA1-based learning in adult animals. Independence from any possible effects of γCaMKII on early brain development was demonstrated by late deletion of γCaMKII (Fig. 2) and adult rescue by γCaMKII delivery in a knockout background (Fig. 4).

As a mechanism for synaptic activity-dependent, cytonuclear communication based on protein translocation\(^{45–48}\), Ca\(^{2+}\)/CaM shuttling can be compared with leading examples of synaptic-nuclear signaling (NFkB, RSK2, Jacob, CRTPC)\(^{11–13}\). It will be interesting to see if these signaling pathways, well described in neuronal cultures, are also critical for the establishment of memory in vivo, separable from other important but confounding functions distinct from memory storage\(^{17,20–23}\). The significance of the γCaMKII/Ca\(^{2+}\)/CaM shuttle could be clearly defined via a specific manipulation of shuttle function (Figs. 3 and 4) and extensive control experiments to rule out morphological, biochemical and behavioral side effects (Supplementary Figs. 1–6).

This addresses a long-standing challenge to studies of LTP: finding key molecular underpinnings, as functionally critical to neuralplasticity as a door hinge is to an opening door\(^{49}\), and linking these processes to behavior.

The strong dominant negative effect of γCaMKII R292P (Fig. 4g, h; Supplementary Fig. 6g) is intriguing as it helps to establish a logical connection between our data and the (heterozygous) human patient. A dominant negative effect could result from competition between wild-type and mutant γCaMKII at various critical steps along the shuttling pathway\(^{19}\): competition for cross-phosphorylation of γCaMKII by βCaMKII, for dephosphorylation by CaN to enable the NLS, or for binding to protein components supporting transport to the nucleus. The dominant negative effect of γCaMKII R292P cannot easily be attributed to loss of catalytic activity because a charge-altering mutation in the homologous position of aCaMKII (K291E) enhances affinity for Ca\(^{2+}\)/CaM 5-fold, via destabilization of the basal CaM-free state\(^{50}\); further, we confirmed that γCaMKII R292P retained kinase activity. Because even kinase-dead γCaMKII is able to support nuclear Ca\(^{2+}\)/CaM delivery\(^{19}\), a
mutation-based deficiency in kinase activity of γCaMKII is unlikely to explain how the R292P mutant interferes with E–T coupling to affect long-term memory. The functional relevance of the γCaMKII/Ca2+/CaM shuttle mechanism is highlighted by human genetics. Each of the molecules along the signaling pathway is encoded by a gene implicated in multiple brain diseases, among them schizophrenia, major depressive disorder, bipolar disorder and autism spectrum disorder. In particular, CAMK2G, the gene encoding γCaMKII, contributes to a genetic cluster that influences memory performance and imaged hippocampal activity in humans, and has cropped up in genetic studies of ASD, and. In rodents, γCaMKII has been implicated in depression-like behavior and as a regulatory target of the NMDA receptor-dependent microRNA miR-219. While the ID-linked R292P mutation causes the most functionally precise lesion so far, it will likely be joined by other genetic modifications that illuminate neuropsychiatric disorders along with fundamental aspects of memory and cognition.

Methods

Data acquisition. All analyses were performed with the experimenter blinded to the genotypes of mice, cultured neurons and acute slices.

Animals. γCaMKII-KO and γCaMKII-cre-KO mice were kindly provided by Dr. Eric N. Olson (UT Southwestern Medical Center) with help of Dr. Johannes Backs and his staff (University of Heidelberg). γCaMKII-Cre mice (Stock no. 005359) were purchased from the Jackson Laboratory and maintained on a C57BL/6J background. The mice (10–14 weeks of age, both genders) were used for behavioral tests. Subject mice were kept in a 20 °C room on a 12-h light/dark cycle (lights off at 6:00) and had access to water and food ad libitum. Mice were subjected for at least 7 d before experiments. All experiments were performed during the dark cycle, after acclimatization to the experimental room for at least 3 d. All experimental procedures involving animals were approved by the Institutional Animal Care and Use Committee at the New York University Langone Medical Center and the Animal Advisory Committee at Zhejiang University.

