The Journal of Neuroscience, November 1, 2002, 22(21):9237–9243

Kainate Receptor-Dependent Short-Term Plasticity of Presynaptic Ca^{2+} Influx at the Hippocampal Mossy Fiber Synapses

Haruyuki Kamiya,[1,2] **Seiji Ozawa,**[2,3] **and Toshiya Manabe**[1,4]

[1]*Division of Cell Biology and Neurophysiology, Department of Neuroscience, Faculty of Medicine, Kobe University, Kobe, Hyogo 650-0017, Japan,* [2]*Department of Physiology, Gunma University School of Medicine, Maebashi, Gunma 371-8511, Japan,* [3]*Core Research for Evolutional Science and Technology, Japan Science and Technology Corporation, Kawaguchi, Saitama 332-0012, Japan, and* [4]*Division of Neuronal Network, Department of Basic Medical Sciences, Institute of Medical Science, University of Tokyo, Tokyo 108-8639, Japan*

Transmitter release at the hippocampal mossy fiber (MF)–CA3 synapse exhibits robust use-dependent short-term plasticity with an extremely wide dynamic range. Recent studies revealed that presynaptic kainate receptors (KARs), which specifically localized on the MF axons, mediate unusually large facilitation at this particular synapse in concert with the action of residual Ca^{2+}. However, it is currently unclear how activation of kainate autoreceptors enhances transmitter release in an activity-dependent manner. Using fluorescence recordings of presynaptic Ca^{2+} and voltage in hippocampal slices, here we demonstrate that paired-pulse stimulation (with 20–200 msec intervals) resulted in facilitation of Ca^{2+} influx into the MF terminals, as opposed to other synapses, such as the Schaffer collateral–CA1 synapse. These observations deviate from typical residual Ca^{2+} hypothesis of facilitation, assuming an equal amount of Ca^{2+} influx per action potential. Pharmacological experiments reveal that the facilitation of presynaptic Ca^{2+} influx is mediated by activation of KARs. We also found that action potentials of MF axons are followed by prominent afterdepolarization, which is partly mediated by activation of KARs. Notably, the time course of the afterdepolarization approximates to that of the paired-pulse facilitation of Ca^{2+} influx, suggesting that these two processes are closely related to each other. These results suggest that the novel mechanism amplifying presynaptic Ca^{2+} influx may underlie the robust short-term synaptic plasticity at the MF–CA3 synapse in the hippocampus, and this process is mediated by KARs whose activation evokes prominent afterdepolarization of MF axons and thereby enhances action potential-driven Ca^{2+} influx into the presynaptic terminals.

Key words: hippocampus; kainate receptor; mossy fiber; paired-pulse facilitation; presynaptic Ca^{2+} influx; short-term plasticity

A prominent feature of transmission at chemical synapses involves modifiability of the strength of information transfer depending on the previous firing history of presynaptic neurons. Both short- and long-lasting forms of use-dependent modifications, referred to as short- and long-term synaptic plasticity, have been described among many central and peripheral synapses (Zucker, 1989; Bliss and Collingridge, 1993; Malenka and Nicoll, 1999). Long-term plasticity might underlie information storage in the CNS, whereas short-term plasticity plays pivotal roles in coding temporal patterns of the activity of the neuronal networks.

In the hippocampus, three principal excitatory connections [perforant path–dentate gyrus synapse, mossy fiber (MF)–CA3 synapse, and Schaffer collateral–CA1 synapse] display very different forms of short- and long-term plasticity (Nicoll and Malenka, 1995; Salin et al., 1996), suggesting that the specific functional roles of each of these synapses in hippocampal information processing may differ. Typically, the amount of transmitter released from hippocampal MF terminals is highly dependent on the frequency of afferent stimulation, and extremely large paired-pulse facilitation (PPF) is an experimental hallmark of MF synaptic transmission (Henze et al., 2000). Because short-term plasticity at this particular synapse displays an unusually wide dynamic range, we hypothesized that some additional mechanism other than the action of residual Ca^{2+} (Zucker, 1989; Zucker and Regehr, 2002) might be involved in activity-dependent tuning of the synaptic strength. The recent demonstration that presynaptic kainate receptors (KARs) (Kamiya and Ozawa, 2000; Kullmann, 2001; Lerma et al., 2001; Schmitz et al., 2001a; Kamiya, 2002) are specifically involved in the frequency facilitation (Schmitz et al., 2001b) prompted us to search for additional mechanisms underlying the unusually large PPF at the MF–CA3 synapse.

In the present study, we used optical measurement of presynaptic Ca^{2+} (Regehr and Tank, 1991; Wu and Saggau, 1994; Kamiya and Ozawa, 1999) and membrane potentials (Sabatini and Regehr, 1996, 1997) in hippocampal slices to elucidate precise cellular mechanisms underlying the robust short-term plasticity at the MF–CA3 synapse. We found that unusually large PPF at this synapse was accompanied by facilitation of stimulus-dependent presynaptic Ca^{2+} influx, as opposed to other synapses, such as Schaffer collateral–CA1 synapses in the hippocampus (Wu and Saggau, 1994; Kamiya and Ozawa; 1998) or parallel fiber synapses in the cerebellum (Regehr and Atluri, 1995; Kre-

Received June 18, 2002; revised Aug. 6, 2002; accepted Aug. 8, 2002.

This work was supported by Grants-in-Aid for Science Research (H.K., S.O., and T.M.), by Special Coordination Funds for Promoting Science and Technology (T.M.) from the Ministry of Education, Science, Sports, Culture and Technology of Japan, and by the grants from the Ichiro Kanehara Foundation and the Novartis Foundation (Japan) for the Promotion of Science (T.M.). We thank Prof. Atsu Aiba for reading this manuscript.

Correspondence should be addressed to Haruyuki Kamiya, Division of Cell Biology and Neurophysiology, Department of Neuroscience, Faculty of Medicine, Kobe University, Kobe, Hyogo 650-0017, Japan. E-mail: hkamiya-kob@umin.ac.jp.

 0270-6474/02/229237-07$15.00/0

itzer and Regehr, 2000). Pharmacological analysis revealed that this novel mechanism amplifying presynaptic Ca^{2+} influx is mediated by kainate autoreceptors specifically localized on the MF axons (Kullmann, 2001; Schmitz et al., 2001a; Kamiya, 2002). It should be noted that this unique autoreceptor system operates substantially by only a single preceding stimulus, as demonstrated by the prominent afterdepolarization of presynaptic axons revealed using voltage-sensitive dye. The evidence for activation of presynaptic kainate receptors by a single stimulus contrasts sharply with the fact that postsynaptic kainate receptors at this synapse are activated substantially only by repeated stimuli (Castillo et al., 1997; Vignes and Collingridge, 1997). Our results suggest that activation of kainate autoreceptors evokes prominent afterdepolarization and thereby modulates action potential-driven Ca^{2+} influx into the presynaptic terminals in an activity-dependent manner.

