

# Attenuated Plasticity of Postsynaptic Kainate Receptors in Hippocampal CA3 Pyramidal Neurons

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Kainate receptor-mediated components of postsynaptic currents at hippocampal mossy fiber synapses have markedly slower kinetics than currents arising from AMPA receptors. Here, we demonstrate that other aspects of kainate and AMPA receptor function at this synapse are distinct; in particular, kainate receptor currents are less sensitive to short- and long-term increases in presynaptic strength. EPSCs arising predominantly from AMPA receptors exhibited well characterized paired-pulse facilitation, frequency facilitation, and NMDA receptor-independent long-term potentiation, whereas isolated kainate receptor synaptic currents (KA-EPSCs) exhibited attenuated facilitation and long-term potentiation. In addition, KA-EPSCs varied in their sensitivity to a low-affinity competitive antagonist, suggestive of a synaptic heterogeneity greater than that of EPSCs comprised predominantly of AMPA receptors. These data suggest that the proportional contribution of AMPA and kainate receptors to ensemble synaptic currents will vary depending on the firing frequency of mossy fiber afferents. These synaptic features may be a mechanism for limiting activation of kainate receptors at mossy fiber synapses, which has been shown to be involved in seizurogenic firing of the CA3 network.

**Key words:** hippocampus; synaptic; transmission; glutamate; plasticity; long-term potentiation

## Introduction

Excitatory synaptic transmission in the mammalian brain is mediated predominantly by three types of ionotropic glutamate receptors, which are comprised of receptor subunits from the AMPA, kainate, and NMDA gene families (Dingledine et al., 1999). AMPA and NMDA receptors mediate EPSCs at most excitatory synapses and are critically involved in most forms of synaptic plasticity, whereas kainate receptors have a distinct and wider variety of functional roles in the brain. Presynaptic kainate receptors modulate both excitatory and inhibitory transmission at a number of synapses (Huettner, 2003; Lerma, 2003). Kainate receptors also mediate postsynaptic currents at a subset of excitatory synapses, usually at significantly lower current densities than AMPA receptors (Huettner, 2003; Lerma, 2003), and likely contribute to temporal summation of synaptic input that can lead to increased neuronal excitability (Frerking and Ohliger-Frerking, 2002). Kainate receptors also are implicated in forms of short- and long-term plasticity of synapses in the hippocampus (Bortolotto et al., 1999; Contractor et al., 2001; Schmitz et al., 2001, 2003), cortex (Kidd and Isaac, 1999), and amygdala (Li et al., 2001).

Postsynaptic kainate receptors were first described functionally at the mossy fiber–CA3 synapse in the hippocampus as small

amplitude, slowly rising and decaying EPSCs that summated during short high-frequency stimulations and that were sensitive to inhibition by high concentrations of the glutamate receptor antagonist CNQX (Castillo et al., 1997; Vignes and Collingridge, 1997). The slow kinetics of mossy fiber kainate receptor synaptic currents (KA-EPSCs) are similar to those at thalamocortical (Kidd and Isaac, 2001), basolateral amygdala (Li and Rogawski, 1998), spinal cord dorsal horn (Li et al., 1999), and cerebellar Golgi neuron synapses (Bureau et al., 2000) but contrast with the rapid activation and deactivation of recombinant kainate receptors expressed in heterologous systems (Herb et al., 1992; Schiffer et al., 1997; Traynelis and Wahl, 1997; Swanson and Heinemann, 1998; Cui and Mayer, 1999) as well as KA-EPSCs in retinal horizontal cells (DeVries and Schwartz, 1999). The underlying mechanism(s) that produces the relatively slow time course of KA-EPSCs at most synapses remains a mystery, although previous studies have tested possible explanations at mossy fiber or other kainate receptor-containing synapses that include extrasynaptic localization, saturation, and receptor desensitization (Castillo et al., 1997; Vignes and Collingridge, 1997; Bureau et al., 2000; Kidd and Isaac, 2001; Cossart et al., 2002). Subunit composition of the postsynaptic receptor complex plays some role in producing these slow receptor kinetics because genetic ablation of the KA-2 kainate receptor subunit increased the KA-EPSC decay rate by ~35% (Contractor et al., 2003); however, this decay rate was still approximately fourfold slower than mossy fiber synaptic currents (MF-EPSCs) that are mediated predominantly by AMPA receptors.

