Calcium stores regulate the polarity and input specificity of synaptic modification

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Activity-induced synaptic modification is essential for the development and plasticity of the nervous system[1–3]. Repetitive correlated activation of pre- and postsynaptic neurons can induce persistent enhancement or decrement of synaptic efficacy, commonly referred to as long-term potentiation or depression[2,3](LTP or LTD). An important unresolved issue is whether and to what extent LTP and LTD are restricted to the activated synapses[4–8]. Here we show that, in the CA1 region of the hippocampus, reduction of postsynaptic calcium influx by partial blockade of NMDA (*N*-methyl-D-aspartate) receptors results in a conversion of LTP to LTD and a loss of input specificity normally associated with LTP, with LTD appearing at heterosynaptic inputs. The induction of LTD at homo- and heterosynaptic sites requires functional ryanodine receptors and inositol triphosphate ($InsP_3$) receptors, respectively. Functional blockade or genetic deletion of type 1 $InsP_3$ receptors led to a conversion of LTD to LTP and elimination of heterosynaptic LTD, whereas blocking ryanodine receptors eliminated only homosynaptic LTD. Thus, postsynaptic Ca^{2+}, deriving from Ca^{2+} influx and differential release of Ca^{2+} from internal stores through ryanodine and $InsP_3$ receptors, regulates both the polarity and input specificity of activity-induced synaptic modification.

Spontaneous and induced theta and gamma oscillations have been implicated in regulating spatial memory in the hippocampus[9]. We first determined that correlated pre- and postsynaptic activation at theta frequency (5 Hz) can induce synaptic modifications in the CA1 region of rat hippocampal slices. Correlated activation comprises a train of stimuli at 5 Hz (for 16 s) delivered to one Schaffer collateral/commissural input, with each stimulus paired with postsynaptic injection of a spike-inducing depolarizing current (2 nA, 2 ms). When the onset of excitatory postsynaptic potentials (EPSPs) preceded the peak of postsynaptic action potentials by 5 ms ('positive' interval), the EPSCs of the stimulated ('homosynaptic') input showed a persistent increase in amplitude after the correlated activation, whereas that of unstimulated ('heterosynaptic' or control) input was not affected (Fig. 1a). When the onset of EPSPs was about 20 ms after ('negative' interval) postsynaptic spiking, the EPSC amplitude of both homo- and heterosynaptic inputs showed a persistent reduction following the correlated activation (Fig. 1b), suggesting induction of LTD in the stimulated pathway and the spread of LTD to the unstimulated pathway. We observed no synaptic modification when the interval between the pre- and postsynaptic activation was larger than 30 ms (Fig. 1c).

We further examined the dependence of synaptic modifications on the relative timing of pre- and postsynaptic activation. At the homosynaptic input, there is a 15-ms window for the induction of LTP and two distinct windows for the induction of LTD at −28 to −16 ms and at +15 to +20 ms (Fig. 2a). At heterosynaptic unstimulated inputs, we found no significant LTP, but two windows for LTD similar to that of the homosynaptic input (Fig. 2b, c). The potentiation window and the depression window at −28 to −16 ms are