Behavioral assays. All behavioral testing occurred between 0800 and 1800 h.

Spatial reference memory was tested using the MWM. A circular tank (diameter: 183 cm) was filled with water at 20 °C and surrounded by uniform blinks and quadrant-specific visual cues. The water was made opaque by adding a sufficient amount of Tempra paint. Animal movement was tracked using a ceiling camera and Ethovision tracking software. Mice were introduced into the pool at pseudorandom drop locations outside of the target quadrant. During the “hidden platform learning task,” a circular escape platform (15 cm diameter) was placed in the middle of a designated target quadrant 1 cm below the water surface. Mice were trained to find the platform by four 90-s trials per day, performed on a duty cycle of 14 min, for 4 consecutive days. During each block of trials, the mice were released from pseudorandomly assigned start locations (NW, NE, SW, and NW). A trial ended either when a subject rested on the hidden platform for 5 s or the end of the trial was reached. Mice that failed to find the platform by the end of the 90-s trial were manually guided to rest on the platform for 15 s. On day 5, a 90-s probe trial was conducted with the escape platform removed.

A modified radial arm maze was used to test working and reference memory at the same time. The floor of the maze was made of white plastic, and the walls (13 cm high) consisted of transparent plexiglass. Each arm (13 × 40 cm) radiated from an octagonal central starting platform (diameter 23 cm) like the spokes of a wheel. The maze was elevated 75 cm above the floor and only four maze arms were baited. The arms were placed randomly throughout the maze, and the time the grid is inverted to the time that the mouse falls off the wire grid (determined visually and measured using a stopwatch) and the holding impulse is the hang time multiplied by the body weight.

Primary cultures of cortical and hippocampal neurons. Hippocampal and cortical neurons were cultured from postnatal day 0 to 1 Sprague-Dawley rat pups or C57BL/6J mice. The hippocampus or frontal cortex were isolated and washed twice in ice-cold modified HBSS (4.2 mM NaHCO3 and 1 mM HEPES, pH 7.35, 300 mOsm) containing 20% fetal bovine serum (FBS; HyClone, Logan, UT) then the meninges and the ice-cold HBSS were discarded. The tissue was digested for 30 min in a papain solution ( Worthington, NJ; 2.5 ml HBSS + 145 U papain + 40 μl DNase) at 37 °C with gentle shaking every 10 min. Digestion was stopped by adding 5 ml of modified HBSS containing 20% FBS. After additional washing, the tissue was dissociated using Pasteur pipettes of decreasing diameter. The cell suspension was filtered twice and filtered with a 70 μm nylon strainer, and plated on 12 mm diameter coverslips coated with poly-l-lysine. The cultures were maintained in Neurobasal (BrainBits, Springfield, IL). Around 30% medium change was performed at 7 days, and once per week thereafter. Neurons were used 12–26 days after plating.

Drug treatments, and stimulation. To induce CREB phosphorylation, Ca2+/CaM/γCaMKII translocation, and other comparable or related events, we stimulated hippocampal and cortical neurons with the indicated high [K+] solution or NMDA-containing solution at 37 °C for 60–600 s, and fixed the cells immediately after the stimulation (in 4% paraformaldehyde in PBS with 20 mM EGTA and 4% (w/v) sucrose). To induce c-Fos expression, we stimulated cells for 300 s, and then moved the coverslip back to the basal media for 40 min, at which point cells were fixed. 5 mM K Tyroide’s of the (in mM): 150 NaCl, 5 KCl, 2 MgCl2, 2 CaCl2, 10 HEPES, 10 glucose, pH 7.4. When stimulating with elevated [K+], Na+ was adjusted to maintain osmolality. All NMDA stimulation conditions were in 5 mM K Tyroide’s containing 25 μM NMDA and 5 μM glycine. All K+–rich and NMDA-containing stimulation solutions contained 0.5 μM TTX (Tocris) to block action potentials, unless otherwise indicated. Where indicated, drugs were added 30 min before and included throughout the stimulation: 10 μM NRBQ (Tocris) to block AMPA receptors, 50 μM D-AP5 (Ascent Scientific) to block NMDA receptors, 10 μM Nimodipine (Abcam) to block CaV1 channels, 4 μM KN93 (Tocris) to block CaM Kinases, 50 μM PD98059 (Tocris) to block MEK1.