In this study, we analyzed additional facets of postsynaptic kainate receptor function at mossy fiber synapses. We focused on comparing the responses of MF- and KA-EPSCs to the well char-

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acterized forms of short- and long-term plasticity exhibited by mossy fiber–CA3 pyramidal neuron synapses. The relatively low basal release probability of these synapses (Salin et al., 1996; Lawrence et al., 2004) and an unusually large readily releasable pool of vesicles in mossy fiber terminals (Hallermann et al., 2003) produce marked potentiation of MF-EPSCs when release probability is increased. We report here that mossy fiber KA-EPSCs show markedly lower sensitivity to changes in presynaptic release probability compared with mixed AMPA/KA-EPSCs (in which AMPA receptors gate the large majority of the peak current). KA-EPSCs also had lower coefficients of variance (CVs) than MF-EPSCs during basal stimulation and were less sensitive to competitive inhibition by the low-affinity antagonist  $\gamma$ -D-glutamylglycine ( $\gamma$ -DGG). These results suggest that cellular mechanisms exist to limit increases in the contribution of kainate receptors to mossy fiber excitatory transmission when the synaptic vesicular release probability increases after the induction of short- and long-term forms of plasticity.

## Materials and Methods

**Slice preparation.** Hippocampal slices were made from postnatal day (P) 14 to P23 129SvEv mice as described previously (Contractor et al., 2001). Briefly, animals were decapitated with a guillotine, and the brain was removed under ice-cold sucrose-rich slicing solution (SRSS) containing 85 mM NaCl, 2.5 mM KCl, 1.25 mM  $\text{NaH}_2\text{PO}_4$ , 25 mM  $\text{NaHCO}_3$ , 25 mM glucose, 75 mM sucrose, 0.5 mM  $\text{CaCl}_2$ , 4 mM  $\text{MgCl}_2$ , 10  $\mu\text{M}$  DL-AP-5, and 100  $\mu\text{M}$  kynurenat. Slices were made with a Vibratome 3000 Plus (Vibratome, St. Louis, MO) at 350  $\mu\text{m}$  and incubated in SRSS that was slowly warmed to 28°C; on reaching this temperature, the slices were slowly returned to room temperature while the SRSS was exchanged for an artificial CSF (ACSF) containing 125 mM NaCl, 2.4 mM KCl, 1.2 mM  $\text{NaH}_2\text{PO}_4$ , 25 mM  $\text{NaHCO}_3$ , 25 mM glucose, 1 mM  $\text{CaCl}_2$ , 2 mM  $\text{MgCl}_2$ , 10  $\mu\text{M}$  DL-AP-5, and 100  $\mu\text{M}$  kynurenat. Both SRSS and ACSF were equilibrated with 95%  $\text{O}_2$ /5%  $\text{CO}_2$ .

**Electrophysiological recordings.** After 1 hr, individual slices were transferred to a recording chamber and perfused continuously with ACSF containing 4 mM  $\text{CaCl}_2$  and 4 mM  $\text{MgCl}_2$  at room temperature. Glass electrodes were pulled from borosilicate glass and had resistances of 2–4 M $\Omega$  when filled with an internal solution containing (in mM) 95 CsF, 25 CsCl, 10 Cs-HEPES, 10 Cs-EGTA, 2 NaCl, 2 Mg-ATP, 10 QX-314, 5 TEA-Cl, and 5 4-AP, with the pH adjusted to 7.3 with CsOH. Whole-cell patch-clamp recordings were made from pyramidal cells in the CA3 region of the hippocampus, visually identified with infrared-differential interference contrast optics. Voltage-clamp recordings were made at a holding potential of  $-70$  mV. Series resistance was maintained at 4–25 M $\Omega$ , and recordings in which series resistance changed significantly were discarded. Synaptic currents were evoked with a monopolar glass electrode positioned in the inner 50  $\mu\text{m}$  of the stratum lucidum. For AMPA-EPSC recordings, we recorded composite MF-EPSCs, which were primarily composed of AMPA-EPSCs ( $\sim 93\%$ ) (Contractor et al., 2003), with ACSF containing 10  $\mu\text{M}$  bicuculline, 50  $\mu\text{M}$  picrotoxin, and 25  $\mu\text{M}$  D-AP-5. We defined synaptic currents as MF-EPSCs if they showed characteristically large paired-pulse facilitation ( $>2.0$  at 40 msec intervals) (Salin et al., 1996), had a rapid rise time and short latency, and were inhibited by  $>70\%$  by the group II mGluR agonist (2S,1'S,2'S)-2-(carboxycyclopropyl)glycine (L-CCG-1) (10  $\mu\text{M}$ ), which was bath applied at the end of each experiment. For KA-EPSC recordings, we recorded from CA3 pyramidal cells in the presence of the ACSF containing the AMPA antagonist GYKI53655 (50  $\mu\text{M}$ ), which was shown not to affect mossy fiber plasticity (Schmitz et al., 2003), in addition to the GABA<sub>A</sub> and NMDA receptor antagonists. To resolve small KA-EPSCs that were obscured by stimulation artifacts, we digitally subtracted a “template” stimulation trace that was acquired after application of CNQX (50  $\mu\text{M}$ ) at the end of the recordings. CV were calculated as the SD/mean amplitude for 8–10 EPSCs.