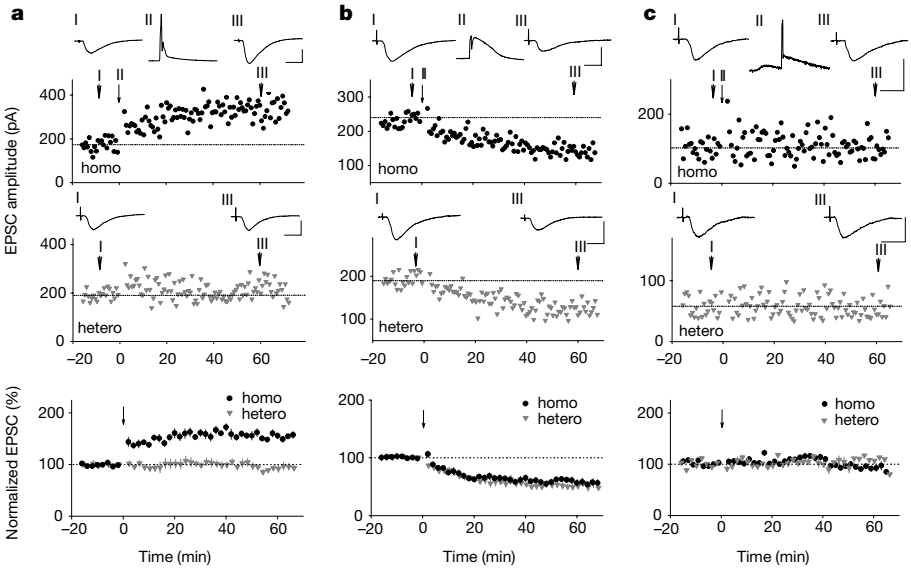

Figure 1 LTP/LTD induced by correlated pre- and postsynaptic activity in hippocampal CA1 pyramidal neurons. **a**, Top and middle, example of induction of LTP in the stimulated (homosynaptic) pathway and absence of LTP in the unstimulated (heterosynaptic) pathway. Data points represent the EPSC amplitude. Sample traces above: EPSCs (I, III) or membrane potential (II) at marked times. Postsynaptic spiking (5 Hz, 16 s) was elicited by depolarizing current pulses (2 nA, 2 ms) in current clamp ~5 ms after presynaptic field activation (5 Hz, 16 s). Scales: 200 pA (or 50 mV) and 20 ms. Bottom, summary of all results using correlated activation with positive intervals (+4 to +6 ms). Data represent mean EPSC amplitude (± s.e.m., $n = 9$). The mean EPSC was significantly elevated over the control ($t < 0$ min) at the homosynaptic ($t = 30–40$ min, $P < 0.001$, Mann–Whitney *U*-test), but not the heterosynaptic pathway. **b**, As in **a**, except that the interval for pre- and postsynaptic activation was −28 to −16 ms (–22 ms in **b** top and middle). Significant reduction of EPSC amplitude was observed in both stimulated and unstimulated pathways ($n = 10$, $P < 0.001$, Mann–Whitney *U*-test) at $t = 30–40$ min. Scales as in **a**. **c**, As in **a**, except that the interval for pre- and postsynaptic activation was < −30 ms or > +30 ms (44 ms in **c** top and middle). No significant change in EPSC amplitude was observed ($n = 6$). Scales: EPSCs, 200 (top) and 100 (middle) pA, 20 ms; potentials, 50 mV, 40 ms.

similar to those reported previously[10,11]. The appearance of an additional depression window may result from a different spatio-temporal pattern of Ca^{2+} elevation or the presence of inhibitory inputs in the slice preparation. The lateral spread of depression at flanking windows may help to reduce the background noise and sharpen the effect of potentiation at selected pathways that are activated within the 15-ms 'potentiation window'. The time interval between the potentiation and depression is close to that of a gamma cycle, a feature that may be related to the idea that sequence recall in a place cell is linked to gamma cycles—on a theta cycle[9].

To further explore the role of Ca^{2+} influx, we examined the effect of a partial blockade of NMDA subtype of glutamate receptors (NMDARs) on synaptic changes induced by the correlated activity (at 5 Hz for 16 s) of positive interval of +4 to +6 ms ('LTP protocol'). Complete block of LTP/LTD was found at 5 and 50 μM AP–5 (D(–)-amino-5-phosphonovaleric acid) (Fig. 3a, b). At 1–3 μM AP5, LTP was reduced or even converted to LTD at the homosynaptic input, consistent with a previous report[12]. Unexpectedly, we consistently observed LTD at the heterosynaptic input at these low AP5 concentrations (Fig. 3a, b). The extent of reduction in postsynaptic NMDAR activity was assayed by measuring NMDA components of EPSCs (Fig. 3c). Input specificity of LTP was lost when NMDAR activity was reduced by about 40% (at 1 μM AP5). These findings are consistent with the Bienenstock–Cooper–Munro (BCM) model

