

Kainate Receptors Are Involved in Short- and Long-Term Plasticity at Mossy Fiber Synapses in the Hippocampus

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Summary

Kainate receptors alter the excitability of mossy fiber axons and have been reported to play a role in the induction of long-term potentiation (LTP) at mossy fiber synapses in the hippocampus. These previous studies have relied primarily on the use of compounds whose selectivity is unclear. In this report, we investigate short- and long-term facilitation of mossy fiber synaptic transmission in kainate receptor knockout mice. We find that LTP is reduced in mice lacking the GluR6, but not the GluR5, kainate receptor subunit. Additionally, short-term synaptic facilitation is impaired in GluR6 knockout mice, suggesting that kainate receptors act as presynaptic autoreceptors on mossy fiber terminals to facilitate synaptic transmission. These data demonstrate that kainate receptors containing the GluR6 subunit are important modulators of mossy fiber synaptic strength.

Introduction

Kainate receptors are a family of ionotropic glutamate receptors (iGluRs) that, in addition to mediating excitatory synaptic transmission at some synapses, can modulate neurotransmitter release (Frerking and Nicoll, 2000). At mossy fiber synapses in the hippocampus, kainate receptors appear to subservise both these functions. Activation of kainate receptors on mossy fibers by exogenous agonists reduces the amplitude of the excitatory postsynaptic current (EPSC), suggesting a presynaptic function for these receptors (Vignes et al., 1998; Contractor et al., 2000; Kamiya and Ozawa, 2000; Schmitz et al., 2000). In addition, postsynaptic kainate receptors contribute to mossy fiber EPSCs (Castillo et al., 1997b; Vignes and Collingridge, 1997), and most recently, kainate receptors were proposed to mediate induction of mossy fiber LTP (Bortolotto et al., 1999). Thus, kainate receptors have multiple roles in transmission at the mossy fiber synapse.

Several studies have reported that kainate receptors are presynaptic modulators of neurotransmitter release, but the mechanism of action of these receptors remains the subject of debate. For example, kainate receptor modulation of inhibitory synaptic transmission at CA1 interneuron–pyramidal cell synapses has been variously ascribed to presynaptic terminal receptors (Clarke et

al., 1997) that may be linked to G proteins (Rodriguez-Moreno and Lerma, 1998; Rodriguez-Moreno et al., 2000), a depletion of the releasable pool of vesicles following depolarization of interneurons by somatodendritic kainate receptors (Cossart et al., 1998; Frerking et al., 1998), activation of GABA_B autoreceptors (Frerking et al., 1999), and postsynaptic shunting of the evoked IPSC (Frerking et al., 1999). At mossy fiber synapses, exogenous activation of kainate receptors depresses excitatory transmission (Vignes et al., 1998; Contractor et al., 2000; Kamiya and Ozawa, 2000; Schmitz et al., 2000). This likely occurs, in part, as a result of kainate receptor depolarization of mossy fiber axons (Kamiya and Ozawa, 2000). Receptors located on presynaptic terminals also may play a role, because the kainate-mediated depression is accompanied by an increase in the paired-pulse ratio of evoked mossy fiber EPSCs (Contractor et al., 2000). In addition, Schmitz et al. (2000) showed that kainate receptor activation by endogenously released glutamate increased the excitability of mossy fiber axons.

Recently, it was reported that kainate receptors play a role in mossy fiber long-term potentiation (Bortolotto et al., 1999). The induction of mossy fiber LTP, which is independent of NMDA receptor activation (Harris and Cotman, 1986), was occluded in the presence of a kainate receptor antagonist reported to be selective for GluR5 subunit-containing receptors (Bortolotto et al., 1999). This result is surprising in light of previous data from a number of researchers that show that mossy fiber LTP was induced in the presence of nonselective AMPA and kainate receptor antagonists (Ito and Sugiyama, 1991; Castillo et al., 1994; Yeckel et al., 1999). Moreover, GluR5 subunit mRNA is sparsely expressed in the dentate gyrus and CA3 pyramidal layer; rather, mRNA for the GluR6 and other receptor subunits predominate (Bahn et al., 1994; Bureau et al., 1999).

We have tried to resolve some of these outstanding issues by studying synaptic plasticity of mossy fibers in kainate receptor knockout mice. Our objectives in this study were 3-fold. First, we tested the hypothesis that kainate receptors are critical for induction of mossy fiber LTP. Second, we explored the subunits that are critical for induction of LTP. Third, we explored short-term forms of plasticity to determine if kainate receptor activation by synaptic release of glutamate influenced the strength of mossy fiber transmission. We found that mossy fiber LTP is reduced, but not absent, in GluR6 knockout mice (GluR6^{-/-}), whereas GluR5 knockout mice (GluR5^{-/-}) exhibit normal LTP at this synapse. Induction of LTP in GluR6^{-/-} mice was occluded completely by an antagonist of group I metabotropic glutamate receptors (mGluRs). Additionally, two short-term forms of mossy fiber plasticity, paired-pulse facilitation and frequency facilitation, were reduced in GluR6^{-/-} mice, suggesting that GluR6-containing kainate receptors act as presynaptic autoreceptors to facilitate excitatory neurotransmission. We conclude from these data that kainate receptors play a critical role in multiple forms of plasticity at the mossy fiber synapse.

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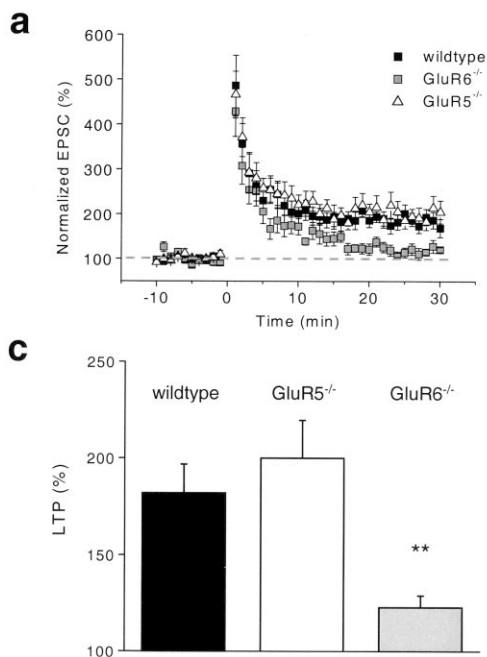


Figure 1. Mossy Fiber LTP Is Impaired in GluR6^{-/-} Mice but Not GluR5^{-/-} Mice

(A) The time course and magnitude of mossy fiber LTP in slices from wild-type, GluR5^{-/-}, and GluR6^{-/-} mice. Mossy fiber LTP was induced in hippocampal slices by tetanic stimulation using 3 episodes of 100 Hz stimulation for 1 s, separated by 10 s intervals, in the presence of the NMDA antagonist D-APV (50 μM). Posttetanic potentiation was observed in both mutant genotypes. However, LTP was impaired significantly in the GluR6^{-/-} mice: wild type, 182% ± 15%, n = 14; GluR5^{-/-} mice, 200% ± 20%, n = 12; and GluR6^{-/-} mice, 123% ± 6%, n = 10.