Data were collected and analyzed with pClamp9 software (Axon Instruments, Union City, CA). All values were represented as mean  $\pm$  SEM;

$n$  values are the number of recordings from individual slice preparations (i.e., slices were not used for more than a single recording). The Student's unpaired  $t$  test or Kolmogorov–Smirnov nonparametric test (for cumulative probability histograms) was used for statistics, and asterisks indicate statistical significance at  $p < 0.05$ .

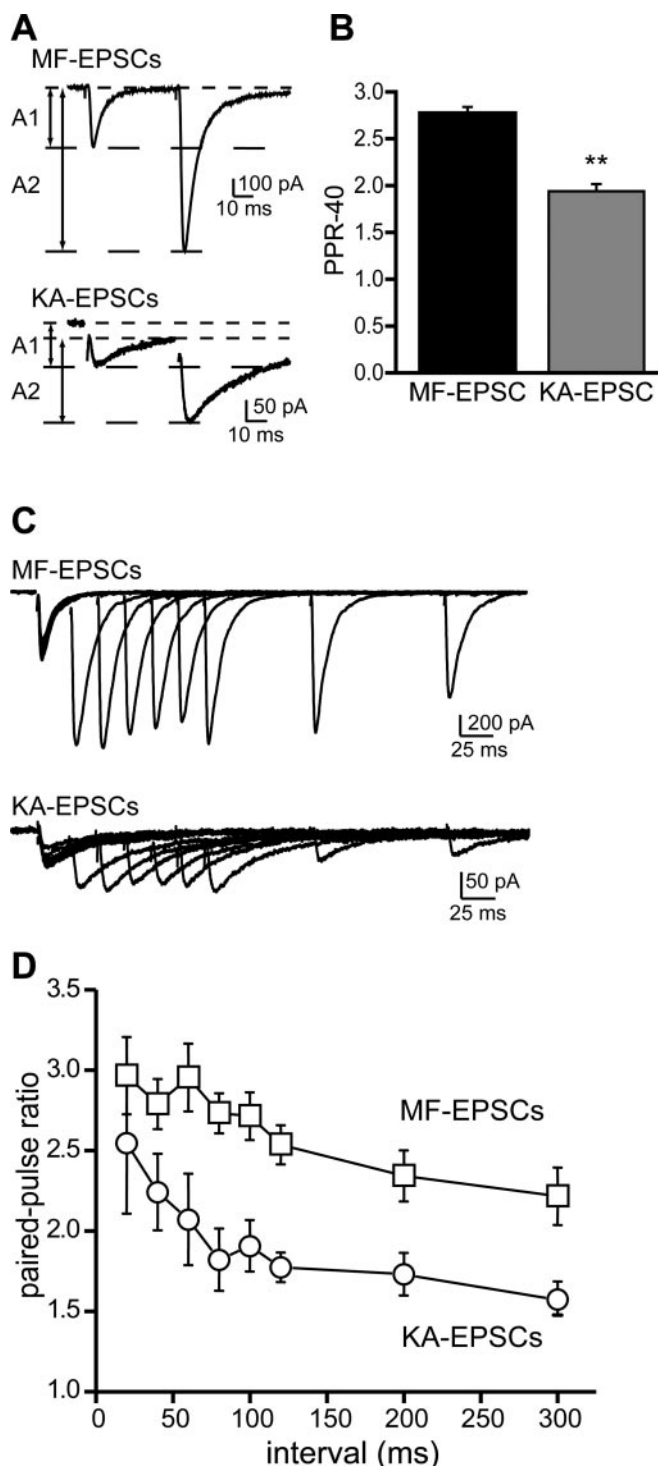
**Stimulation protocols.** Control test pulses were given every 20 sec. In the paired-pulse stimulation protocol, two pulses were delivered with 20–300 msec intervals. For frequency facilitation, synaptic stimulation was delivered for 2 min at 0.2–2 Hz, and the degree of facilitation was calculated by averaging the size of control EPSCs measured just before the frequency facilitation protocol (for 5 min) and comparing it to the average size of the eight EPSCs at peak facilitation. For induction of long-term potentiation (LTP), we used a long high-frequency stimulation (L-HFS) in which 100 Hz stimuli for 1 sec were delivered three times at 10 sec intervals. The degree of LTP was calculated by averaging the size of the control EPSCs measured just before the L-HFS for 10 min and comparing it to the average size of the EPSCs between 25 and 30 min after the L-HFS. Forskolin (FSK; 10  $\mu\text{M}$ ) and  $\gamma$ -DGG (1 mM) were bath applied.