Lentiviral transduction of cultured neurons. To produce lentivirus, the pLVT/TH shRNA or pCDH-EF1-γCaMKII constructs were transduced into 293FT cells along with the packaging plasmids pSPAX2 and the envelope plasmid pMD2.G, kindly provided by Dr. D. Trono. After 16 h, the medium was changed. The supernatant was collected 24 h later and cleared of cell debris by filtering through a 0.45 mm filter. The viral particles were concentrated by centrifuging the filtrate at 70,000 x g for 2 h at 4 °C using a Beckman SW28 rotor. The viral pellet was then resuspended in sterile PBS, aliquoted, and stored at 80 °C. Lentivirus particles (0.5–1 ml of viral stock diluted in 20 ml of PBS per coverslip) were added to cultured neurons containing 500 μl of medium the day after plating. Twenty-four hours later, the cultures were fed with 1 ml of medium and used 4–5 days later, at which point infection efficiency was nearly 100%.
Stereotaxic surgery. Mice (postnatal 80–90 days) were anesthetized with isoflurane and the brain was quickly dissected out after decapitation. Transverse acute slices (350 μm thick) were cut in a Corinna cryostat carried out in cold sucrose cutting solution (in mM: 260 Sucrose, 26 NaHCO3, 11 Glucose, 2.5 KCl, 1 NaH2PO4, 20 MgCl2, 0.5 CaCl2). Then hippocampal slices were incubated in 95% O2:5% CO2 aerated ACSF (in mM: 122 NaCl, 3 KCl, 10 Glucose, 1.25 NaH2PO4, 1.5 MgCl2, 2 CaCl2, 26 NaHCO3) at 32 °C to recover for 1 h, and then left at room temperature for 1 h for further recovery for use up to 5 h later.

Electrophysiology. Field excitatory postsynaptic potential (EPSP) recordings were performed in area CA1 from 350 μm transverse acute slices. Recording was in an interface chamber at 32 °C, perfused with artificial cerebrospinal fluid (ACSF). Slices were allowed 20–30 min to equilibrate in ACSF before recording. Recordings were made with glass pipettes (3–5 MΩ) filled with ACSF and placed in the stratum radiatum of CA1. Intracellular twitch stimulation electrode was placed between area CA3 and CA1 for stimulation of the Schaffer collaterals. The intensity of the stimulating pulse was adjusted to produce responses 30–50% of the maximum and 30 min of baseline recording was performed to ensure the stability of responses. Late long-term potentiation (LTP) was induced by three 1 s trains of 100 Hz stimulation at 5 min intervals. Stimulus intensity was equivalent in test and induction responses.

CA1 pyramidal cell membrane properties were determined with whole-cell clamp recordings, performed with an intracellular solution containing (in mM): 140 K-Gluconate, 10 HEPES, 0.3 Mg-ATP, 0.3 Na-GTP and 0.1% bioyctin. After maintenance of whole-cell recording for 15–20 min, slices were transferred to a 4% paraformaldehyde fixative solution overnight, then washed and stained with Alexa Fluor 488 streptavidin (1:700, Molecular Probes). Cells were imaged with a Zeiss confocal microscope and analyzed for dendritic morphology.

Field stimulation and calcium imaging. Following the cutting and recovery of hippocampal slices, they were incubated for 30 min at 37 °C, followed by 15 min at RT, in a 1:1 dilution of Fluo-4 (Life Technologies) in ACSF to load slices with the Ca2+ indicator. Following loading, slices were placed on a stage, submerged in fresh oxygenated ACSF. Two platinum electrodes were positioned with a distance of 10 mm on either side of the slice. The acute slices were field stimulated at 100 Hz for 5 s with square waves (1 ms per pulse) at 15 V. Cells were imaged with a Zeiss LSM 510 meta Imager.M1 confocal microscope at 1.25X. ΔF/ ΔF Ca2+ responses were analyzed using Icy software.