(B) Representative mossy fiber EPSCs from single recordings from mice from all three genotypes before and after LTP induction. Calibration: x axis, 10 ms; y axis, 500 pA.

(C) Histogram summary of LTP calculated as the percent increase in the mean EPSC amplitude between 20–30 min after tetanus compared to the mean amplitude in the 10 min control period. LTP in GluR6^{-/-} mice was significantly lower compared to wild-type and GluR5^{-/-} mice (p < 0.01).

Results

Kainate Receptors Mediate Mossy Fiber Long-Term Potentiation

In order to test the hypothesis that kainate receptors are necessary for induction of mossy fiber LTP, we compared the magnitude of LTP in hippocampi from wild-type mice to that in GluR5^{-/-} and GluR6^{-/-} mice (Mulle et al., 1998, 2000). Stimulation in stratum lucidum evoked monosynaptic mossy fiber EPSCs that were identified according to several well-defined criteria (see Experimental Procedures). Mossy fiber LTP was induced in wild-type and knockout mice using a tetanus consisting of three 1 s trains at 100 Hz frequency given at an interval of 10 s (L-HFS-3) (Urban and Barrionuevo, 1996). The time course of potentiation for the three mouse genotypes is shown in Figure 1A; representative traces from single experiments are shown in Figure 1B. The percent increase in EPSC amplitude (measured as the percent increase of the mean EPSC amplitude between 20 and 30 min after induction compared to the mean control amplitude) was 182% ± 15% in wild-type mice (Figure 1C, n = 14). However, the magnitude of LTP in GluR6^{-/-} mice was significantly less (123% ± 6%, n = 10, p < 0.01 compared to wild-type LTP). Mossy fiber LTP in GluR5^{-/-} mice was indistinguishable from that in wild-type mice (200% ± 20%, n = 12, p > 0.05; Figure 1).

It is possible that kainate receptors activation might be indirectly involved in mossy fiber LTP. In light of previous results that showed that mossy fiber LTP is impaired by antagonists of β-adrenergic compounds (Huang and Kandel, 1996), it could be argued that activation of kainate receptors on noradrenergic terminals located in this region of the hippocampus might increase the release of norepinephrine and thereby facilitate LTP induction. In order to test this possibility, we made recordings from wild-type mice in the presence of the β-adrenergic antagonist propranolol (1 μM). However,

we found in these control experiments that we could elicit normal mossy fiber LTP during β-adrenergic blockade (223% ± 24%, n = 3, p > 0.05, compared to wild type without propranolol), suggesting that β-adrenergic receptor activation is not necessary for induction of mossy fiber LTP in disinhibited hippocampal slice preparations from mice. These results are in agreement with an earlier study which found that β-adrenergic receptor activation only played a role in mossy fiber LTP in slices in which inhibition was intact (Hopkins and Johnston, 1988). These experiments strongly support the hypothesis that kainate receptors at mossy fiber synapses play a critical role in mossy fiber LTP.

PKA-Dependent Potentiation Is Intact in GluR6^{-/-} Mice

Activation of cAMP-dependent protein kinase (PKA) is a critical downstream component of mossy fiber long-term potentiation. Application of forskolin (50 μM), which activates adenylyl cyclase, enhances mossy fiber transmission and occludes further tetanus-induced potentiation (Huang et al., 1994; Weisskopf et al., 1994). To determine if ablation of the GluR6 subunit altered PKA-dependent enhancement of mossy fiber transmission, we compared forskolin-induced potentiation of mossy fiber EPSCs in wild-type and GluR6^{-/-} mice. There was no significant difference in the magnitude of potentiation induced by forskolin in wild-type and GluR6^{-/-} mice (wild type, 205% ± 31%, n = 10; GluR6^{-/-}, 179% ± 19%, n = 10, p > 0.05; Figure 2). These results suggest that activation of mossy fiber kainate receptors occurs at a point upstream of PKA in the signaling cascade that gives rise to LTP.

Paired-Pulse Facilitation Is Reduced in GluR6^{-/-} Mice

Pairing stimuli to mossy fibers facilitates the amplitude of the second evoked EPSC; this form of short-term

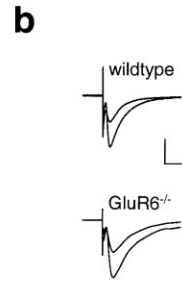
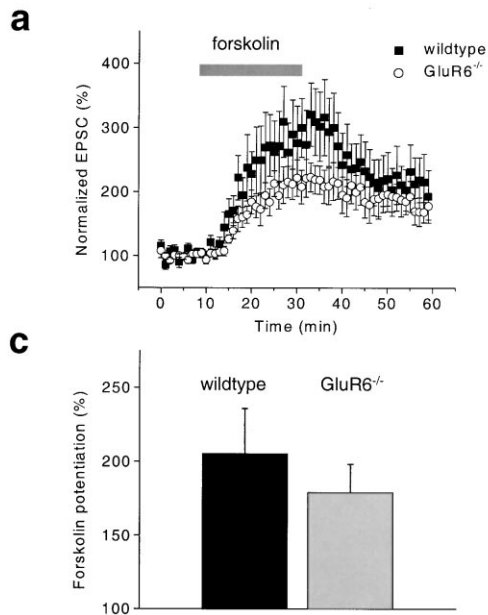


Figure 2. Forskolin Enhancement of Mossy Fiber Transmission Is Normal in $\text{GluR6}^{-/-}$ Mice

(A) Time course and magnitude of forskolin-induced potentiation in wild-type and $\text{GluR6}^{-/-}$ mice. After an initial 10 min control period, forskolin ($50 \mu\text{M}$) was applied for 20 min (indicated by the bar).

(B) Representative traces from one experiment showing mossy fiber EPSCs in wild-type and $\text{GluR6}^{-/-}$ mice before and after forskolin-induced potentiation. Calibration—wild type: x axis, 400 pA, y axis, 10 ms; $\text{GluR6}^{-/-}$: x axis, 200 pA, y axis, 10 ms.