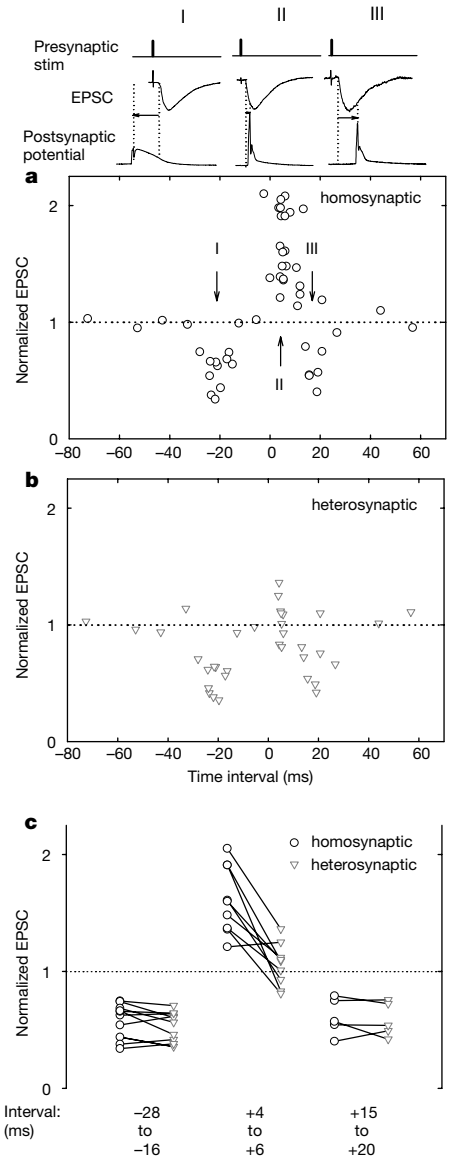

Figure 2 Critical time windows for the induction of LTP/LTD by correlated pre- and postsynaptic activation. Normalized mean EPSC amplitudes at 30–40 min after correlated activation are plotted against the interval between pre- and postsynaptic activation. The time interval refers to the time between the onset of the EPSC (top) and the peak of the postsynaptic action potential during each correlated activation. **a**, Summary of changes in the homosynaptic pathway ($n = 50$). **b**, Summary of changes in the heterosynaptic (unstimulated) pathway ($n = 34$). Data are from a subset of experiments such as those shown in **a**, in which the control pathway was monitored throughout the experiment. **c**, Changes of synaptic strength at the homo- and heterosynaptic pathways for experiments using time intervals of –28 to –16, +4 to +6 and +15 to +20 ms. Lines connect data points from the same experiment.

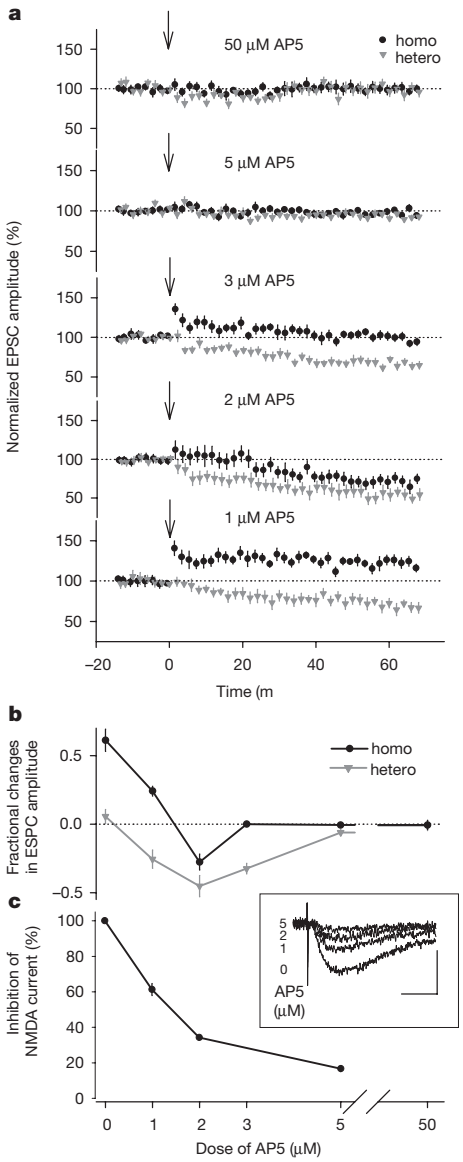

Figure 3 Effects of partial blockade of NMDA receptors. **a**, Experiments similar to that in Fig. 1a, except for the presence of AP5 (1–50 μM, $n = 5$ each). Correlated activity of positive intervals (+4 to +6 ms; 'LTP protocol') was applied at the time marked by the arrow. **b**, Dependence of synaptic modifications on the dose of AP5. The percentage change in the mean EPSC amplitude (at $t = 40$–50 min) was plotted for homo- and heterosynaptic pathways (same data as in **a**). **c**, Percentage reduction of the NMDA component of EPSCs in CA1 pyramidal neurons after sequential application of AP5 from 0–5 μM (in CNQX and bicuculline, 20 μM each, with 1 mM QX-314 in patch pipettes; (s.e.m., $n = 5$). Data are normalized to that observed at 0 μM AP5. Stimulus strength is the same as in LTP experiments (100–200 pA in the absence of CNQX and bicuculline). Inset, sample traces of NMDA currents. Scales: 20 pA, 50 ms.

of synaptic modification[13–15], assuming that heterosynaptic spread of LTD is due to the spread of postsynaptic Ca^{2+} elevation from homo- to heterosynaptic sites.