Perfusion and fixation of tissue. Animals were anesthetized with a lethal dose of pentobarbital (100 mg/kg) and underwent intracardiac perfusion with 50 ml of ice-cold PBS. The brain was removed from the skull, the right side was immersed or perfused with ice-cold solution made up of 4% PFA in PBS, pH 7.4 and the left side was dissected into hippocampus and cortex that were snap-frozen and stored at −80 °C until they were used for biochemical analyses. Twenty-four hours PFA-fixed hemispheres were transferred to 30% sucrose until tissue sank, then OCT-embedded and stored at −80 °C until performance of further tissue sectioning and immunohistochemical analyses.

Morphology analysis. Six male mice (littermates aged P60-65, 3 mice per genotype) were used for morphological analysis. FD Rapid Golgi Stain Kit (FD Neurolucida software, Microbright USA). After the sections had dried (2 h), the tissue was stained with a solution (1:1 dilution of Fluo-4 (Life Technologies) in ACSF to load slices with the Ca2+ indicator. Following loading, slices were placed on a stage, submerged in fresh oxygenated ACSF. Two platinum electrodes were positioned with a distance of 10 mm on either side of the slice. The acute slices were field stimulated at 100 Hz for 5 s with square waves (1 ms per pulse) at 15 V. Cells were imaged with a Zeiss LSM 510 meta Imager.M1 confocal microscope at 1.25X. ΔF/ΔF Ca2+ responses were analyzed using Icy software.

Measurement of CaMKII dissociation rate from CaMKII. CaMKII (C75) was labeled with IAEDANS (Thermofisher) as previously described with small modifications. Briefly, 50 nm CaMKII was incubated with 0.5 mM IAEDANS (urea not present) overnight followed by dialysis using PD MiniTrap G-25 against 50 mM MOPS, pH 7.0, 150 mM KCl, 0.1 mM EGTA, 0.5 mM CaCl2, and 0.1 mg/ml BSA. 1 mM MgATP was added into the reaction solution containing 0.5 mM Ca2+, CaMKII(C75) and yCaMKII or yCaMKII R292P to enable phosphorylation at Thr 287. After incubation at room temperature for 5 min, a 75-fold excess of unlabeled CaMKII was added to exchange with labeled CaMKII(C75)Ae. For fluorescence measurements, excitation was at 345 nm and emission at 465 nm was monitored at 3 Hz using a Flex station. The experimental data was fitted with a two phase decay function (V = A1*exp(-t/τ1) + A2*exp(-t/τ2) + Vb).

CaMKII activity assay. The kinase activity of yCaMKII was measured using the Cylex Calmodulin kinase II Assay Kit (Cat # CY-E1173, Cyclex). Briefly, purified yCaMKII (or yCaMKII R292P, equal amounts) was added to the kinase reaction buffer provided with the kit and incubated for 30 min at 30 °C in 96-well plates. Wells were washed 5 times for wash buffer and 10 μl of the horseradish peroxidase-conjugated detection antibody MS-666 was added. After 60 min incubation at room temperature, samples were washed five times, and the substrate reagent (100 μl) was added. After 15 min incubation at room temperature, the reaction was stopped by the addition of a stop solution and the absorbance read at 450 nm with a microplate reader.