(C) Summary graph of forskolin-induced potentiation in wild-type and $\text{GluR6}^{-/-}$ mice. The facilitation was measured 20–30 min after forskolin had been washed off and was compared to the EPSC amplitude during control recording. No significant difference was observed between wild-type and $\text{GluR6}^{-/-}$ mice ($205\% \pm 31\%$, $n = 10$ versus $179\% \pm 19\%$, $n = 10$, respectively, $p > 0.5$).

synaptic plasticity occurs because residual Ca^{2+} in the presynaptic terminal increases the release probability for subsequent EPSCs (Regehr et al., 1994). We were interested in determining if paired-pulse facilitation (PPF) at this synapse was altered in kainate receptor knockout mice. A previous study of $\text{GluR6}^{-/-}$ mice reported that there was no change in the mossy fiber paired-pulse ratio (PPR) measured at a 40 ms interstimulus interval (Mulle et al., 1998). We first compared wild-type mossy fiber PPF at a range of interstimulus intervals to PPF in $\text{GluR5}^{-/-}$ and $\text{GluR6}^{-/-}$ mice (Figure 3). At intervals of less than 60 ms, the paired-pulse ratio was

significantly lower in the $\text{GluR6}^{-/-}$ mice as compared to wild-type mice (Figure 3A), whereas $\text{GluR5}^{-/-}$ mice showed no deficit. EPSCs paired at a 20 ms interval facilitated by 2.3 ± 0.2 -fold in wild-type mice ($n = 16$) and 2.0 ± 0.3 -fold ($n = 6$) in $\text{GluR5}^{-/-}$ mice, whereas the PPR at this interval was significantly smaller than wild type in the $\text{GluR6}^{-/-}$ mice (1.8 ± 0.1 , $n = 15$, $p < 0.05$). In these experiments, the PPR at an interval of 40 ms (PPR-40) was also significantly smaller in $\text{GluR6}^{-/-}$ mice (wild type, 2.5 ± 0.3 , $n = 16$; $\text{GluR5}^{-/-}$, 2.2 ± 0.4 , $n = 6$; and $\text{GluR6}^{-/-}$, 1.7 ± 0.1 , $n = 15$, $p < 0.05$). At interstimulus intervals of 60–500 ms, no significant

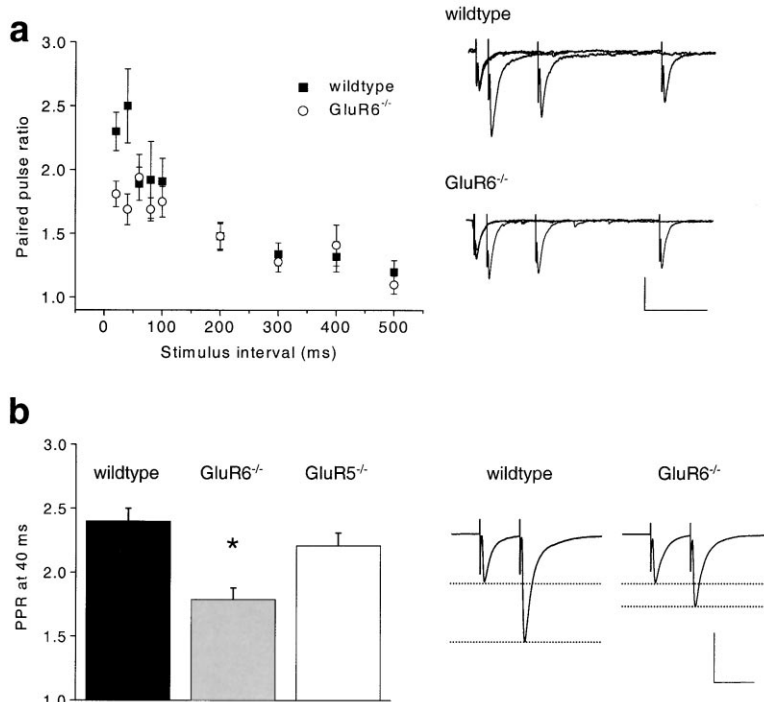


Figure 3. Mossy Fiber Paired-Pulse Facilitation Is Impaired in $\text{GluR6}^{-/-}$ Mice

(A) Left panel: Graph of paired-pulse ratio of mossy fiber EPSCs at increasing interstimulus intervals for wild-type ($n = 16$) and $\text{GluR6}^{-/-}$ mice ($n = 15$). A significant difference in PPR was observed at short intervals less than 60 ms (20 ms, $p < 0.05$; 40 ms, $p < 0.05$). Right panel: Representative paired mossy fiber EPSCs evoked at intervals of 20 ms, 100 ms, and 300 ms in slices from wild-type and $\text{GluR6}^{-/-}$ mice. Calibration: x axis, 100 ms; y axis, (wild type) 300 pA, ($\text{GluR6}^{-/-}$) 512.5 pA.

(B) Left panel: Paired-pulse ratio at an interval of 40 ms measured for up to 30 events for each recording in wild-type (2.4 ± 0.1 , $n = 21$), $\text{GluR6}^{-/-}$ mice (1.8 ± 0.1 , $n = 16$), and $\text{GluR5}^{-/-}$ mice (2.2 ± 0.1 , $n = 15$). Right panel: Representative EPSCs evoked at 40 ms interval in wild-type and $\text{GluR6}^{-/-}$ mice. Calibration: x axis, 40 ms; y axis, (wild type) 500 pA, ($\text{GluR6}^{-/-}$) 675 pA.