Release of Ca^{2+} from internal stores, triggered either by postsynaptic Ca^{2+} influx[16] or by activation of metabotropic glutamate receptors (mGluRs)[17] is involved in activity-induced synaptic modification. We thus examined the effect of a monoclonal antibody (18A10) against the type 1 $InsP_3$ receptor ($InsP_3R$), a predominant subtype of $InsP_3Rs$ found in CA1 pyramidal neurons and localized primarily in the dendritic shaft and soma[18]. The function-blocking activity of 18A10 was shown by its blocking effect on $InsP_3$-induced Ca^{2+} release from hippocampal microsomal fractions (see Supplementary Information). After postsynaptic loading of 18A10 (160 μg ml^{-1}), repetitive correlated activation with an interval of −24 to −20 ms ('LTD protocol') resulted in homosynaptic LTP, without significant heterosynaptic effect (Fig. 4a). Experiments in which control immunoglobulin-γ (IgG; 160 μg ml^{-1}) was used instead of 18A10 showed normal homo- and heterosynaptic LTD. Loading of 18A10 did not block the induction of LTP by the LTP protocol, but increased the extent of LTP (Fig. 5a). Unexpectedly, in 3 out of 6 cases, significant LTP was found also in the heterosynaptic pathway (Fig. 5a), indicating that there may be a loss of input specificity. Furthermore, using slices obtained from $InsP_3R$-deficient mice[19], we found that LTD protocol resulted in a slight LTP at the homosynaptic input and no synaptic change at the heterosynaptic site (Fig. 4b), whereas LTP protocol resulted in an elevated LTP at the homosynaptic input and a slight potentiation at the heterosynaptic input (Fig. 5b). Parallel recordings using slices from wild-type mice (Figs 4b, 5b) yielded results identical to those obtained from rat slices (Fig. 1a, b). Together, these results indicate that $InsP_3R$ activity determines the polarity and the extent of synaptic changes and is responsible for the heterosynaptic spread of LTD. The finding that heterosynaptic LTD normally triggered by tetanic stimulation in the CA1 area of hippocampus of the wild-type mice was completely absent in the type-1 $InsP_3R$ deficient mice (K.K., unpublished data) is also consistent with this idea.

Metabotropic GluR-dependent LTD may be induced at Schaffer collateral-CA1 synapses[20]. One of the downstream pathways of mGluR activation is the production of $InsP_3$ and the resultant Ca^{2+} release through $InsP_3Rs$[21]. After bath-application of an antagonist of mGluRs, (*S*)-α-methyl-4-carboxyphenylglycine (MCPG, 1 mM), stimulation of Schaffer collateral/commissural-CA1 synapses with the LTD protocol induced significant LTP in both homo- and heterosynaptic pathways, with the mean EPSC amplitude elevated to 142 ± 10 and 133 ± 13% (s.e.m., n = 7) of the control level, respectively, similar to that induced by antibody blockade or genetic deletion of $InsP_3Rs$. This is consistent with a MCPG-induced reduction of $InsP_3R$ activity, although the effect of MCPG on presynaptic mGluRs and other downstream effectors[20] cannot be excluded.