MATERIALS AND METHODS

Transverse hippocampal slices (~400 μm thick) were prepared from BALB/c mice (14–20 d of age). All experiments were performed according to the guidelines laid down by the Animal Care and Experimentation Committee of Gunma University and Kobe University. Mossy fibers were stimulated at the stratum granulosum in the dentate gyrus, and the resultant field EPSPs were recorded from the stratum lucidum in the CA3 region. Slices were continuously superfused with the solution composed of the following (in mM):127 NaCl, 1.5 KCl, 1.2 KH_2PO_4, 2.4 $CaCl_2$, 1.3 $MgSO_4$, 26 $NaHCO_3$, and 10 glucose. The solution was equilibrated with 95% O_2 and 5% CO_2. Fluorescence recordings of presynaptic Ca^{2+} were made as described previously (Kamiya and Ozawa, 1999). Briefly, rhod-2 AM (Dojindo Laboratory, Kumamoto, Japan), a membrane-permeable Ca^{2+} indicator, was loaded into the MF terminals without severing the axons (Regehr and Tank, 1991). The dye was injected locally into the stratum lucidum, resulting in selective labeling of the mossy fibers (Kamiya and Ozawa, 1999). The fluorescence (excitation at 510–560 nm and monitoring above 580 nm) from the area (~100 μm diameter) containing the labeled terminals was measured with a single photodiode (S2281–01; Hamamatsu Photonics, Hamamatsu, Japan). The $\Delta F/F$ value evoked by a single electrical stimulus was used as a measure of $[Ca^{2+}]_i$ increase during an action potential. For the optical measurement of presynaptic voltage, fluorescent voltage-sensitive dye (di-8-ANEPPS; Molecular Probes, Eugene, OR) was injected locally into the axon bundles (stratum lucidum) (see Fig. 7*A*). Four to 6 hr after the injection, the fluorescence transient was also measured with the photodiode. The fluorescence (monitored in the same wavelength range as noted above) decreased transiently in response to the stimulation of MF. In Figures 7*B* and 8*A*, the decrease in fluorescence was illustrated as an upward deflection. The output of the photodiode was *I–V* converted, amplified, and filtered at 500 Hz with an eight-pole Bessel filter (FLA-1; Cygnus Technology, Delaware Water Gap, PA). The signal was then digitized with a 12 bit analog-to-digital converter (Digidata 1200A; Axon instruments, Foster City, CA) and acquired at 10 kHz using pClamp8 software (Axon Instruments). The values in the text and figures are expressed as mean ± SEM (the number of experiments). Statistical analysis was performed using the paired *t* test, and $p < 0.05$ was accepted for statistical significance.

RESULTS

Short-term plasticity of presynaptic Ca^{2+} transients at the MF–CA3 synapse

First we addressed why the MF synapse exhibits unusually large PPF. One obvious possibility is that the amount of presynaptic Ca^{2+} influx per action potential is modified in an activity-dependent manner (Jackson et al., 1991; Borst and Sakmann, 1998; Cuttle et al., 1998). To test this possibility directly, we optically measured the amount of presynaptic Ca^{2+} influx at the MF–CA3 synapse (Kamiya and Ozawa, 1999). Figure 1*A* shows representative presynaptic Ca^{2+} transients (F_{Ca}) when single or paired stimulation was given to the mossy fibers. The amplitude

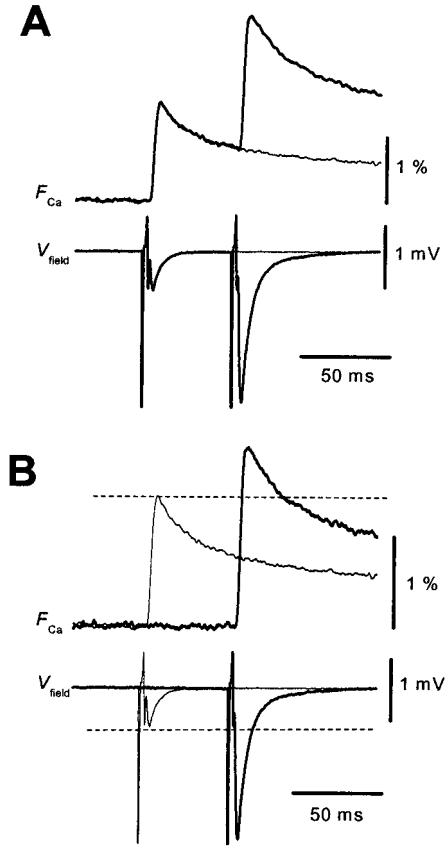

Figure 1. PPF of presynaptic Ca^{2+} transients at the MF–CA3 synapse. *A*, Representative records of presynaptic Ca^{2+} transients (F_{Ca}) and simultaneously recorded field EPSPs (V_{field}) evoked by single (*thin traces*) or paired-pulse stimulation (*thick traces*) with an ISI of 50 msec. *Traces* are the average of 10 sweeps. *B*, The second response to the paired stimulation was extracted by subtracting that evoked by the single stimulus and superimposed with the single response for comparison. Note that the second response was considerably larger than the first one.

of the fluorescence transient elicited in response to the second stimulus was considerably larger than that elicited by the first stimulus (Fig. 1*B*). This effect lasted for several hundreds of milliseconds (Fig. 2*A*,*B*). At interstimulus intervals (ISI) of 50 msec, the ratio of the second response to the first one (F_2/F_1) was 121 ± 2% ($n = 22$). This result was in contrast with that found for the Schaffer collateral–CA1 synapse (Wu and Saggau, 1994; Kamiya and Ozawa; 1998), in which the ratio never exceeds 1. It should be noted that EPSPs showed substantial PPF at ISIs longer than 300 msec, whereas the facilitation of Ca^{2+} transients disappeared completely at the time. This finding suggests that facilitation is attributable, at least in part, to mechanisms other than the increase in F_{Ca}.