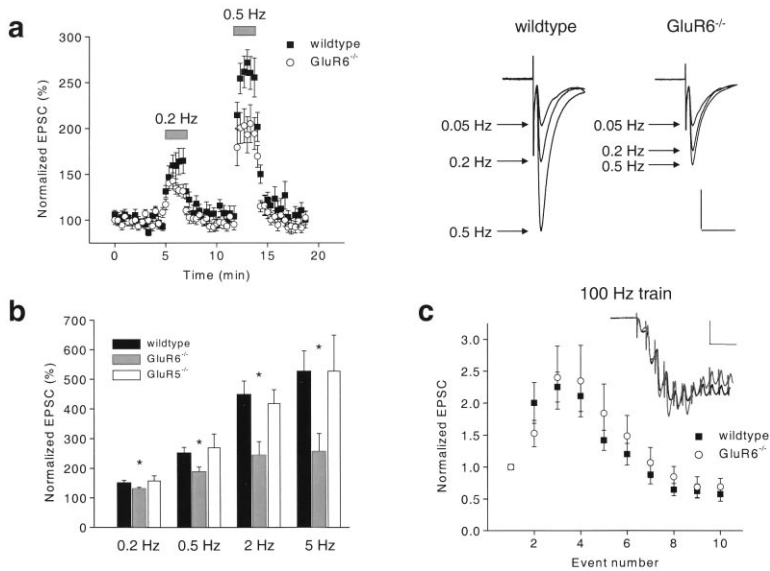


Figure 4. Frequency Facilitation Is Impaired in *GluR6*^{-/-} Mice

(A) Left panel: Time course of facilitation at stimulation frequencies of 0.2 Hz and 0.5 Hz. The frequency of stimulation during the control was 0.05 Hz. Normalized EPSC amplitudes are shown for 18 experiments from wild type and nineteen experiments from *GluR6*^{-/-} mice. For clarity, data points are shown only at 20 s intervals. Right panel: representative EPSCs from one experiment from wild type and *GluR6*^{-/-} mice. Calibration: x axis, 10 ms; y axis, (wild type) 250 pA, (*GluR6*^{-/-}) 380 pA. (B) Summary of frequency facilitation experiments showing percentage facilitation of the normalized amplitude for wild-type, *GluR6*^{-/-}, and *GluR5*^{-/-} mice at 0.2 Hz (wild type, 151% ± 8%, n = 18; *GluR6*^{-/-}, 131% ± 5%, n = 19; *GluR5*^{-/-}, 157% ± 18%, n = 10), at 0.5 Hz (wild type, 252% ± 19%, n = 18; *GluR6*^{-/-}, 189% ± 15%, n = 19; *GluR5*^{-/-}, 269% ± 46%, n = 10), at 2 Hz (wild type, 449% ± 45%, n = 10; *GluR6*^{-/-}, 245% ± 45%, n = 8; *GluR5*^{-/-}, 418% ± 46%, n = 6),

and at 5 Hz (wild type, 527% ± 70%, n = 15; *GluR6*^{-/-}, 257% ± 60%, n = 7; *GluR5*^{-/-}, 527% ± 123%, n = 4). (C) Facilitation of the first 10 EPSCs evoked during a 100 Hz train delivered to mossy fibers in slices from wild-type (n = 17) and *GluR6*^{-/-} mice (n = 13). EPSCs are normalized to the amplitude of the first EPSC in each train. Inset: Representative recording of the first 10 EPSCs during a 100 Hz train for wild-type (black) and *GluR6*^{-/-} (gray) mice. Calibration: x axis, 30 ms; y axis, (wild type) 1000 pA, (*GluR6*^{-/-}) 575 pA.

differences were detected in the mossy fiber PPR of the wild-type and knockout mice (Figure 3A). Because mossy fiber EPSCs exhibit a large trial-to-trial variation in amplitude and paired-pulse ratio, we confirmed the difference in short-term plasticity by averaging 15–30 paired EPSCs evoked with an interstimulus interval of 40 ms. We again found that PPR in the *GluR6*^{-/-} mice was significantly smaller than that in wild-type or *GluR5*^{-/-} mice (wild type, 2.4 ± 0.1, n = 21; *GluR5*^{-/-}, 2.2 ± 0.1, n = 15; *GluR6*^{-/-}, 1.8 ± 0.1, n = 16, p < 0.01; Figure 3B). These data suggest that activation of kainate receptors contributes to facilitation of mossy fiber EPSCs paired at short intervals.

Mossy Fiber Frequency Facilitation Is Impaired in *GluR6*^{-/-} Mice

Mossy fiber synaptic transmission is acutely sensitive to the frequency at which synaptic currents are evoked (Salin et al., 1996). To determine if kainate receptors contribute to increase in EPSC amplitudes elicited by higher frequencies of stimulation (termed frequency facilitation), we recorded mossy fiber EPSCs while stimulating at a range of frequencies (Figure 4). The stimulation frequency was increased to 0.2, 0.5, 2, or 5 Hz from a baseline stimulation frequency of 0.05 Hz. In wild-type mice, increasing the stimulation frequency to 0.2 and 0.5 Hz for 2 minutes resulted in a 151% ± 8.0% and 252% ± 19% increase in EPSC amplitudes (n = 18). The facilitation of transmission was not different in *GluR5*^{-/-} mice (157% ± 18% and 269% ± 46% increases at 0.2 and 0.5 Hz, respectively, n = 10, p > 0.05) but was significantly lower in *GluR6*^{-/-} mice (131% ± 5.2% and 189% ± 15%, n = 19, p < 0.05) (Figures 4A and 4B).

We next tested whether the reduced frequency facilitation in *GluR6*^{-/-} mice persisted at higher stimulation frequencies. The stimulation frequency was increased from 0.05 to 2 Hz for 1 min or to 5 Hz for 15 s. In wild-

type mice these frequencies of stimulation increased the amplitude of mossy fiber EPSCs by 449% ± 45% (2 Hz, n = 10) and 527% ± 70% (5 Hz, n = 15). The *GluR6*^{-/-} mice showed a large deficit in facilitation at these higher frequencies, as EPSC amplitudes increased by only 245% ± 45% and 257% ± 60% at 2 Hz and 5 Hz (n = 8 and 7, respectively, p < 0.05) (Figure 4B). Furthermore, we observed no difference in frequency facilitation in the *GluR5*^{-/-} mice at 2 Hz (418% ± 46%, n = 6, p > 0.05) or at 5 Hz (527% ± 123%, n = 4, p > 0.05) (Figure 4B). These results strongly support the hypothesis that *GluR6*-containing kainate receptors act as presynaptic autoreceptors to augment frequency-dependent facilitation of synaptic transmission at mossy fiber terminals.