The above studies showed that reduction of functional $InsP_3Rs$ resulted in marked changes in the polarity and extent of synaptic modification: correlated activation of negative intervals led to LTP instead of LTD, whereas that of positive intervals led to a larger LTP. What is the cellular mechanisms underlying these changes? We found that the resting potential, membrane resistance, time constant, firing threshold, and the height and half-width of action potentials were not significantly different between the type 1 $InsP_3R$-deficient and wild-type mice (data not shown). However, we observed a marked reduction of spike frequency adaptation[22] in response to a long depolarizing step in mutant slices (see Supplementary Information), suggesting reduced K^+ channel activity in mutant mice. Ca^{2+}-activated K^+channels can be activated through Ca^{2+} released from internal stores[23]. Dysfunction of $InsP_3Rs$, therefore, may cause the inactivation of Ca^{2+}-activated K^+channels, resulting in an increased postsynaptic excitability. We also found that synaptic NMDA currents near the resting membrane potential, as recorded in the presence of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and bicuculline, were significantly higher in mutant slices or in wild-type slices after loading of the specific type-1 $InsP_3R$ antibody 18A10 (see Supplementary Information), suggesting a reduced blockade of NMDARs by Mg^{2+}. Thus, a higher level of local Ca^{2+} influx during correlated activation may be responsible for the observed changes in synaptic modification.

In addition to Ca^{2+} release mediated by $InsP_3Rs$, internal Ca^{2+} release through RyRs is also involved in the induction of LTD by a low frequency stimulation[24]. Here we found postsynaptic loading with ryanodine (100 μM), which blocks ryanodine receptors (RyRs) in the internal stores, resulted in a failure of standard LTD

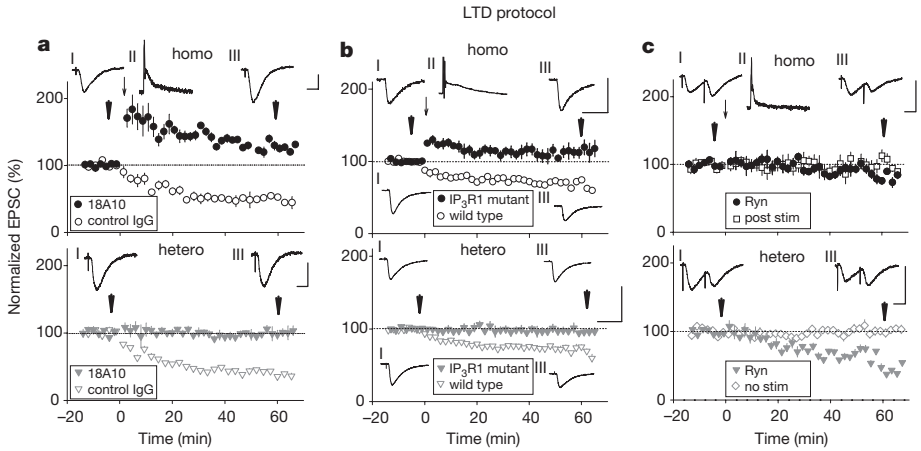

Figure 4 Effects of blocking Ca^{2+} release from internal stores on the induction of LTD. **a**, Postsynaptic neurons were loaded with a function-blocking antibody (18A10, 160 μg ml^{-1}; n = 6) against the type 1 $InsP_3R$ or control IgG (160 μg ml^{-1}; n = 3). For 18A10 loading, homosynaptic LTP instead of LTD (upper graph) and no heterosynaptic change (lower graph) were observed. For control IgG loading, significant LTD was observed in both pathways. Scales: 100 pA (30 mV), 20 ms. **b**, LTD protocol applied to slices obtained from $InsP_3R$-deficient mice (IP_3R1 mutant; n = 6) resulted in a slight LTP at the homosynaptic input (upper graph) and no heterosynaptic change (lower graph). The induction protocol was the same as that used for rat slices (Fig. 1b), except that the initial EPSC amplitude was in the range of 50–70 pA. Control experiments using slices from wild-type mice (n = 6) showed significant LTD in both homo- and heterosynaptic pathways. Scales: 100 pA (or 200 mV), 50 ms. **c**, LTD protocol applied to slices with postsynaptic loading of ryanodine (Ryn; 100 μM) resulted in no synaptic change at the homosynaptic pathway (upper graph) but LTD at heterosynaptic pathway (lower graph). Samples of paired EPSCs above show responses induced by sequential stimulation (interval 50 ms) of the homo- then heterosynaptic pathways (upper graph), and the hetero- then homosynaptic pathways (lower graph), respectively. Scales: 200 pA (or 100 mV), 20 ms. Control experiments: post stim, postsynaptic stimulation alone (n = 6); no stim, no pre- or postsynaptic activation (n = 4).

protocol in inducing homosynaptic LTD (Fig. 4c). Surprisingly, LTD was observed at the heterosynaptic input. These results were not due to the loading of ryanodine, as, for neurons loaded with ryanodine, repetitive postsynaptic activation alone ('post stim') or in the absence of any stimulation ('no stim') resulted in no change in synaptic efficacy (Fig. 4c). Thus, Ca^{2+} release from RyRs is essential for the induction of homosynaptic LTD by the correlated activity. Furthermore, after postsynaptic loading of ryanodine, standard LTP protocol induced an elevated level of homosynaptic LTP and a significant heterosynaptic LTD (Fig. 5c).