**Drugs.** All drugs were purchased from Tocris Cookson (Bristol, UK), except inorganic salts, QX-314, TEA-Cl, and 4-AP (Sigma-Aldrich, St. Louis, MO). GYKI53655 was custom synthesized by Tocris-Cookson.

## Results

To compare short-term plasticity of AMPA- and kainate receptor-mediated currents at mossy fiber synapses, we recorded EPSCs from CA3 pyramidal neurons in mouse hippocampal slices while selectively stimulating mossy fibers with pairs of stimuli separated by a 40 msec interval. MF-EPSCs were identified by their brief latency, relatively large paired-pulse ratio (PPR), and sensitivity to group II mGluR agonist L-CCG-1 (10  $\mu\text{M}$ ), as described previously (Contractor et al., 2001). In the presence of the NMDA receptor antagonist, MF-EPSCs arise predominantly ( $>90\%$ ) from activation of AMPA receptors with a minor kainate receptor component (Contractor et al., 2003). In this study, we used the mixed MF-EPSCs as a measure of AMPA-EPSC function because selective antagonists that inhibit KA-EPSCs are not commercially available and would inhibit presynaptic kainate receptors that modulate glutamate release. KA-EPSCs were isolated after application of 50  $\mu\text{M}$  GYKI 53655, a selective AMPA receptor antagonist (Paternain et al., 1995), and were detectable only in a subset of recordings. After recording KA-EPSCs, CNQX (50  $\mu\text{M}$ ) was applied to completely inhibit the KA-EPSCs and isolate the stimulation artifact, which in most cases was digitally subtracted from KA-EPSC recordings to resolve more precisely the small synaptic currents mediated by kainate receptors.

We first determined the PPR at 40 msec (PPR40) for MF- and KA-EPSCs (Fig. 1). The amplitudes of second EPSCs were divided by the amplitudes of the first EPSCs to calculate the PPR40. In addition, the amplitude of the second KA-EPSCs during the paired stimulation was measured after correcting the baseline for the relatively slow decay of the first EPSC, as shown in Figure 1A. In agreement with previous studies, we found that the PPR40 was  $2.77 \pm 0.07$  for MF-EPSCs ( $n = 74$ ); however, the PPR40 for KA-EPSCs was significantly lower at  $1.94 \pm 0.08$  ( $n = 54$ ) (Fig. 1B). The difference between MF- and KA-EPSCs was observed over a wide range of interstimulus intervals (Fig. 1C). Paired-pulse facilitation at mossy fiber synapses is long lasting (Salin et al., 1996), and accordingly, we observed significant facilitation of MF-EPSCs at intervals of up to 300 msec. At each interval measured, with the exception of the shortest (20 msec), the PPRs of KA-EPSCs were lower than MF-EPSCs (MF-EPSC and KA-EPSC PPRs: 20 msec,  $3.0 \pm 0.2$  and  $2.5 \pm 0.4$ ; 40 msec,  $2.8 \pm 0.2$  and  $2.2 \pm 0.2$ ; 60 msec,  $2.9 \pm 0.2$  and  $2.1 \pm 0.3$ ; 80 msec,  $2.7 \pm 0.1$  and  $1.8 \pm 0.2$ ; 100 msec,  $2.7 \pm 0.1$  and  $1.0 \pm 0.2$ ; 120 msec,  $2.5 \pm 0.1$