This reduction in the frequency-dependent enhancement of mossy fiber EPSCs in *GluR6*^{-/-} mice, combined with the observed deficit in mossy fiber long-term potentiation, raised the question of whether a similar deficit in facilitation of EPSCs during the 100 Hz train used to evoke LTP occurred in *GluR6*^{-/-} mice. The locus of induction of mossy fiber LTP is still controversial (e.g., Weisskopf and Nicoll, 1995; Yeckel et al., 1999), and *GluR6*-containing kainate receptors are located both pre- and postsynaptically (Mulle et al., 1998; Contractor et al., 2000). A reduction in facilitation during the initial phase of the LTP tetanus in *GluR6*^{-/-} mice would provide suggestive evidence for a presynaptic kainate receptor contribution to induction. To test this possibility, we normalized the first 10 EPSCs in a 100 Hz stimulation train to the amplitude of the first event in wild-type and *GluR6*^{-/-} mice (amplitudes of individual EPSCs were measured from a baseline current amplitude immediately preceding the stimulus artifact for each EPSC; see inset in Figure 4C for representative current recordings). No significant difference in the normalized amplitudes of the first 10 EPSCs evoked at 100 Hz in wild-type and

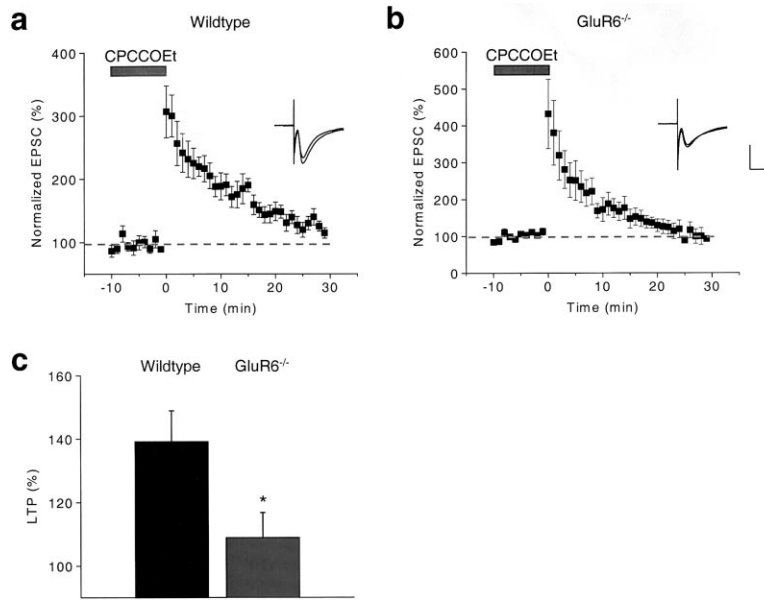


Figure 5. Residual LTP in $\text{GluR6}^{-/-}$ Mice Is Mediated by Activation of Group I mGluRs

(A) The time course and magnitude of mossy fiber LTP in wild-type mice is significantly impaired when the selective group I mGluR antagonist CPCCOEt (100 μM) is present during induction ($139\% \pm 10\%$, $n = 11$, $p < 0.05$). Representative EPSC traces from one recording before and after tetanic stimulation are shown inset.

(B) In $\text{GluR6}^{-/-}$ mice, potentiation after tetanic stimulation of mossy fibers in the presence of CPCCOEt is abolished completely ($109\% \pm 8\%$, $n = 8$).

(C) Summary graphs of the potentiation observed in the presence of CPCCOEt. LTP induced during mGluR blockade was impaired but still present in wild-type mice; however, LTP was not observed in $\text{GluR6}^{-/-}$ mice. The percentage change from control was significantly different to that in wild-type mice in the presence of CPCCOEt ($p < 0.01$) (calibration: 300 pA, 10 ms).

$\text{GluR6}^{-/-}$ mice was observed ($n = 17$ and 13, respectively; Figure 4C). These results demonstrated that an initial deficit in facilitation does not underlie the reduced mossy fiber LTP in $\text{GluR6}^{-/-}$ mice.

Kainate Receptor-Independent LTP Is Mediated by Group 1 Metabotropic Glutamate Receptors

We were interested in determining what receptor systems might mediate the small amount of LTP observed in the $\text{GluR6}^{-/-}$ mice. Previously, Yeckel and coworkers (1999) were able to block mossy fiber LTP by the simultaneous application of both kynurenate and an antagonist of group I mGluRs. To determine if kainate receptor-independent LTP was mediated by group I mGluRs, we induced LTP in the presence of the noncompetitive group I mGluR antagonist CPCCOEt (100 μM). In wild-type mice, the magnitude of LTP was significantly reduced in the presence of CPCCOEt ($139\% \pm 10\%$, $n = 11$, $p < 0.05$, compared to control LTP) (Figure 5). In $\text{GluR6}^{-/-}$ mice, LTP was completely abolished by application of CPCCOEt during induction ($109\% \pm 8\%$, $n = 8$, $p = 0.3$). These data support the finding that there are two pathways for the induction of mossy fiber LTP: one pathway requires activation of $\text{GluR6}^{-/-}$ -containing kainate receptors, while the other involves activation of mGluRs.

Discussion

In this study we have made two principal observations concerning the involvement of kainate receptors in synaptic transmission at the mossy fiber to CA3 synapse in the hippocampus. First, comparisons of mossy fiber LTP between wild-type and kainate receptor knockout mice provide strong support for the hypothesis that kainate receptors containing the GluR6 subunit play an integral role in the induction of LTP at this synapse. Forskolin-induced enhancement of mossy fiber transmission is normal in $\text{GluR6}^{-/-}$ mice, suggesting that

kainate receptors mediate their action upstream of the intracellular effector protein kinase A. Second, deficits in short-term plasticity in the $\text{GluR6}^{-/-}$ mice suggest that kainate receptors function as autoreceptors that are activated by release of endogenous glutamate and facilitate mossy fiber transmission.

Kainate Receptors and Mossy Fiber LTP

Mossy fiber LTP appears to be mechanistically distinct from other forms of LTP at excitatory synapses in the hippocampus. As is the case at other synapses, consensus has been reached on some aspects of mossy fiber LTP, but many elements are still the subject of debate. It is accepted that induction of LTP does not depend on NMDA receptor activation (Harris and Cotman, 1986) and that LTP is maintained by a long-lasting enhancement of mossy fiber release probability (Zalutsky and Nicoll, 1990). In contrast, the mechanisms of induction of LTP at this synapse are not generally agreed upon; there is evidence both for and against roles of postsynaptic Ca^{2+} signaling (Zalutsky and Nicoll, 1990; Castillo et al., 1994; Yeckel et al., 1999), activation of mGluRs (Bashir et al., 1993; Conquet et al., 1994; Manzoni et al., 1994; Hsia et al., 1995), and activation of ionotropic glutamate receptors (including kainate receptors) (Harris and Cotman, 1986; Ito and Sugiyama, 1991; Castillo et al., 1994; Bortolotto et al., 1999). The recent finding by Bortolotto and coworkers (1999) that kainate receptors mediate LTP at mossy fibers was difficult to reconcile with previous observations made by several different researchers, because these studies showed that inhibition of AMPA and kainate receptors, using the low-affinity antagonist kynurenate, did not occlude the induction of mossy fiber LTP (Ito and Sugiyama, 1991; Castillo et al., 1994). However, a recent report by Yeckel et al. (1999) showed that kynurenate occluded induction of mossy fiber LTP in a subset of recordings (3 of 8 CA3 neurons) after a tetanus identical to that used in our study was delivered to the mossy fibers. Furthermore,

when both ionotropic and group I metabotropic glutamate receptors were inhibited, LTP was not elicited. These data provide suggestive evidence for the existence of two pathways for induction of mossy fiber LTP.