We next explored the mechanism of PPF of F_{Ca}. This phenomenon may reflect increased Ca^{2+} influx during paired stimuli (Jackson et al., 1991; Borst and Sakmann, 1998; Cuttle et al., 1998; Brody and Yue, 2000; Lee et al., 2000; DeMaria et al., 2001; Currie and Fox, 2002; Tsujimoto et al., 2002). Alternatively, it may be attributable to saturation of the endogenous mobile high-affinity Ca^{2+} buffer (Neher, 1998). Modulation of the degree of saturation of endogenous Ca^{2+} buffers by the Ca^{2+} influx elicited by the first action potential could result in enhanced transmitter release simply by an increased increment of intraterminal Ca^{2+}. In fact, it has been demonstrated that such a supralinear summa-

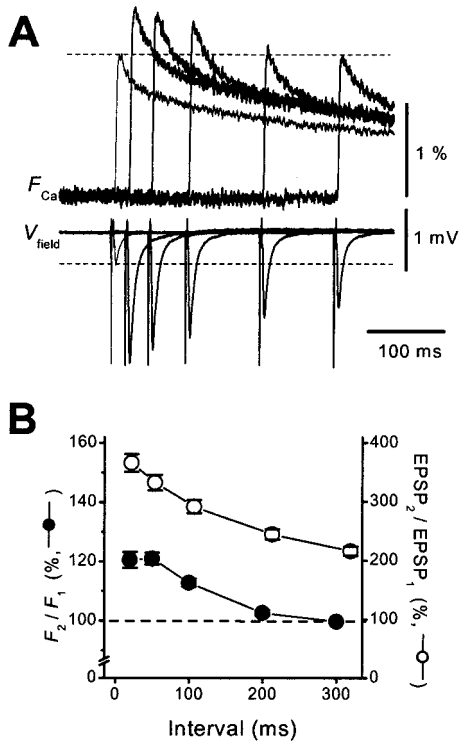

Figure 2. Time course of facilitation of F_{Ca}. *A*, The first and second responses were calculated and displayed as in Figure 1*B*. *B*, Ratios of F_{Ca} (F_2/F_1; ●) and field EPSPs ($EPSP_2/EPSP_1$; ○) were plotted against ISI (20–300 msec; $n = 22$).

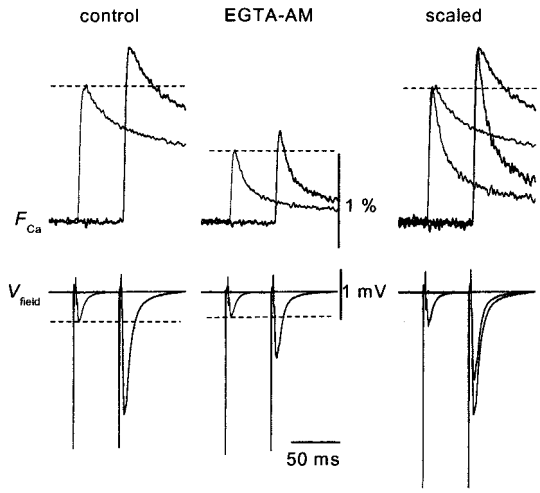

Figure 3. Effect of 100 μM EGTA AM, a membrane-permeable Ca^{2+} chelator, on PPF of F_{Ca} (50 msec ISI). Application of EGTA AM reduced the amplitude and accelerated the decay time course of the Ca^{2+} signal, confirming the loading of the presynaptic terminals with EGTA in these experimental conditions. However, the facilitation ratio did not change significantly, as demonstrated by the peak-scaled traces in the *right panels*.

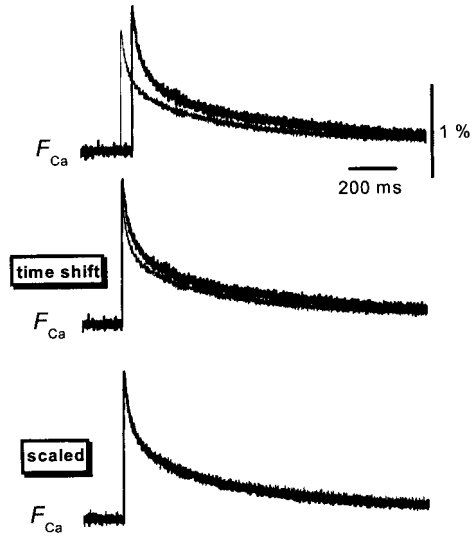

Figure 4. Comparison of the time course of the F_{Ca} evoked by the first and second stimuli delivered at 50 msec ISI. The *top traces* are the superimposition of the first and second responses calculated as in Figure 1*B*. In the *middle traces*, the second response is shifted for 50 msec to adjust for the timing of the stimulus. Note the lack of obvious difference in the time course of these signals, as demonstrated in the *bottom traces*, in which the peak amplitudes were scaled.

tion of Ca^{2+} transients in response to repetitive depolarizing pulses occurs in cerebellar Purkinje cells (Maeda et al., 1999), which express a high-affinity Ca^{2+} binding protein calbindin D_{28k}. This possibility of saturable Ca^{2+} buffer must be carefully explored, because calbindin D_{28k} is exclusively expressed in hippocampal MF terminals, and knock-out mice of this protein exhibit reduced PPF at MF–CA3 synapses but not at CA1 synapses (Klapstein et al., 1998). Altered short-term plasticity has also been reported recently in knock-out mice of parvalbumin (Caillardet al., 2000), which is another Ca^{2+} binding protein with an EF-hand motif. To test the possibility of Ca^{2+} buffer saturation, we used the membrane-permeable slow Ca^{2+} chelator EGTA AM (Atluri and Regehr, 1996; Salin et al., 1996) to perturb intraterminal Ca^{2+} buffering. Bath application of 100 μM EGTA AM for 20 min reduced the amplitude of the EPSP and F_{Ca} to 81 ± 8 and 63 ± 4% of the control levels ($n = 8$) (Fig. 3), respectively. The slight inhibition of the first EPSP might suggest that the release sites are not in the immediate vicinity of the Ca^{2+} channels at this particular synapse (Salin et al., 1996). On the other hand, the ratio F_2/F_1 was not changed significantly by application of EGTA AM (121 ± 4 and 119 ± 3% in the absence and presence of EGTA AM, respectively; $n = 8$). This result suggests that saturation of endogenous Ca^{2+} buffer during PPF is not significant at this synapse, and observed facilitation of F_{Ca} is likely to be explained by genuine facilitation of presynaptic Ca^{2+} influx. In line with this notion, the time course of the facilitated F_{Ca} was not significantly different from that of the unconditioned responses (Fig. 4).