Immunocytochemistry. Cultured cells were fixed in ice-cold 4% paraformaldehyde (PFA) in PBS supplemented with 20 mM EGTA and 4% (w/v) sucrose. Fixed cells were then permeabilized with 0.1% Triton X-100, blocked with 6% (cells) or 10% (10 μm hippocampal slices) normal donkey serum, and incubated overnight (cells) or two nights (10 μm hippocampal slices) at 4 °C in primary antibodies: rabbit anti-pCaMKIV (1:500, sc-28443-R, Santa Cruz Biotechnology); mouse anti-CaMKII (1:500, 05-173, Millipore); rabbit anti-pCREB (1:333, 1918, Cell Signaling Technology); rabbit anti-c-Fos (1:500, 2250, Cell Signaling Technology); rabbit anti-BDNF (1:1000, sc-546, Santa Cruz); rabbit anti-Arc (1:1000, sc-17839, Santa Cruz); mouse anti-HA (1:1000, sc-37789, Santa Cruz); mouse anti-α-tubulin (1:1000, MMS-101P, Covance); mouse anti-GAP43 (1:1000, H9658, Sigma-Aldrich); goat anti-Rsk2 (1:1000, sc-1430, Santa Cruz); rabbit anti-CaM kinase II (1:1000, A10089, Bethyl); mouse anti-CaMKII (1:2000, 13-7300, ThermoFisher). The next day, cells were washed with PBS, incubated at room temperature for 45 min (for cells) or 2 h (hippocampal slices) with Alexa-tagged secondary antibodies (1:1000 for cells, 1:500 for hippocampal slices, Molecular Probes), washed with PBS and mounted using ProLong Gold (Invitrogen). The coverslips were then imaged on a Zeiss Axiovert 200M microscope using a Zeiss AxioObserver microscope with a Plan-Apochromat 63×/1.4 oil objective and analyzed with AxioVision.
Western blot. SDS-PAGE loading buffer and β-mercaptoethanol were added to the lysate (25 and 10% of the total lysate volume, respectively), and the mixture was heated at 95 °C for 5 min. Cellular protein was separated on a 10% SDS-PAGE gel and transferred to Immobilon transfer membrane (Millipore). The membrane was then blocked at room temperature for 1 h in Odyssey blocking buffer (Li-Cor Biosciences, Lincoln, NE). The membrane was incubated overnight with antibodies as the γCaMKII antibody (rabbit, 1:1000) was raised against γCaMKII (amino acids 441-460) and its specificity was tested in γCaMKII 

moiety; rabbit anti-Lamin B (1:1000, 12568, Cell Signaling Technology); rabbit anti-GAPDH (1:1000, 5174, Cell Signaling Technology); mouse anti-α-Tub (1:1000, H9685, Sigma); mouse anti-CaM (1:500, 95-173, Millipore); rabbit anti-c-Fos (1:500, 2250, Cell Signaling Technology); rabbit anti-CaMKII (1:1000, sc-546, Santa Cruz); rabbit anti-CaMKII (1:1000, sc-17835, Santa Cruz); mouse anti-αCaMKII (1:2000, 13-7300, Thermofisher); mouse anti-pβCaMKII (1:1000, 13980, Invitrogen); rabbit anti-pCaMKII (1:1000, 13-7300, abcam); rabbit anti-pPKA (1:500, sc-377575, Santa Cruz, to track PKA activation); rabbit anti-p44/42 MAPK (1:1000, 4370, Cell Signaling Technology); rabbit anti-p38 MAPK (1:1000, 921, Cell Signaling Technology); rabbit anti-NKCC1 (1:2000, AB5609P, Chemicon International); rabbit anti-IKBα (1:2000, 07-432, Chemicon International); rabbit anti-GABA_R (1:2000, Chemicon International); rabbit anti-GluR1 (1:500, Millipore, Rabbit). The specificity of BDNF antibody for WB or ICC was tested previously59 and the band (~16kDa) was examined for WB. The following day, the membrane was washed with 0.1% Tween 20 in PBS and incubated with an IRDye-labelled secondary antibody (Li-Cor) for 1 h. After incubation, the membrane was washed with PBS and imaged with Li-Cor Odyssey imaging system. All the bands were analyzed with Image Studio. For nuclear extraction, the nuclear proteins of cortical neurons (DIV13-15) were isolated with NE-PER Nuclear and Cytoplasmic Extraction Reagent Kit (Thermo Scientific) before the gel electrophoresis. For CaM overlay, CaM (Enzo Life Sciences) was added at a final concentration of 0.5 μg/ml in the 5 mM K+ Tyrode’s and incubated with membrane for 1 h before addition of the primary antibody for CaM62. Images in the main text have been cropped for presentation. Full size images are presented in Supplementary Figs. 7–9.