On the basis of these previous data and our current results, we propose that two separate receptors systems, kainate receptors and mGluRs, mediate the induction of mossy fiber LTP. We found that mossy fiber LTP is significantly reduced in GluR6^{-/-} mice and was completely eliminated in these mice using a group I mGluR antagonist. These results suggest that when a single pathway is inhibited, either pharmacologically or in knockout mice, a lower degree of potentiation can still be attained due to activation of the alternative pathway.

Although our study in kainate receptor knockout mice supports the involvement of kainate receptors in mossy fiber LTP, we found that LTP is impaired only in GluR6^{-/-} mice, rather than GluR5^{-/-} mice. We therefore arrive at different conclusion regarding the subunit composition of mossy fiber kainate receptors than Bortolotto and coworkers (1999), who proposed that GluR5 is a critical subunit based on the pharmacological selectivity of the receptor antagonist LY382884. Because this compound is not generally available, our divergent results cannot be addressed experimentally at the current time. In principle, however, the kainate receptor knockout mice represent very useful tools for validating the pharmacological selectivity of new compounds. Finally, it should be noted that the lack of any apparent alteration of LTP in the GluR5^{-/-} mice does not rule out the participation of GluR5 subunit-containing kainate receptors in mossy fiber synaptic plasticity. It remains possible that the previous pharmacological study by Bortolotto et al. (1999) uncovered a role for these type of receptors that was not obvious in our comparative studies.

Presynaptic Kainate Receptors and Mossy Fiber Synaptic Transmission

A number of studies have shown that activation of kainate receptors on mossy fiber axons or terminals can depress excitatory synaptic transmission to CA3 pyramidal neurons (Vignes et al., 1998; Contractor et al., 2000; Kamiya and Ozawa, 2000). These studies, as well as early autoradiographic data (Represa et al., 1987), demonstrated that kainate receptors are present on mossy fibers, but did not resolve how activation of presynaptic kainate receptors by synaptically released glutamate affects excitatory transmission. Most recently, it was reported that heterosynaptic activation of kainate receptors by spillover from neighboring mossy fiber and associational-commissural inputs depressed mossy fiber transmission (Schmitz et al., 2000).

We demonstrated that activation of kainate receptors by synaptic glutamate facilitated mossy fiber transmission by examining the magnitude of short-term plasticity phenomena in the kainate receptor knockout mice. We saw reductions in two forms of short-term plasticity, paired-pulse facilitation and frequency facilitation, in the GluR6^{-/-} mice. Paired-pulse facilitation at short interstimulus intervals (of less than 60 ms) was smaller by ~25% in GluR6^{-/-} mice, a result apparently at odds with a previous study in these mice (Mulle et al., 1998). The reasons why our data diverge from those of Mulle

et al. is not clear, although it is worth noting that the paired-pulse ratios we observed in the wild-type mice, which were similar to those in previous reports (Salin et al., 1996; Castillo et al., 1997a; Domenici et al., 1998), were significantly lower than those in the report by Mulle et al., suggesting that there might have been experimental condition(s) that underlay the different results. Our data suggest that GluR6^{-/-}-containing kainate receptors on mossy fiber terminals are activated by a single release event and can contribute to the facilitation of subsequent release of glutamate, if a second stimulus arrives within a narrow window of coincidence.

Kainate receptors also facilitate transmission if mossy fibers are stimulated at low frequencies for extended trains. The frequency dependence of increases in mossy fiber EPSC amplitudes arises from presynaptic mechanisms that share characteristics with paired-pulse facilitation (Salin et al., 1996). We found that frequency facilitation was reduced significantly in GluR6^{-/-} mice at stimulation frequencies that ranged from 0.2–5 Hz. At a stimulation frequency of 5 Hz, GluR6^{-/-} mice had a ~2-fold lower magnitude of facilitation compared to wild-type mice. In contrast, frequency facilitation in GluR5^{-/-} mice was similar to that in wild-type mice at all the frequencies we tested. These data demonstrate that one important physiological function of kainate receptors is homosynaptic frequency-dependent augmentation of mossy fiber transmission.

In light of data from a number of groups that showed that kainate receptor activation can depress mossy fiber transmission (Vignes et al., 1998; Contractor et al., 2000; Kamiya and Ozawa, 2000; Schmitz et al., 2000), it is somewhat surprising that we only observed facilitation of transmission by synaptic activation of kainate receptors. However, it should be noted that we examined frequencies of activation of up to only 5 Hz. It remains possible that at high-stimulation frequencies synaptic glutamate may activate kainate receptors to a degree that results in depression, rather than facilitation, of mossy fiber transmission. Alternatively, glutamate released during high-frequency stimulation may access different populations of kainate receptors with functionally distinct consequences.

Does the Deficit in Kainate Receptor-Mediated Facilitation Account for the Impairment of LTP in GluR6^{-/-} Mice?

It was possible that the observed reduction in mossy fiber LTP in the GluR6^{-/-} mice arose either from the deficit in the presynaptic frequency response or from postsynaptic mechanisms, because GluR6-containing kainate receptors are located at both pre- and postsynaptic sites at the mossy fiber synapse (Mulle et al., 1998; Contractor et al., 2000). In an initial attempt to address this issue, we analyzed EPSCs evoked during the first 100 Hz train used to induce mossy fiber LTP. Comparison of the amplitudes of each of the first ten EPSCs to that of the first EPSC in the train did not reveal differences between wild-type and GluR6^{-/-} mice. This finding suggests that differences in facilitation do not underlie the reduced LTP in the GluR6^{-/-} mice but is not conclusive. Further studies will be required to ascertain if kainate receptor-dependent LTP is due to activation

of presynaptic receptors, postsynaptic receptors, or receptors localized to each side of the synapse.