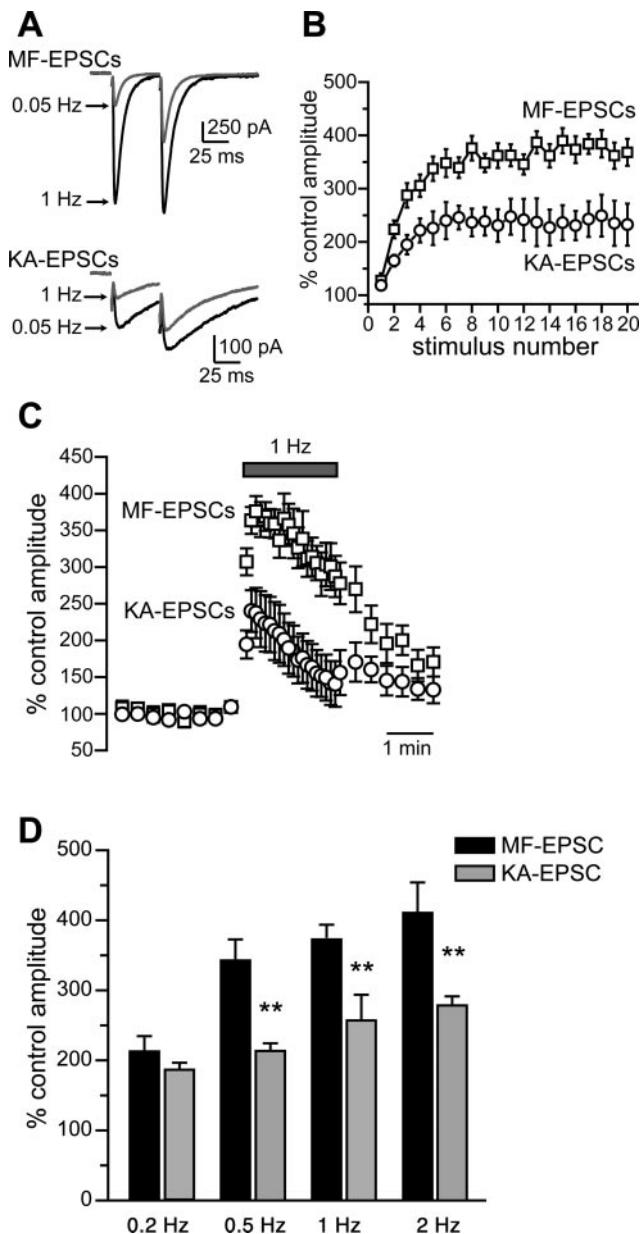


**Figure 1.** KA-EPSCs have lower PPRs compared with MF-EPSCs. *A*, Representative traces of MF-EPSCs (top) and KA-EPSCs (bottom) in response to pairs of stimuli separated by an interval of 40 msec. *A1* and *A2* indicate the amplitudes of the first and second EPSCs, respectively. KA-EPSCs decay with a relatively slow time course, and therefore to measure the amplitude of the second KA-EPSCs, the baseline was adjusted to the current immediately preceding the stimulation artifact. *B*, Summary of PPR40 values. The value of PPR40 for KA-EPSCs ( $1.94 \pm 0.08$ ;  $n = 54$ ) is significantly smaller than that for MF-EPSCs ( $2.77 \pm 0.07$ ;  $n = 74$ ;  $**p < 0.01$ ). *C*, Superimposed pairs of MF-EPSCs (top) and KA-EPSCs (bottom) stimulated at intervals of 20, 40, 60, 80, 100, 120, 200, and 300 msec. *D*, Summary of PPRs for MF-EPSCs and KA-EPSCs. KA-EPSCs show significantly lower paired-pulse facilitation compared with MF-EPSCs at all intervals, except 20 msec.