Image analysis. For cultured neurons, images for protein quantification were analyzed using a custom-written macro in ImageJ (NIH). The nuclear marker DAPI and an antibody against the neuron-specific protein MAP2 were used to delineate the nucleus and cytoplasm, respectively. A region of interest adjacent to each neuron analyzed was used as an ‘off-cell’ background. In cortical cultures, the protein level in each neuron was calculated by subtracting the average intensity in the ‘off-cell’ background region of interest from the average intensity in the appropriate region of interest for each neuron. The nuclear/cytoplasmic ratio was calculated by comparing nuclear immunoreactivity with apical dendrite immunoreactivity (immunoreactivity of the membrane profile was not included). For hippocampal slices, two images were taken in the CA1 from every slice (two slices per animal). Using ImageJ, ROIs were chosen at each DAPI+nucleus in a given section. The average pixel intensity of staining for the signal-of-interest was obtained within the boundary of each nuclear ROI. From this value, a background value (taken in the stratum radiatum) was subtracted. For c-Fos staining, an arbitrary threshold of 50 intensity units was set and applied to all slices, and the percentage of cells with c-Fos staining >50 was calculated for each image.

DNA constructs. The point mutation was produced using QuikChange Lightning Site Directed Mutagenesis Kit (Agilent) with pCDH-EF1a-HA-γCaMKII, as template and the following primer and its reverse-complement (mutated bases in lower case bold):

\[ \gamma\text{CaMKII R}292\text{P: }GA\text{AGG TCG GAT GGC GGT GTC AAG AAA AGG AAG GAC ATG TCC AGG GTG CAC CTA ATG GAC CAG} \]

All γCaMKII constructs in this study utilized the human γCaMKII isoform of γCaMKII.

Data availability. The data that support the findings of this study are available from the corresponding author upon reasonable request.