In summary, this study confirms that kainate receptor activation is central to induction of LTP at the mossy fiber synapse. Our observations also demonstrate the kainate receptors act as autoreceptors to facilitate transmission during rapid afferent stimulation. These results reveal a novel role for iGluRs as facilitators of short- and long-term plasticity of the mossy fiber synapse.

Experimental Procedures

Transverse hippocampal slices (350 μm) were made from P16–P26 mice. Animals were anaesthetized with isoflurane and decapitated. The brain was removed under ice-cold sucrose slicing ACSF containing: 85 mM NaCl, 2.5 mM KCl, 1.25 mM NaH_2PO_4 , 25 mM NaHCO_3 , 25 mM glucose, 75 mM sucrose, 0.5 mM CaCl_2 , and 4 mM MgCl_2 , equilibrated with 95% O_2 /5% CO_2 . Slices were incubated at 28°C for 30 min and then the sucrose slicing solution was exchanged for a normal ACSF containing: 125 mM NaCl, 2.4 mM KCl, 1.2 mM NaH_2PO_4 , 25 mM NaHCO_3 , 25 mM glucose, 1 mM CaCl_2 , and 2 mM MgCl_2 . Ten micromolar D, L-APV, and 100 μM kynurenate were included in the slicing and incubation solutions. After slices were transferred to a recording chamber, they were continuously perfused with an ACSF containing 2 mM CaCl_2 and 1 mM MgCl_2 . Whole-cell patch-clamp recordings were made from visually identified pyramidal cells in the CA3 region of the hippocampus. All experiments were done at room temperature. Glass electrodes were pulled from borosilicate glass and had resistances of 3–4 M Ω when filled with internal solution containing: 95 mM CsF, 25 mM CsCl, 10 mM Cs-HEPES, 10 mM Cs-EGTA, 2 mM NaCl, 2 mM Mg-ATP, 10 mM QX-314, 5 mM TEA-Cl, 5 mM 4-AP, pH adjusted to 7.3 with CsOH.

Synaptic currents were evoked with a monopolar glass electrode positioned in the stratum lucidum. Data collection and analysis were done with pClamp 8 software. To isolate AMPA/kainate-mediated EPSCs, slices were bathed in ACSF with 10 μM bicuculline, 50 μM picrotoxin, and 50 μM D-AP5. Slices in which continuous spontaneous epileptic discharge occurred were not used. Mossy fiber EPSCs were distinguished by their characteristically large paired-pulse facilitation (Salin et al., 1996), rapid rise time, and short latency (Yeckel et al., 1999) and by the inhibition of transmission by the group II mGluR agonist L-CCG-1 (10 μM) (Kamiya et al., 1996), which was applied at the end of each experiment. Recordings were considered to be mossy fibers if LCCG-1 inhibited the evoked EPSC by >70%. EPSCs were evoked every 20 s. For LTP induction, we used 3 trains of high-frequency stimulation at 100 Hz for 1 s separated by 10 s. The degree of LTP was calculated by averaging the size of the EPSC during a 10 min control and comparing it to the average size of the EPSC between 20 and 30 min after the tetanus. In some experiments, we blocked group I mGluRs by applying 100 μM CPCCOEt for 10 min prior to and during tetanic stimulation. Frequency facilitation experiments were carried out by increasing the stimulation frequency from 0.05 Hz to 0.2 Hz and 0.5 Hz for 2 min or to 2 Hz for 1 min and 5 Hz for 15 s. For analysis of EPSCs evoked at 100 Hz, the current amplitude immediately preceding the stimulus artifact for each event was set as the baseline amplitude. Data are presented as mean \pm SEM. Parameters were compared using the Student's unpaired t test and $p < 0.05$ was considered significant.

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References

- Bahn, S., Volk, B., and Wisden, W. (1994). Kainate receptor gene expression in the developing rat brain. *J. Neurosci.* *14*, 5525–5547.
- Bashir, Z.I., Bortolotto, Z.A., Davies, C.H., Berretta, N., Irving, A.J., Seal, A.J., Henley, J.M., Jane, D.E., Watkins, J.C., and Collingridge, G.L. (1993). Induction of LTP in the hippocampus needs synaptic activation of glutamate metabotropic receptors. *Nature* *363*, 347–350.
- Bortolotto, Z.A., Clarke, V.R.J., Delany, C.M., Parry, M.C., Smolders, I., Vignes, M., Ho, K.H., Miu, P., Brinton, B.T., Fantaske, R., et al. (1999). Kainate receptors are involved in synaptic plasticity. *Nature* *402*, 297–301.
- Bureau, I., Bischoff, S., Heinemann, S.F., and Mulle, C. (1999). Kainate receptor-mediated responses in the CA1 field of wild-type and GluR6-deficient mice. *J. Neurosci.* *19*, 653–663.
- Castillo, P.E., Weisskopf, M.G., and Nicoll, R.A. (1994). The role of Ca^{2+} channels in hippocampal mossy fiber synaptic transmission and long-term potentiation. *Neuron* *12*, 261–269.
- Castillo, P.E., Janz, R., Sudhof, T.C., Tzounopoulos, T., Malenka, R.C., and Nicoll, R.A. (1997a). Rab3A is essential for mossy fibre long-term potentiation in the hippocampus. *Nature* *388*, 590–593.
- Castillo, P.E., Malenka, R.C., and Nicoll, R.A. (1997b). Kainate receptors mediate a slow postsynaptic current in hippocampal CA3 neurons. *Nature* *388*, 182–186.
- Clarke, V.R., Ballyk, B.A., Hoo, K.H., Mandelzys, A., Pellizzari, A., Bath, C.P., Thomas, J., Sharpe, E.F., Davies, C.H., Ornstein, P.L., et al. (1997). A hippocampal GluR5 kainate receptor regulating inhibitory synaptic transmission. *Nature* *389*, 599–603.
- Conquet, F., Bashir, Z.I., Davies, C.H., Daniel, H., Ferraguti, F., Bordi, F., Franz-Bacon, K., Reggiani, A., Matarese, V., Conde, F., et al. (1994). Motor deficit and impairment of synaptic plasticity in mice lacking mGluR1. *Nature* *372*, 237–243.
- Contractor, A., Swanson, G.T., Sailer, A., O'Gorman, S., and Heinemann, S.F. (2000). Identification of the kainate receptor subunits underlying modulation of excitatory synaptic transmission in the CA3 region of the hippocampus. *J. Neurosci.* *20*, 8269–8278.
- Cossart, R., Esclapez, M., Hirsch, J.C., Bernard, C., and Ben-Ari, Y. (1998). GluR5 kainate receptor activation in interneurons increases tonic inhibition of pyramidal cells. *Nat. Neurosci.* *1*, 470–478.
- Domenici, M.R., Berretta, N., and Cherubini, E. (1998). Two distinct forms of long-term depression coexist at the mossy fiber-CA3 synapse in the hippocampus during development. *Proc. Natl. Acad. Sci. USA* *95*, 8310–8315.
- Frerking, M., and Nicoll, R.A. (2000). Synaptic kainate receptors. *Curr. Opin. Neurobiol.* *10*, 342–351.
- Frerking, M., Malenka, R.C., and Nicoll, R.A. (1998). Synaptic activation of kainate receptors on hippocampal interneurons. *Nat. Neurosci.* *1*, 479–486.
- Frerking, M., Petersen, C.C., and Nicoll, R.A. (1999). Mechanisms underlying kainate receptor-mediated disinhibition in the hippocampus. *Proc. Natl. Acad. Sci. USA* *96*, 12917–12922.
- Harris, E.W., and Cotman, C.W. (1986). Long-term potentiation of guinea pig mossy fiber responses is not blocked by N-methyl-D-aspartate antagonists. *Neurosci. Lett.* *70*, 132–137.
- Hopkins, W.F., and Johnston, D. (1988). Noradrenergic enhancement of long-term potentiation at mossy fiber synapses in the hippocampus. *J. Neurophysiol.* *59*, 667–687.
- Hsia, A.Y., Salin, P.A., Castillo, P.E., Aiba, A., Abeliovich, A., Tonegawa, S., and Nicoll, R.A. (1995). Evidence against a role for metabotropic glutamate receptors in mossy fiber LTP: the use of mutant mice and pharmacological antagonists. *Neuropharmacology* *34*, 1567–1572.
- Huang, Y.Y., and Kandel, E.R. (1996). Modulation of both the early and the late phase of mossy fiber LTP by the activation of beta-adrenergic receptors. *Neuron* *16*, 611–617.
- Huang, Y.Y., Li, X.C., and Kandel, E.R. (1994). cAMP contributes to mossy fiber LTP by initiating both a covalently mediated early phase