and  $1.8 \pm 0.1$ ; 200 msec,  $2.3 \pm 0.2$  and  $1.7 \pm 0.1$ ; 300 msec,  $2.2 \pm 0.2$  and  $1.6 \pm 0.1$ ;  $n = 10$ –15 for MF-EPSCs and 3–6 for KA-EPSCs). These results demonstrate that KA-EPSCs are consistently less facilitated than MF-EPSCs during paired stimulation of mossy fiber synapses.

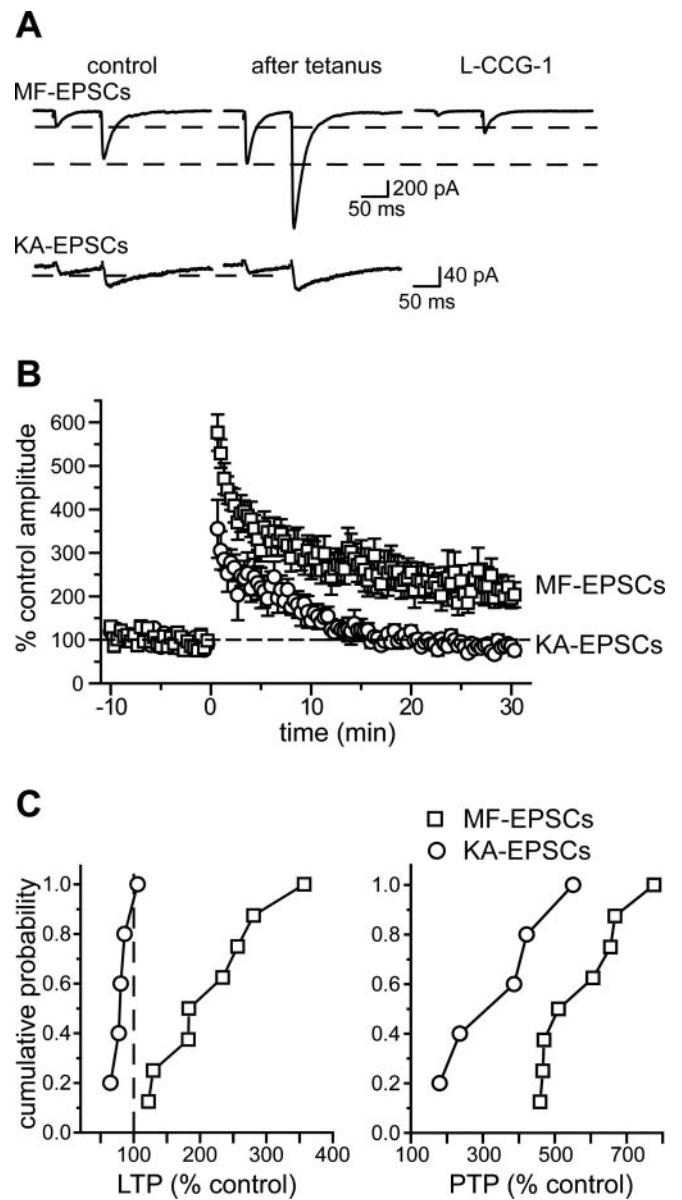
We next tested whether MF-EPSCs and KA-EPSCs were differentially sensitive to an alternate form of presynaptic short-term synaptic plasticity, frequency-dependent facilitation. Trains of relatively low-frequency stimuli rapidly increase mossy fiber release probability (Salin et al., 1996), in part through activation of presynaptic kainate receptors (Contractor et al., 2001; Lauri et al., 2001a; Schmitz et al., 2001). Representative currents at basal frequencies and at 1 Hz, shown in Figure 2*A*, demonstrate that both types of EPSCs increased in amplitude when the stimulation frequency was increased, but KA-EPSC amplitudes did not show the same degree of facilitation as MF-EPSCs. The mean percentage amplitudes for the first 20 MF- and KA-EPSCs during 1 Hz stimulation trains are shown in Figure 2*B*. The rate of increase was similar for the EPSCs, but the peak facilitation was significantly larger for MF-EPSCs. Relatively long trains of 1 Hz stimulation (2 min, 120 stimuli) produced facilitation in both MF- and KA-EPSCs that slowly attenuated (Fig. 2*C*). KA-EPSCs showed greater depression at the end of the train, which returned to control levels on resumption of the basal stimulation frequency, suggesting that the receptors might have been accumulating in desensitized states. PPRs of MF- and KA-EPSCs during stimulation trains were reduced to similar values ( $1.14 \pm 0.14$  and  $0.91 \pm 0.10$ , respectively) despite their initial difference during basal stimulation, suggesting that the increased release probability reaches a “ceiling” during 1 Hz stimulation and that KA-EPSCs show less facilitation because their presynaptic terminals have higher basal release probability. The average facilitation at the four stimulation frequencies are shown in Figure 2*D*; as was observed at 1 Hz, MF-EPSCs facilitated to a significantly larger degree than KA-EPSCs at all stimulation frequencies tested (MF-EPSC facilitation: 0.2 Hz,  $212 \pm 22\%$ ; 0.5 Hz,  $342 \pm 30\%$ ; 1 Hz,  $373 \pm 17\%$ ; 2 Hz,  $411 \pm 44\%$ ;  $n = 5$ –17; KA-EPSCs: 0.2 Hz,  $186 \pm 10\%$ ; 0.5 Hz,  $213 \pm 11\%$ ; 1 Hz,  $257 \pm 38\%$ ; 2 Hz,  $278 \pm 23\%$ ;  $n = 3$ –9). These data suggest that limitations exist at mossy fiber synapses to prevent a marked increase in kainate receptor synaptic strength equivalent to that of AMPA receptor-mediated EPSCs.