From our results, we propose the following model for the interplay of localized Ca^{2+} influx and Ca^{2+} release from internal stores (Fig. 6a). Ca^{2+} influx through NMDARs and voltage-dependent Ca^{2+} channels, together with mGluR activation, triggers $InsP_3R$-dependent Ca^{2+} release from internal stores, which in turn causes further Ca^{2+} release through RyRs[17,25]. When a high-level of Ca^{2+} transient is created, for example, under conditions that induce LTP, $InsP_3Rs$ become desensitized[26]. Furthermore, Ca^{2+} release from internal stores may reduce postsynaptic neuronal excitability through Ca^{2+}-activated K^+ channels[23] and activated $InsP_3Rs$ may also reduce NMDAR activity through an unknown mechanism. Consistent with the idea that the polarity of homosynaptic modification depends on the level of postsynaptic Ca^{2+}—a high-level Ca^{2+} triggers LTP, whereas a modest-level Ca^{2+} triggers LTD (see refs 27, 28)—reduction of NMDAR activity (which reduces Ca^{2+} influx) results in a reduced LTP or even a conversion to LTD (Fig. 3). Inhibition or deletion of $InsP_3Rs$ elevates neuronal excitability and NMDAR activity, resulting in a higher Ca^{2+} level and thus elevated LTP (Figs 4b, 5b). Inhibition of RyRs may also result in a higher Ca^{2+}, through reduced activation of K^+ channels[25], leading to the absence of homosynaptic LTD following LTD protocol, but an increased homosynaptic LTP following LTP protocol.

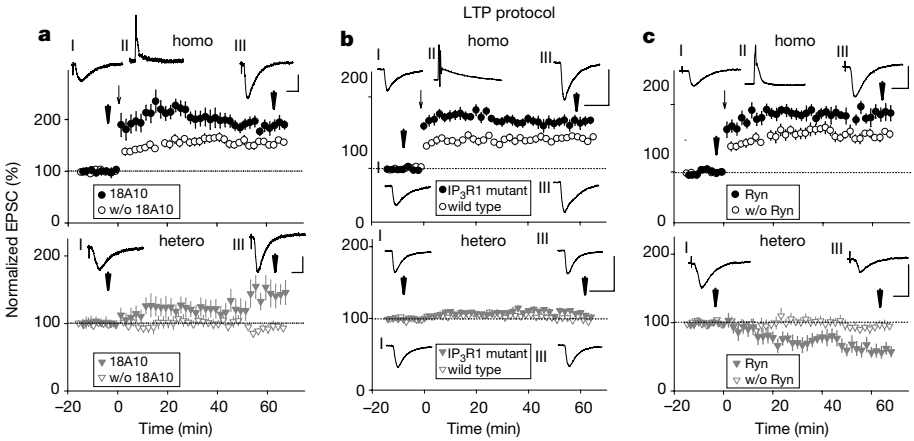

Figure 5 Effects of blocking internal Ca^{2+} release on the induction of LTP. **a**, As in Fig. 4a, except that LTP protocol was used (with 18A10 loading, $n = 6$). The extent of potentiation at $t = 30–40$ min was significantly greater ($P < 0.03$, Mann–Whitney U-test) than that found in the absence of antibody loading (w/o 18A10, same data as in Fig. 1a, bottom). Slight potentiation was also found in the heterosynaptic pathway. Scales: 100 pA (or 30 mV), 20 ms. **b**, As in Fig. 4b, except that LTP protocol was used ($n = 6$). Significant enhancement of homosynaptic LTP was observed (164 ± 8% and 142 ± 2% of the control for mutant and wild-type mice at $t = 30–40$ min, respectively, $P < 0.05$, t-test). No significant synaptic change was observed for the heterosynaptic input ($n = 6$, $P = 0.077$, t-test). **c**, Postsynaptic loading of ryanodine resulted in a greater extent of homosynaptic LTP after the standard LTP induction protocol ($n = 5$, $P < 0.03$, Mann–Whitney U-test) than that found in the absence of ryanodine (same data set as in Fig. 1, top). Marked depression was found in the heterosynaptic pathway. Scales as in **a**.