Another possible mechanism is the facilitation of the Ca^{2+} current attributable to either the acceleration of activation (Borst and Sakmann, 1998; Cuttle et al., 1998; Tsujimoto et al., 2002) or the relief of G-protein inhibition (Park and Dunlap, 1998; Brody and Yue, 2000). MF terminals express several G-protein-coupled autoreceptors whose activation leads to inhibition of presynaptic Ca^{2+} currents. Among them, adenosine A_1 and $GABA_B$ receptors are activated tonically, whereas group II metabotropic glutamate (mGlu) receptors are not (Yamamoto et al., 1993; Kamiya et al., 1996; Vogt and Nicoll, 1999). Therefore voltage-dependent relief of tonic inhibition of Ca^{2+} channels through A_1 or $GABA_B$ receptors might occur during paired stimuli. To test this possibility, we next examined the effect of pharmacological activation of these G-protein-coupled autoreceptors. Application of a selective agonist of A_1 receptors, 2-chloro-adenosine (2-CA) at 10 μM,

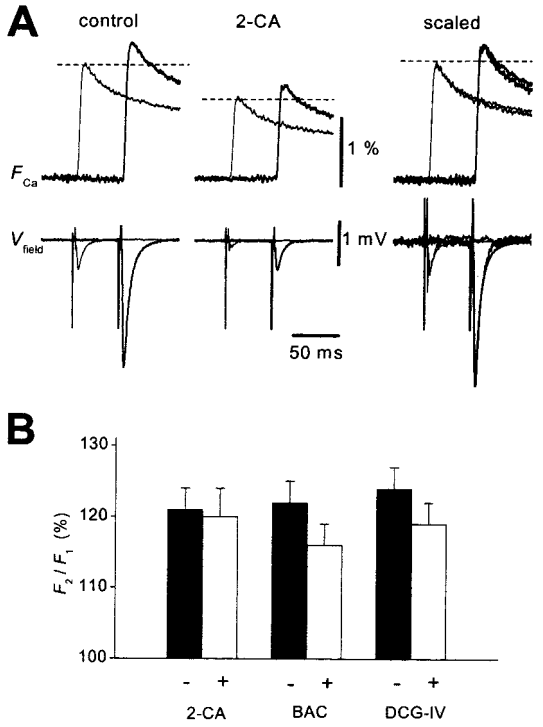

Figure 5. PPF of F_{Ca} is unchanged during inhibition of Ca^{2+} channels via G-protein-coupled metabotropic receptors. *A*, Representative records of F_{Ca} evoked by paired-pulse stimulation (50 msec ISI) before (*left*) and after (*middle*) application of 10 μM 2-CA, an agonist of adenosine A_1 receptor. 2-CA reduced both the first and second responses to a similar degree, whereas the facilitation ratio did not change significantly (scaled traces; *right*). *B*, Summary graph for the effects of 2-CA (10 μM; $n = 7$), the $GABA_B$ receptor agonist baclofen (*Bac*; 10 μM; $n = 6$), and the group II metabotropic glutamate receptor agonist DCG-IV (1 μM; $n = 6$) on the facilitation ratio of the F_{Ca} (F_2/F_1).

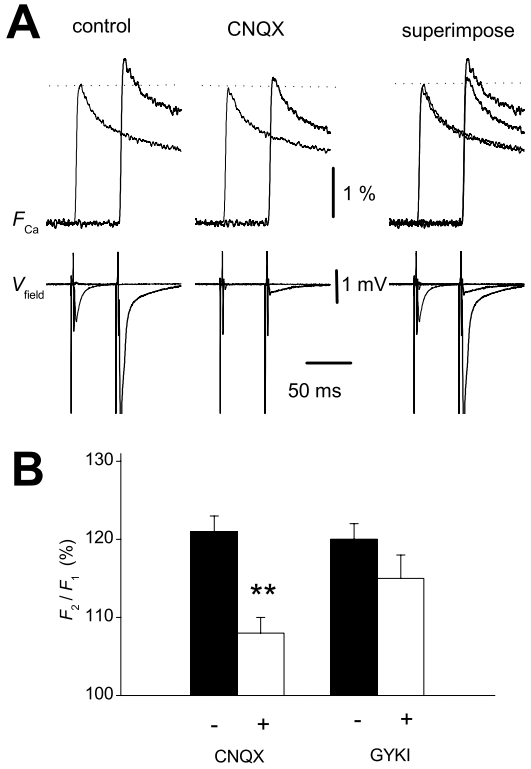

Figure 6. KAR involvement in PPF of F_{Ca}. *A*, Effect of CNQX on PPF of F_{Ca}. Application of 10 μM CNQX suppressed the second response, whereas the first one was little affected. Selective inhibition of the facilitation ratio by CNQX is revealed in the superimposed (*right*) traces. *B*, Summary graph for the effect of 10 μM CNQX ($n = 10$) and the AMPA receptor-selective antagonist GYKI 52466 (100 μM; $n = 7$; $^{**}p < 0.01$).

reduced the amplitude of the first EPSP and F_{Ca} to 22 ± 4 and 62 ± 4% of the control value ($n = 7$), respectively. However, the ratio of the second response to the first one (F_2/F_1) did not change significantly (121 ± 3 and 120 ± 4% in the absence and presence of 2-CA, respectively; $n = 7$) (Fig. 5*A*,*B*). Similar results were obtained for the $GABA_B$ receptor agonist baclofen and the group II mGlu receptor agonist (2*S*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl)glycine (DCG-IV). Baclofen at 10 μM decreased the first EPSP and F_{Ca} to 14 ± 4 and 60 ± 3% of the control value ($n = 6$), respectively, but the ratio F_2/F_1 did not change significantly (122 ± 2 and 116 ± 3% in the absence and presence of baclofen, respectively; $n = 6$) (Fig. 5*B*). DCG-IV at 1 μM also decreased the first EPSP and F_{Ca} to 11 ± 5 and 64 ± 3% of the control value ($n = 6$), but the ratio did not change significantly (124 ± 3 and 119 ± 3% in the absence and presence of DCG-IV, respectively; $n = 6$) (Fig. 5*B*). Thus, it is unlikely that relief of G-protein inhibition of Ca^{2+} channels (Park and Dunlap, 1998; Brody and Yue, 2000) is involved in the PPF of F_{Ca} at this synapse.