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References

1. Davis, H. P. & Squire, L. R. Protein synthesis and memory: a review. Psychol. Bull. 96, 518–539 (1984).
2. Alberini, C. M. & Kandel, E. R. The regulation of transcription in memory consolidation. Cold Spring Harb. Perspect. Biol. 7, a012741 (2015).
3. Kandel, E. R., Dudai, Y. & Mayford, M. R. The molecular and systems biology of memory. Cell 157, 163–186 (2014).
4. Frey, U. & Morris, R. G. Synaptic tagging and long-term potentiation. Nature 385, 533–536 (1997).
5. Nguyen, P. V., Abel, T. & Kandel, E. R. Requirement of a critical period of transcription for induction of a late phase of LTP. Science 265, 1104–1107 (1994).
6. Moosmang, S. et al. Role of hippocampal Cav1.2 Ca2+ channels in NMDA receptor-independent synaptic plasticity and spatial memory. J. Neurosci. 25, 9890–9892 (2005).
7. Greenberg, M. E., Ziff, E. B. & Greene, L. A. Stimulation of neuronal acetylcholine receptors induces rapid gene transcription. Science 234, 80–83 (1986).
8. Bourchuladze, R. et al. Deficient long-term memory in mice with a targeted mutation of the cAMP-responsive element-binding protein. Cell 79, 59–68 (1994).
9. Guzowski, J. F. & McGaugh, J. L. Antisense oligodeoxynucleotide-mediated disruption of hippocampal cAMP response element binding protein levels impairs consolidation of memory for water maze training. Proc. Natl Acad. Sci. USA 94, 2693–2698 (1997).
10. Guzowski, J. F., Setlow, B., Wagner, E. K. & McGaugh, J. L. Experience-dependent gene expression in the rat hippocampus after spatial learning: a comparison of the immediate-early genes Arc, c-fos, and zif268. J. Neurosci. 21, 5089–5098 (2001).
11. Adams, J. P. & Dudek, S. M. Late-phase long-term potentiation: getting to the nucleus. Nat. Rev. Neurosci. 6, 737–743 (2005).
12. Meffert, M. K., Chang, J. M., Wiltgen, B. J., Fanselow, M. S. & Baltimore, D. Calcium-calmodulin-dependent protein kinase II activity in the dentate gyrus during memory memory formation or reactivation increases memory strength without compromising memory quality. J. Neurosci. 32, 17857–17868 (2012).
13. Deisseroth, K., Heist, E. K. & Tsien, R. W. Translocation of calmodulin to the nucleus supports CREB phosphorylation in hippocampal neurons. Nature 392, 198–202 (1998).
14. Boersma, M. C. et al. A novel function for KLF7 in the development of and plasticity-associated synaptic downregulation. J. Neurosci. 31, 5414–5425 (2011).
15. Morice, E. et al. Defective synaptic transmission and structure in the dentate gyrus and selective fear memory impairment in the Rsk2 mutant mouse model of Coffin-Lowry syndrome. Neurobiol. Dis. 58, 156–168 (2013).
16. Spiller, C. et al. A Jacob/Nfs1 gene knockout results in hippocampal dysfunction and impaired BDNF signaling in dentgenesis. PLoS Genet. 12, e1005907 (2016).
17. Li, X., Zhang, C., Takemori, H., Zhou, Y. & Xiong, Z. Q. TORC1 regulates activity-dependent CREB-target gene transcription and dendritic growth of cortical neurons. J. Neurosci. 29, 2334–2343 (2009).
18. Limback-Stokin, K., Korzus, E., Nagaoka-Yasuda, R. & Mayford, M. Nuclear calcium/calmodulin regulates memory consolidation. J. Neurosci. 24, 10858–10867 (2004).
19. Kang, H. et al. An important role of neural activity-dependent CaMKIV signaling in the consolidation of long-term memory. Cell 106, 771–783 (2001).
20. Wei, F. et al. Calcium calmodulin-dependent protein kinase IV is required for fear memory. Nat. Neurosci. 5, 573–579 (2002).
21. Cohen, S. M. et al. Excitation–transcription coupling in parvalbumin–positive interneurons employs a novel Gαq CaM kinase-dependent pathway distinct from excitatory neurons. Neuron 90, 292–307 (2016).
22. de Ligt, J. et al. Diagnostic exome sequencing in persons with severe intellectual disability. N. Engl. J. Med. 367, 1921–1929 (2012).
23. Backs, J. et al. The gamma isoform of CaMKII controls mossy fiber activation by regulating cell cycle resumption. Proc. Natl Acad. Sci. USA 107, 81–86 (2010).
24. Morris, R. G., Garrud, P., Rawlins, J. N. & O’Keefe, J. Place navigation impaired in rats with hippocampal lesions. Nature 297, 681–683 (1982).
25. Tsien, J. Z., Huerta, P. T. & Tonegawa, S. The essential role of hippocampal Ca1 NMDA receptor-dependent synaptic plasticity in spatial memory. Cell 87, 1327–1338 (1996).
32. Imprey, S. et al. Induction of CRE-mediated gene expression by stimuli that generate long-lasting LTP in area CA1 of the hippocampus. Neuron 16, 973–982 (1996).
33. Abel, T. et al. Genetic demonstration of a role for PKA in the late phase of LTP and in hippocampus-based long-term memory. Cell 88, 615–626 (1997).