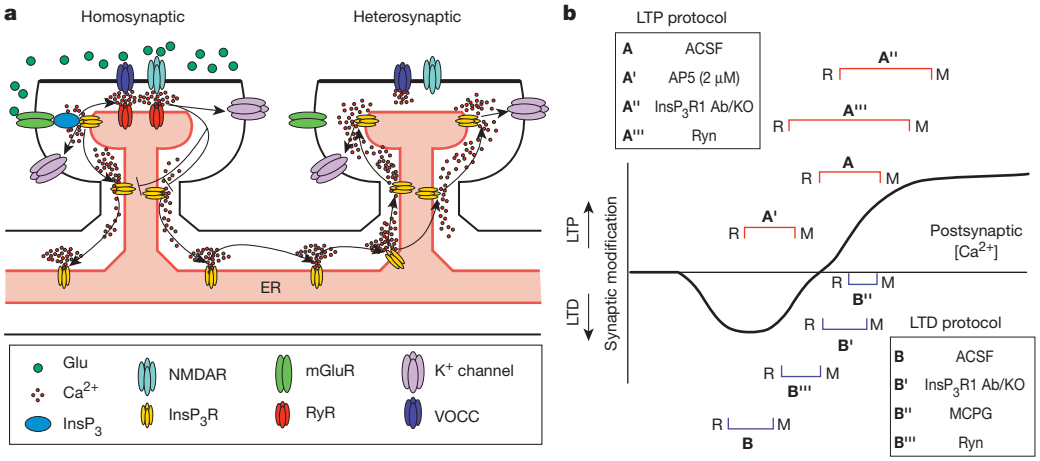

Figure 6 A model for the roles of postsynaptic Ca^{2+} signalling in synaptic modification. **a**, Postsynaptic Ca^{2+} regulation. Influx of Ca^{2+} through NMDARs and voltage-operated Ca^{2+} channels (VOCCs), together with mGluR-dependent Ca^{2+} release from $InsP_3Rs$, triggers further Ca^{2+} release from RyRs and $InsP_3R$-dependent propagation of Ca^{2+} waves in the dendrite. Localized high-level Ca^{2+} transients in the spine may desensitize $InsP_3Rs$, preventing propagation of Ca^{2+} waves. Release of Ca^{2+} from internal stores also activates plasma membrane K^+ channels. **b**, Dependence of synaptic modification on postsynaptic Ca^{2+}. The LTP and LTD induced by correlated activity of positive and negative intervals, respectively, at homosynaptic (M) and heterosynaptic (R) pathways are indicated by the bracket. For different experimental conditions, the polarity, degree, and input-specificity of synaptic modification are shifted in accord with the prediction of the BCM model (solid curve), assuming postsynaptic Ca^{2+} levels to be the determining variable. A–A‴ and B–B‴ (boxed) denote different experimental conditions. ACSF, artificial cerebrospinal fluid; Ab, antibody; KO, knock-out.

To account for the lack of input specificity for LTD and the dependence of input specificity on postsynaptic Ca^{2+}, we propose that synaptic activation by correlated activity results in Ca^{2+} elevation that spreads from the active synapses to distant unstimulated heterosynaptic sites by propagating Ca^{2+} waves[29] mediated by $InsP_3Rs$, which are localized primarily to the soma and dendritic shafts[18]. As illustrated by the BCM model for the induction of LTP/LTD[13–15] (Fig. 6b), standard LTP protocol (A) results in a high level of Ca^{2+} and LTP only at the homosynaptic input. No change is induced at the heterosynaptic site, because high-level Ca^{2+} elevation at the homosynaptic site had caused RyR-dependent desensitization of $InsP_3Rs$. Standard LTD protocol (B) results in a modest elevation of Ca^{2+} that leads to homo- and heterosynaptic LTD, the latter owing to $InsP_3R$-mediated long-range spread of Ca^{2+}. For modification induced by the LTP protocol, partial reduction of NMDAR-mediated Ca^{2+} influx results in a downward slide and LTD at both inputs (A′). Reduction of functional $InsP_3Rs$ led to an upward slide, with larger homosynaptic LTP and a loss of input specificity (A″). For the LTD protocol, an upward slide occurs after reduction of functional InsP3Rs by antibody blockade, genetic deletion (B′), or by MCPG (B″), leading to a conversion of LTD to LTP at the homosynaptic input and corresponding changes at the heterosynaptic site. Although blocking RyRs by ryanodine resulted in a higher level of local Ca^{2+}, and so upward shift of homosynaptic modification (A‴ and B‴), the lack of RyR-dependent desensitization of $InsP_3Rs$ allowed $InsP_3R$-dependent spread of LTD to heterosynaptic sites. By this account, input specificity in synaptic modification is not an intrinsic property associated with LTP or LTD, but a dynamic variable linked to the pattern of long-range Ca^{2+} signalling in the postsynaptic dendrite.