and macromolecular synthesis-dependent late phase. *Cell* 79, 69–79.

Ito, I., and Sugiyama, H. (1991). Roles of glutamate receptors in long-term potentiation at hippocampal mossy fiber synapses. *Neuroreport* 2, 333–336.

Kamiya, H., and Ozawa, S. (2000). Kainate receptor-mediated presynaptic inhibition at the mouse hippocampal mossy fibre synapse. *J. Physiol. Lond.* 523, 653–665.

Kamiya, H., Shinozaki, H., and Yamamoto, C. (1996). Activation of metabotropic glutamate receptor type 2/3 suppresses transmission at rat hippocampal mossy fibre synapses. *J. Physiol. Lond.* 493, 447–455.

Manzoni, O.J., Weisskopf, M.G., and Nicoll, N. (1994). MCPG antagonizes metabotropic glutamate receptors but not long term potentiation in the hippocampus. *Eur. J. Neurosci.* 6, 1050–1054.

Mulle, C., Sailer, A., Pérez-Otano, I., Dickinson-Anson, H., Castillo, P.E., Bureau, I., Maron, C., Gage, F.H., Mann, J.R., Bettler, B., and Heinemann, S.F. (1998). Altered synaptic physiology and reduced susceptibility to kainate-induced seizures in GluR6-deficient mice. *Nature* 392, 601–605.

Mulle, C., Sailer, A., Swanson, G.T., O’Gorman, S., Bettler, B., and Heinemann, S.F. (2000). Subunit composition of kainate receptors in hippocampal interneurons. *Neuron* 28, 475–484.

Regehr, W.G., Delaney, K.R., and Tank, D.W. (1994). The role of presynaptic calcium in short-term enhancement at the hippocampal mossy fiber synapse. *J. Neurosci.* 14, 523–537.

Represa, A., Tremblay, E., and Ben-Ari, Y. (1987). Kainate binding sites in the hippocampal mossy fibers: localization and plasticity. *Neuroscience* 20, 739–748.

Rodriguez-Moreno, A., and Lerma, J. (1998). Kainate receptor modulation of GABA release involves a metabotropic function. *Neuron* 20, 1211–1218.

Rodriguez-Moreno, A., Lopez-Garcia, J.C., and Lerma, J. (2000). Two populations of kainate receptors with separate signaling mechanisms in hippocampal interneurons. *Proc. Natl. Acad. Sci. USA* 97, 1293–1298.

Salin, P.A., Scanziani, M., Malenka, R.C., and Nicoll, R.A. (1996). Distinct short-term plasticity at two excitatory synapses in the hippocampus. *Proc. Natl. Acad. Sci. USA* 93, 13304–13309.

Schmitz, D., Frerking, M., and Nicoll, R.A. (2000). Synaptic activation of presynaptic kainate receptors on hippocampal mossy fiber synapses. *Neuron* 27, 327–338.

Urban, N.N., and Barrionuevo, G. (1996). Induction of hebbian and non-hebbian mossy fiber long-term potentiation by distinct patterns of high-frequency stimulation. *J. Neurosci.* 16, 4293–4299.

Vignes, M., and Collingridge, G.L. (1997). The synaptic activation of kainate receptors. *Nature* 388, 179–182.

Vignes, M., Clarke, V.R., Parry, M.J., Bleakman, D., Lodge, D., Ornstein, P.L., and Collingridge, G.L. (1998). The GluR5 subtype of kainate receptor regulates excitatory synaptic transmission in areas CA1 and CA3 of the rat hippocampus. *Neuropharmacology* 37, 1269–1277.

Weisskopf, M.G., and Nicoll, R.A. (1995). Presynaptic changes during mossy fibre LTP revealed by NMDA receptor-mediated synaptic responses. *Nature* 376, 256–259.

Weisskopf, M.G., Castillo, P.E., Zalutsky, R.A., and Nicoll, R.A. (1994). Mediation of hippocampal mossy fiber long-term potentiation by cyclic AMP. *Science* 265, 1878–1882.

Yeckel, M.F., Kapur, A., and Johnston, D. (1999). Multiple forms of LTP in hippocampal CA3 neurons use a common postsynaptic mechanism. *Nat. Neurosci.* 2, 625–633.

Zalutsky, R.A., and Nicoll, R.A. (1990). Comparison of two forms of long-term potentiation in single hippocampal neurons. *Science* 248, 1619–1624.