Involvement of kainate autoreceptors in PPF of presynaptic Ca^{2+} transients

Schmitz et al. (2001b) reported that presynaptic KARs (Kullmann, 2001; Schmitz et al., 2001a; Kamiya, 2002), unique autoreceptors whose activation leads to the enhancement of transmitter release (Turecek and Trussell, 2001), might specifically contribute to frequency facilitation at this synapse. We therefore examined the involvement of KARs in PPF of presynaptic Ca^{2+} influx. For this purpose, we tested the effect of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), a non-NMDA receptor antagonist. As reported previously (Kamiya and Ozawa, 1999), CNQX at 10 μM, which suppressed field EPSPs completely, did not affect the F_{Ca} in response to a single stimulus (97 ± 2% of control; $n = 10$), suggesting that the fluorescence signals originated exclusively from the presynaptic structure in these measurements. In contrast, PPF of F_{Ca} was selectively reduced by application of CNQX (121 ± 2 and 108 ± 2% in the absence and presence of CNQX, respectively; $n = 10$; $p < 0.01$) (Fig. 6*A*,*B*). The AMPA receptor-selective blocker 1-(4-aminophenyl)-4-methyl-7,8-methylene-dioxy-5H-2,3-benzodiazepine (GYKI 52466) hydrochloride at 100 μM, which also abolished field EPSP completely, did not mimic this effect (120 ± 2 and 115 ± 3% in the absence and presence of GYKI 52466; $n = 7$; $p = 0.10$) (Fig. 6*B*), suggesting that activation of KARs underlies PPF of presynaptic Ca^{2+} influx. The observation that blocking KARs affect PPF of the F_{Ca} raised the question of whether presynaptic KARs are activated substantially during paired-pulse protocols. To examine whether the preceding "single" stimulus is able to activate presynaptic KARs and to cause substantial axonal depolarization, we used optical measurement after selective labeling of MF with the voltage-sensitive dye di-8-ANEPPS (Sabatini and Regehr, 1996, 1997) as in Figure 7*A*. The presynaptic voltage transient (F_v) consists of fast and slow components (Fig. 7*B*), possibly representing action potential and afterdepolarization of MF axons (Geiger and Jonas, 2000). Application of 10 μM CNQX reduced the amplitudes of the slow but not the fast components (52 ± 2 and 96 ± 1% of control, respectively; $n = 12$;

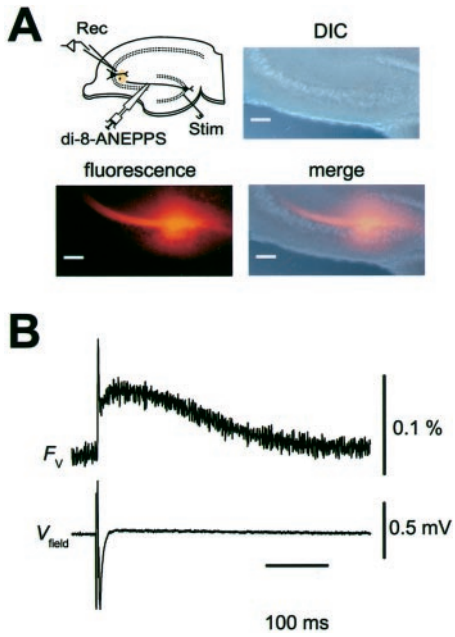

Figure 7. Optical recordings of presynaptic voltage transient (F_v) at MF–CA3 synapse. *A*, Selective loading of MF with the voltage-sensitive dye di-8-ANEPPS. Locally injected di-8-ANEPPS diffused along MF axons in the stratum lucidum. Changes in fluorescence intensity (F_v) were measured at the synaptic area distant from the injection site. Scale bars, 100 μm. *Rec*, Recording electrode; *Stim*, stimulating electrode. *B*, Representative records of presynaptic voltage transient (F_v) and simultaneously recorded V_{field}. *Traces* are the average of 16 trials. F_v consisted of fast and slow components, possibly representing action potential and afterdepolarization of MF axons.

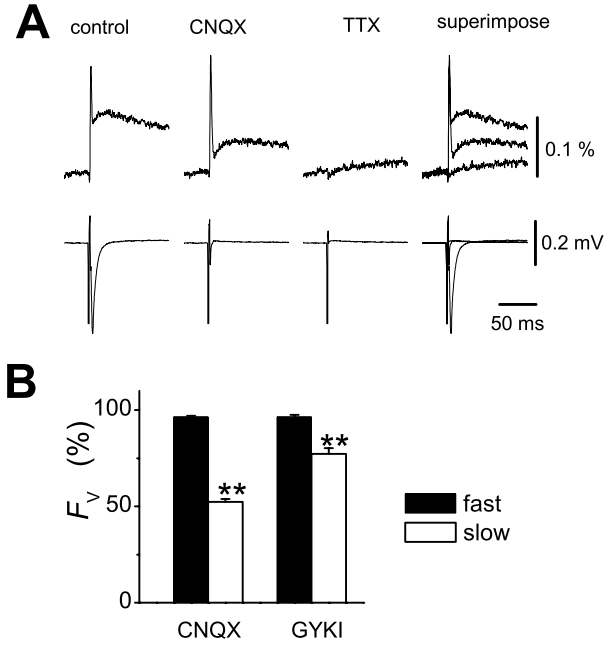

Figure 8. Evidence for activation of kainate autoreceptors by a single stimulus. *A*, Effects of CNQX on F_V. Application of 10 μM CNQX selectively reduced the amplitude of the slow but not the fast components of F_v. *B*, Summary graph for the effects of 10 μM CNQX ($n = 12$) and 100 μM GYKI 52466 ($n = 5$) on the fast and slow components of F_v.

$p < 0.01$) (Fig. 8*A*,*B*), whereas 1 μM TTX blocked both components of the signal. GYKI 52466 at 100 μM suppressed the slow component to 77 ± 3%, whereas the fast component was little affected (96 ± 1%; $n = 5$; $p < 0.01$) (Fig. 8*B*). The effect of GYKI 52466 on the slow component of F_v was weaker than that of CNQX (77 ± 3 and 52 ± 2%, respectively; $p < 0.01$). These results indicate that kainate autoreceptors mediate a prominent part of afterdepolarization of MF axons and thereby modulate the amount of presynaptic Ca^{2+} influx elicited by a subsequent stimulus. In support of this notion, the time course of the slow component of F_v (Fig. 7*B*) approximates to that of the PPF of F_{Ca} (Fig. 2*B*, *filled circles*).