34. Stubble-Weatherly, L., Harding, J. W. & Wright, J. W. Effects of discrete kainic acid-induced hippocampal lesions on spatial and contextual learning and memory in rats. Brain Res. 716, 29–38 (1996).
35. Frey, U., Krug, M., Reymann, K. G. & Matthies, H. Anisomycin, an inhibitor of protein synthesis, blocks late phases of LTP phenomena in the hippocampal CA1 region in vitro. Brain Res. 452, 57–65 (1988).
36. Alarcon, J. M. et al. Chromatin acetylation, memory, and LTP are impaired in CRBP–/– mice: a model for the cognitive deficit in Rubinstein-Taybi syndrome and its amelioration. Neuroreport 42, 947–959 (2004).
37. Huang, Y. Y. & Kandel, E. R. Recruitment of long-lasting and protein kinase A-dependent long-term potentiation in the CA1 region of hippocampus requires repeated tetanization. Learn. Mem. 1, 74–82 (1994).
38. Adams, J. P., Hudgins, E., Lundquist, J. I., Zhao, M. & Dudek, S. M. in Synaptic Plasticity and Transsynaptic Signaling (eds Stanton, P. K., Bramham, C. & Scharfman, H. E.) (Springer US, New York, 2005).
39. Wu, G. Y., Deisseroth, K. & Tsien, R. W. Activity-dependent CREB phosphorylation: convergence of a fast, sensitive calmodulin kinase pathway and a slow, less sensitive mitogen-activated protein kinase pathway. Proc. Natl Acad. Sci. USA 98, 2808–2813 (2001).
40. de Quervain, D. J. & Papassotiropoulos, A. Identification of a genetic cluster influencing memory performance and hippocampal activity in humans. Proc. Natl Acad. Sci. USA 103, 4270–4274 (2006).
41. Singla, S. I., Hudmon, A., Goldberg, J. M., Smith, J. L. & Schulman, H. Neuronal CA2+–/+ calmodulin-dependent protein kinase II: the role of structure and autoregulation in cellular function. Annu. Rev. Biochem. 71, 473–510 (2002).
42. Meyer, T., Hanson, P. I., Stryer, L. & Schulman, H. Calcium/calmodulin-dependent protein kinase II activity in hippocampal CA1 region in vitro. Neuron 276, 2935–2940 (2001).
43. Hudmon, A. & Schulman, H. Neuronal CA2+–/+ calmodulin-dependent protein kinase II: a model of NMDA-induced LTP and in hippocampus-based long-term memory. Neuron 16, 973–982 (1996).
44. Imprey, S. et al. Induction of CRE-mediated gene expression by stimuli that generate long-lasting LTP in area CA1 of the hippocampus. Neuron 16, 973–982 (1996).
45. Bader, P. L. et al. Mouse model of Timothy syndrome recapitulates triad of transcriptional defects. Proc. Natl Acad. Sci. USA 108, 15432–15437 (2011).
46. Peixoto, L. L. et al. Memory acquisition and retrieval impact different SW1/SNF complexes. Mol. Cell 60, 1020–1034 (2015).
47. Taubenfeld, S. M., Wiig, K. A., Bear, M. F. & Alberini, C. M. A molecular correlate of memory and amnesia in the hippocampus. Nat. Neurosci. 2, 309–310 (1999).
48. Putkey, J. A. & Waxham, M. N. A peptide model for calmodulin trapping by calcium/calmodulin-dependent protein kinase II. J. Biol. Chem. 271, 29619–29623 (1996).
49. Matsumoto, T. et al. Biosynthesis and processing of endogenous BDNF: CNS neurons store and secrete BDNF, not pro-BDNF. Nat. Neurosci. 11, 131–133 (2008).
50. Braun, D. J., Kalinin, S. & Feinstein, D. L. Conditional deletion of hippocampal brain-derived neurotrophic factor exacerbates neuropathology in a mouse model of Alzheimer’s disease. ASN Neuro 9, 17599011796161 (2017).
51. Tanantakii, T. et al. The impact of Bdnf gene deficiency to the memory impairment and brain pathology of APP{+}PS1{ΔE9} mouse model of Alzheimer’s disease. PLoS ONE 8, e68722 (2013).
52. Mukherji, S. & Soderling, T. R. Mutational analysis of Ca(2+)-independent autophosphorylation of calcium/calmodulin-dependent protein kinase II. J. Biol. Chem. 270, 14062–14067 (1995).
53. Waxham, M. N., Tsai, A. L. & Putkey, J. A. A mechanism for calmodulin (CaM) trapping by CaM-kinase II defined by a family of CaM-binding peptides. J. Biol. Chem. 273, 17579–17584 (1998).

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Author contributions

H.M. and R.W.T. conceived the project. S.M.C. performed calcium imaging, immunostaining for rescuing experiments in WT and exc-KO mice, and cell culture experiments related to NMDA signaling. B.S. performed LTP recordings and N.N.T. performed other electrophysiology experiments. X.H. did IA experiments and immunostaining after the tests. N.N.T. and C.M. performed the experiments to assess neuronal morphology. A.S. performed viral injections. H.M. performed all the other experiments with the assistance of X.W., G.Z., S.W., X.H., I.K., and S.S., H.M., S.M.C., and R.W.T. wrote the manuscript.

Additional information

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