We have shown three important features of bi-directional synaptic modification induced by correlated activity in the CA1 region of the hippocampus. First, correlated activity of the same low frequency of 5 Hz can induce both LTP and LTD, depending on the precise timing of pre- and postsynaptic activation, suggesting a synaptic mechanism for processing and storage of information with a time resolution in the order of 10 ms. Second, the polarity and magnitude of synaptic changes are readily modified by manipulations of Ca^{2+} influx or Ca^{2+} release from internal stores in the postsynaptic neuron. Third, the input specificity depends on the pattern of postsynaptic Ca^{2+} and $InsP_3R$-mediated Ca^{2+} release is essential for the long-range propagation of LTD. Together, these results underscore the importance of spatiotemporal patterns of postsynaptic Ca^{2+}, for which the differential dendritic localization of $InsP_3$- and ryanodine-sensitive stores is critical. □

Methods

Hippocampal slice preparation

We prepared hippocampal slices according to the standard procedure[30]. Male albino rats (26–33 days), mice lacking type1 $InsP_3Rs$ and wild-type littermates (20–23 days) were anaesthetized with halothane and decapitated using a guillotine. Mouse genotypes were identified using a described procedure[19]. Hippocampi were dissected rapidly and placed in gassed (95% O_2 / 5% CO_2) extracellular solution at 10 °C containing (in mM): 124 NaCl, 3 KCl, 2.6 $CaCl_2$ (2.0 for mice), 1.3 $MgSO_4$ (2.0 for mice), 1.25 NaH_2PO_4, 22 $NaHCO_3$ and 10 glucose. Transverse slices (500-μm thick) were cut with a rotary tissue slicer (DTY7700) and maintained in an incubation chamber for at least 2 h at room temperature. For experiments, individual slices were transferred to a submersion recording chamber and perfused continuously with extracellular solution (~4.0–4.5 ml min^{-1}) at room temperature (23–26 °C).

Whole-cell recordings

Whole-cell recordings were made in the cell body layer of CA1 by the 'blind' patch-clamp method, using an Axopatch 200B amplifier (Axon Inst.). Stimuli were applied at 0.05 Hz and alternated between two non-overlapping Schaffer collateral/commissural inputs, using bipolar electrodes (MCE-100, RMI). The non-overlapping nature of the two inputs was confirmed by applying paired-pulse test stimuli (with 50-ms intervals) alternately to show the absence of cross facilitation between the two inputs (see traces in Fig. 4c). Constant current pulses (amplitude, 6–14 μA; duration, 300 μs) normally evoked EPSCs with amplitudes in the range of 100–200 pA. We considered only data with initial EPSC amplitude > 70 pA, as synaptic modification induced by correlated activity in these slices differs significantly between inputs with strength more than and less than 70 pA (M.N., *et al.*, unpublished data). Data were filtered at 2 kHz and digitized at 10 kHz. Patch electrodes were pulled from borosilicate glass (1.2-mm optical density) and had a resistance of ~3.5–6 MΩ. Pipettes were normally filled with a solution containing (in mM): 130 caesium methanesulphonate, 10 tetraethylammonium chloride, 5 NaCl, 0.25 1,2-bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid (BAPTA), 10 HEPES, 4 Mg-ATP, with pH adjusted to 7.35 (using CsOH). The series resistance was typically 10–14 MΩ and was compensated 50–80% during the experiment. Drugs were purchased from RBI (AP5), Calbiochem (ryanodine) or Tocris Cookson (MCPG). For intracellular loading of ryanodine and anti-$InsP_3R$ antibody, correlated activation was applied 15–20 min after break in.

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