DISCUSSION

Activity-dependent plasticity of presynaptic Ca^{2+} influx at the MF-CA3 synapse

Although the residual Ca^{2+} hypothesis has often been postulated to explain PPF (Zucker, 1989), recent evidence suggests that considerable revision of this hypothesis may be needed for some synapses (Zucker and Regehr, 2002). One aspect of the revision is involvement of a different Ca^{2+}-dependent process from exocytosis (Kamiya and Zucker, 1994; Atluri and Regehr, 1996; Zucker and Regehr, 2002). Another consideration is whether the amount of presynaptic Ca^{2+} influx per action potential is modified in an activity-dependent manner (Jackson et al., 1991; Borst and Sakmann, 1998; Cuttle et al., 1998; Brody and Yue, 2000; Lee et al., 2000; DeMaria et al., 2001; Currie and Fox, 2002; Tsujimoto et al., 2002). We demonstrated here that unusually large PPF at the hippocampal MF–CA3 synapse was accompanied by facilitation of presynaptic Ca^{2+} transients (F_{Ca}), in contrast to the Schaffer collateral–CA1 synapse (Wu and Saggau, 1994; Kamiya and Ozawa, 1998).

One may argue that recruitment of more fibers with the second stimulus underlies the observed facilitation of F_{Ca}. Afterdepolarization of MF axons (Fig. 7) (Geiger and Jonas, 2000) may lower threshold for the second stimulus. However, Schmitz et al. (2000) demonstrated that the site of activation of KA autoreceptors are restricted in the stratum lucidum (MF termination zone) but not in the granule cell layer. Because the stimulating electrode was placed in the granule cell layer in the present study, recruitment of subthreshold fibers is less likely to contribute to the results. In support of this idea, the amplitude of presynaptic fiber volley was not augmented by paired stimuli, as illustrated in Figure 1*A*.

Other possible artifacts, e.g., saturation of the Ca^{2+} indicator or polarization of stimulating electrode, would be expected to counteract the facilitation of F_{Ca}. Consistent with this notion, presynaptic Ca^{2+} transients at the CA1 synapse, which exhibits smaller PPF than at the MF–CA3 synapse, decrease slightly in response to paired stimuli attributable to saturation of the high-affinity dye (Wu and Saggau, 1994).

The effect of EGTA AM was complicated by the fact that it affects both the time course and the amplitude of the fluorescence transients. Bath application of 100 μM EGTA AM reduced the amplitude of F_{Ca} by 37% and that of EPSPs by 19% on average. Analysis of the quantitative relationships between EPSPs and F_{Ca} by changing extracellular Ca^{2+} concentrations (Kamiya and Ozawa, 1999) revealed a supralinear relationship at this particular synapse. Therefore, we suppose that sublinear dependency of EPSPs on F_{Ca} during EGTA AM treatment does not imply that transmitter release at this synapse is very weakly sensitive to Ca^{2+} but rather reflects the limited spatial and temporal resolution of the recording system. Our methods do not allow detection of the localized peak Ca^{2+} transients at active zone that is responsible for transmitter release but only provide a measure for

the volume-averaged Ca^{2+} changes within the whole presynaptic terminals. Because of this limitation, a slow Ca^{2+} chelator such as EGTA would be expected to preferentially suppress the volume-averaged Ca^{2+} transient (which we measured in this study) with relatively small effects on the large, brief calcium increases that trigger release. In support of this idea, Atluri and Regehr (1996) also reported sublinear dependency (decrease in peak Ca^{2+} by 45% and reduction of EPSC by 42%) during EGTA AM application at 100 μM (the same concentration as in this study) in the similar multifiber presynaptic Ca^{2+} measurement in cerebellar synapses using lower affinity dyes, which is expected to reflect undistorted Ca^{2+} transients. More importantly, EGTA did not affect the facilitation ratio of the F_{Ca} (Fig. 3), suggesting that PPF of the F_{Ca} is less likely attributable to the possible saturation of the endogenous Ca^{2+} buffer.

With these considerations, we conclude that the activity-dependent short-term plasticity of F_{Ca} at the MF–CA3 synapses is most likely interpretable by facilitation of presynaptic Ca^{2+} influx. This novel mechanism of amplifying Ca^{2+} signaling within the presynaptic MF terminals supports an extremely wide dynamic range of activity-dependent regulation of the synaptic efficacy (Salin et al., 1996; Henze et al., 2000) in concert with the action of residual Ca^{2+} (Regehr et al., 1994).

Kainate autoreceptor involvement in PPF of presynaptic Ca^{2+} influx

We demonstrated pharmacologically that KARs are involved in PPF of F_{Ca} and prominent afterdepolarization of MFs. However, it should be noted that GYKI 52466 at 100 μM weakly reduced both PPF of Ca^{2+} signals (although statistically not significant) and presynaptic afterdepolarization, as shown in Figures 6*B* and 8*B*. These findings may reflect relatively poor selectivity of this antagonist for AMPA versus KA receptors. Although GYKI 52466 is the most selective commercially available AMPA receptor-selective antagonist, it was reported that 100 μM GYKI 52466 weakly inhibited KARs (to ~70–80% of control) while almost completely blocking AMPA receptors in cultured hippocampal neurons (Paternain et al., 1995).

One missing link in this study is whether facilitation of F_{Ca} mediates synaptic PPF. The suppression of facilitation of F_{Ca} by CNQX does, however, strongly support a causal relationship. Although CNQX blocked field EPSPs and therefore may not be used to examine the effect on synaptic PPF, Schmitz et al. (2001b) bypassed this problem by measuring NMDA receptor-mediated EPSCs ($EPSC_{NMDA}$) at positive membrane potential and found that CNQX reduces facilitation of $EPSC_{NMDA}$ during 25 Hz (40 msec ISI) train (close to our conditions of 50 msec ISI) (Fig. 6) without affecting the first responses. The similar (but not identical) time course between them (Fig. 2*B*) also strongly suggests that F_{Ca} facilitation underlies synaptic PPF.

How does activation of kainate autoreceptors lead to facilitation of presynaptic Ca^{2+} influx? It is possible that depolarization of MF axons (Geiger and Jonas, 2000) may inactivate K^+ channels shaping repolarization of presynaptic action potentials, thereby increasing Ca^{2+} influx. However, the results obtained by direct whole-terminal recordings from MF boutons (Geiger and Jonas, 2000) suggests that broadening of action potentials is minimal with the PPF protocol used in this study (e.g., 1.3% prolongation per action potential at 50 Hz). In fact, the duration of the fast component of F_V was not prolonged by paired stimuli delivered at 50 msec ISI (H. Kamiya, unpublished observation).

Another possible mechanism is the facilitation of presynaptic Ca^{2+} channels. Whole-cell recordings from the calyx-type presynaptic terminals in the brainstem have revealed that depolarizing prepulses resulted in shot-term facilitation of the presynaptic Ca^{2+} current (Borst and Sakmann, 1998; Cuttle et al., 1998; Currie and Fox, 2002). It was demonstrated that calmodulin (Lee et al., 2000; DeMaria et al., 2001) or neuronal calcium sensor 1 (Tsujimoto et al., 2002) is involved in this action. Because fluorescence measurement of presynaptic voltage revealed prominent afterdepolarization of MF axons after a single stimulus (Fig. 7*B*) (Geiger and Jonas, 2000), the first action potential as well as the subsequent afterdepolarization may modify the state of Ca^{2+} channels and thereby facilitate Ca^{2+} current in response to the second action potential. It should be noted that, although it has been proposed that relief of G-protein inhibition of Ca^{2+} channels is involved in short-term plasticity in cultured hippocampal neurons (Brody and Yue, 2000), this mechanism was not responsible for facilitation of presynaptic Ca^{2+} influx observed in this study, because the pharmacological activation of G-protein-coupled metabotropic receptors failed to affect this phenomenon significantly (Fig. 5). Slight decrease in the F_2/F_1 ratio by 2-CA, baclofen, or DCG-IV, although statistically insignificant, might be explained by the reduction in glutamate release and subsequent activation of KA autoreceptors.

The novel mechanism of short-term plasticity revealed in this study may also be important for the induction of long-term potentiation (LTP) and long-term depression (LTD) at this synapse, because these forms of long-term plasticity depend on Ca^{2+} accumulation within MF terminals (Castillo et al., 1994; Kobayashi et al., 1996) (but see Yeckel et al., 1999). In support of this notion, it has been demonstrated that MF–LTP is impaired in GluR6-deficient mice (Contractor et al., 2001) or by GluR5 antagonist LY 382884 (Bortolotto et al., 1999; Lauri et al., 2001), although there remains a substantial debate about this issue (Nicoll et al., 2000).

Activity-dependent regulation of signal transfer at the MF–CA3 synapse is extremely complex, i.e., homosynaptic and heterosynaptic activity-dependent presynaptic modulation mediated via mGlu- (Kamiya et al., 1996; Vogt and Nicoll, 1999), $GABA_B$- (Vogt and Nicoll, 1999), and NMDA receptor-independent forms of LTP (Zalutsky and Nicoll, 1990) and LTD (Kobayashi et al., 1996). The novel mechanism of presynaptic plasticity involving the kainate autoreceptor system, as revealed in this study, must be also taken into account. The multiple autoreceptor systems, as well as the structural peculiarity of the MF–CA3 synapse (Henze et al., 2000), support an especially large dynamic range of activity-dependent tuning of the synaptic strength and therefore is important for information processing in the hippocampus.

REFERENCES

Atluri PP, Regehr WG (1996) Determinants of the time course of facilitation at the granule cell to Purkinje cell synapse. J Neurosci 16:5661–5671.

Bliss TVP, Collingridge GL (1993) A synaptic model of memory: long-term potentiation in the hippocampus. Nature 361:31–39.

Borst JG, Sakmann B (1998) Facilitation of presynaptic calcium currents in the rat brainstem. J Physiol (Lond) 513:149–155.

Bortolotto ZA, Clarke VR, Delany CM, Parry MC, Smolders I, Vignes M, Ho KH, Miu P, Brinton BT, Fantaske R, Ogden A, Gates M, Ornstein PL, Lodge D, Bleakman D, Collingridge GL (1999) Kainate receptors are involved in synaptic plasticity. Nature 402:297–301.

Brody DL, Yue DT (2000) Relief of G-protein inhibition of calcium channels and short-term synaptic facilitation in cultured hippocampal neurons. J Neurosci 20:889–898.

Caillard O, Moreno H, Schwaller B, Llano I, Celio MR, Marty A (2000) Role of the calcium-binding protein parvalbumin in short-term synaptic plasticity. Proc Natl Acad Sci USA 97:13372–13377.

Castillo PE, Weisskopf MG, Nicoll RA (1994) The role of Ca^{2+} channels in hippocampal mossy fiber synaptic transmission and long-term potentiation. Neuron 12:261–269.
Castillo PE, Malenka RC, Nicoll RA (1997) Kainate receptors mediate a slow postsynaptic current in hippocampal CA3 neurons. Nature 388:182–186.
Contractor A, Swanson G, Heinemann SF (2001) Kainate receptors are involved in short- and long-term plasticity at mossy fiber synapses in the hippocampus. Neuron 29:209–216.
Currie KP, Fox AP (2002) Differential facilitation of N- and P/Q-type calcium channels during trains of action potential-like waveforms. J Physiol (Lond) 539:419–431.
Cuttle MF, Tsujimoto T, Forsythe ID, Takahashi T (1998) Facilitation of presynaptic calcium current at an auditory synapse in rat brainstem. J Physiol (Lond) 512:723–729.
DeMaria CD, Soong TW, Alseikhan BA, Alvania RS, Yue DT (2001) Calmodulin bifurcates the local Ca^{2+} signal that modulates P/Q-type Ca^{2+} channels. Nature 411:484–489.
Geiger JRP, Jonas P (2000) Dynamic control of presynaptic Ca^{2+} inflow by fast-inactivating K^+ channels in hippocampal mossy fiber boutons. Neuron 28:927–939.
Henze DA, Urban NN, Barrionuevo G (2000) The multifarious hippocampal mossy fiber pathway: a review. Neuroscience 98:407–427.
Jackson MB, Konnerth A, Augustine GJ (1991) Action potential broadening and frequency-dependent facilitation of calcium signals in pituitary nerve terminals. Proc Natl Acad Sci USA 88:380–384.
Kamiya H (2002) Kainate receptor-dependent presynaptic modulation and plasticity. Neurosci Res 42:1–6.
Kamiya H, Ozawa S (1998) Kainate receptor-mediated inhibition of presynaptic Ca^{2+} influx and EPSP in area CA1 of the rat hippocampus. J Physiol (Lond) 509:833–845.
Kamiya H, Ozawa S (1999) Dual mechanism for presynaptic modulation by axonal metabotropic glutamate receptor at the mouse mossy fibre-CA3 synapse. J Physiol (Lond) 518:497–506.
Kamiya H, Ozawa S (2000) Kainate receptor-mediated presynaptic inhibition at the mouse hippocampal mossy fibre synapse. J Physiol (Lond) 523:653–665.
Kamiya H, Zucker RS (1994) Residual Ca^{2+} and short-term synaptic plasticity. Nature 371:603–606.
Kamiya H, Shinozaki H, Yamamoto C (1996) Activation of metabotropic glutamate receptor type 2/3 suppresses transmission at rat hippocampal mossy fibre synapse. J Physiol (Lond) 493:447–455.
Klapstein GJ, Vietla S, Lieberman DN, Gray PA, Airaksinen MS, Thoenen H, Meyer M, Mody I (1998) Calbindin-D_{28k} fails to protect hippocampal neurons against ischemia in spite of its cytoplasmic calcium buffering properties: evidence from calbindin-D_{28k} knockout mice. Neuroscience 85:361–373.
Kobayashi K, Manabe T, Takahashi T (1996) Presynaptic long-term depression at the hippocampal mossy fiber-CA3 synapse. Science 273:648–650.
Kreitzer AC, Regehr WG (2000) Modulation of transmission during trains at a cerebellar synapse. J Neurosci 20:1348–1357.
Kullmann DM (2001) Presynaptic kainite receptors in the hippocampus: slowly emerging from obscurity. Neuron 32:561–564.
Lauri SE, Bortolotto ZA, Bleakman D, Ornstein PL, Lodge D, Isaac JT, Collingridge GL (2001) A critical role of a facilitatory presynaptic kainite receptor in mossy fiber LTP. Neuron 32:697–709.
Lee A, Scheuer T, Catterall WA (2000) Ca^{2+}/calmodulin-dependent facilitation and inactivation of P/Q-type Ca^{2+} channels. J Neurosci 20:6830–6838.
Lerma J, Paternain AV, Rodríguez-Moreno A, López-García JC (2001) Molecular physiology of kainate receptors. Physiol Rev 81:971–998.
Maeda H, Ellis-Davies GC, Ito K, Miyashita Y, Kasai H (1999) Supralinear Ca^{2+} signaling by cooperative and mobile Ca^{2+} buffering in Purkinje neurons. Neuron 24:989–1002.
Malenka RC, Nicoll RA (1999) Long-term potentiation—a decade of progress. Science 285:1870–1874.
Neher E (1998) Vesicle pools and Ca^{2+} microdomains: new tools for understanding their roles in neurotransmitter release. Neuron 20:389–399.
Nicoll RA, Malenka RC (1995) Contrasting properties of two forms of long-term potentiation in the hippocampus. Nature 377:115–118.
Nicoll RA, Mellor J, Frerking M, Schmitz D (2000) Kainate receptors and synaptic plasticity. Nature 406:957.
Park D, Dunlap K (1998) Dynamic regulation of calcium influx by G-proteins, action potential waveform, and neuronal firing frequency. J Neurosci 18:6757–6766.
Paternain AV, Morales M, Lerma J (1995) Selective antagonism of AMPA receptors unmasks kainate receptor-mediated responses in hippocampal neurons. Neuron 14:185–189.
Regehr WG, Atluri PP (1995) Calcium transients in cerebellar granule cell presynaptic terminals. Biophys J 68:2156–2170.
Regehr WG, Tank DW (1991) Selective fura-2 loading of presynaptic terminals and nerve cell processes by local perfusion in mammalian brain slice. J Neurosci Methods 37:111–119.
Regehr WG, Delaney KR, Tank DW (1994) The role of presynaptic calcium in short-term enhancement at the hippocampal mossy fiber synapse. J Neurosci 14:523–537.
Sabatini BL, Regehr WG (1996) Timing of neurotransmission at fast synapses in the mammalian brain. Nature 384:170–172.
Sabatini BL, Regehr WG (1997) Control of neurotransmitter release by presynaptic waveform at the granule cell to Purkinje cell synapse. J Neurosci 17:3425–3435.
Salin PA, Scanziani M, Malenka RC, Nicoll RA (1996) Distinct short-term plasticity at two excitatory synapses in the hippocampus. Proc Natl Acad Sci USA 93:13304–13309.
Schmitz D, Frerking M, Nicoll RA (2000) Synaptic activation of presynaptic kainate receptors on hippocampal mossy fiber synapses. Neuron 27:327–338.
Schmitz D, Mellor J, Frerking M, Nicoll RA (2001a) Presynaptic kainate receptors at hippocampal mossy fiber synapses. Proc Natl Acad Sci USA 98:11003–11008.
Schmitz D, Mellor J, Nicoll RA (2001b) Presynaptic kainate receptor mediation of frequency facilitation at hippocampal mossy fiber synapses. Science 291:1972–1976.
Tsujimoto T, Jeromin A, Saitoh N, Roder JC, Takahashi T (2002) Neuronal calcium sensor 1 and activity-dependent facilitation of P/Q-type calcium currents at presynaptic nerve terminals. Science 295:2276–2279.
Turecek R, Trussell LO (2001) Presynaptic glycine receptors enhance transmitter release at a mammalian central synapse. Nature 411:587–590.
Vignes M, Collingridge GL (1997) The synaptic activation of kainate receptors. Nature 388:179–182.
Vogt KE, Nicoll RA (1999) Glutamate and gamma-aminobutyric acid mediate a heterosynaptic depression at mossy fiber synapses in the hippocampus. Proc Natl Acad Sci USA 96:1118–1122.
Wu LG, Saggau P (1994) Presynaptic calcium is increased during normal synaptic transmission and paired-pulse facilitation, but not in long-term potentiation in area CA1 of hippocampus. J Neurosci 14:645–654.
Yamamoto C, Sawada S, Ohno-Shosaku T (1993) Quantal analysis of modulating action of adenosine on the mossy fiber synapse in hippocampal slices. Hippocampus 3:87–92.
Yeckel MF, Kapur A, Johnston D (1999) Multiple forms of LTP in hippocampal CA3 neurons use a common postsynaptic mechanism. Nat Neurosci 2:625–633.
Zalutsky RT, Nicoll RA (1990) Comparison of two forms of long-term potentiation in single hippocampal neurons. Science 248:1619–1624.
Zucker RS (1989) Short-term synaptic plasticity. Annu Rev Neurosci 12:13–31.
Zucker RS, Regehr WG (2002) Short-term synaptic plasticity. Annu Rev Physiol 64:355–405.