MF-EPSCs also exhibit several well characterized forms of long-term synaptic plasticity that are expressed as a stable increase in release probability (Zalutsky and Nicoll, 1990; Weisskopf et al., 1994; Urban and Barrionuevo, 1996; Yeckel et al., 1999). To determine how KA-EPSCs respond to this form of mossy fiber plasticity, we compared the potentiation of MF- and KA-EPSCs after relatively strong tetanic stimulation (three 1 sec trains of 100 Hz stimulation at 10 sec intervals), which reliably elicits LTP of MF-EPSCs. Representative currents from both MF- and KA-EPSCs are shown in Figure 3*A*. MF-EPSCs exhibited large post-tetanic potentiation (PTP;  $576 \pm 42\%$ ), followed by relaxation of synaptic strength to a stable potentiation of  $218 \pm 28\%$  ( $n = 8$ ) 30 min after tetanus (Fig. 3*B*, squares). In contrast, KA-EPSCs were modestly potentiated immediately after tetanus ( $311 \pm 70\%$ ) and stabilized at an amplitude lower than that of control ( $83 \pm 7\%$ ;  $n = 5$ ;  $p < 0.05$  compared with MF-EPSC potentiation) (Fig. 3*B*, circles). Cumulative probability histograms for LTP and PTP data are shown in Figure 3*C*. This slight reduction in KA-EPSC amplitudes compared with the baseline control initially suggested to us that KA-EPSCs were depressed rather than potentiated by the tetanic stimulation, but this



**Figure 2.** Low-frequency trains of stimuli facilitate KA-EPSCs less than MF-EPSCs. *A*, Representative MF-EPSCs (top) and KA-EPSCs (bottom) are shown at basal frequency (0.05 Hz) and during 1 Hz stimulation. Gray traces are currents at the basal frequency of 0.05 Hz, and black traces were collected during stimulation at 1 Hz. *B*, Graph of the mean relative amplitudes of the first 20 MF- and KA-EPSCs at the start of 1 Hz stimulation. *C*, Mean amplitudes of MF- and KA-EPSCs during 1 Hz frequency for 2 min. For the sake of clarity, data were averaged over 6 sec segments during the 1 Hz stimulation. The gray bar shows the period of higher-frequency stimulation. EPSC amplitudes in *B* and *C* were normalized to the mean EPSC amplitudes in the 5 min control period preceding 1 Hz stimulation. *D*, Summary histogram of frequency facilitation. KA-EPSCs were less facilitated compared with MF-EPSCs at all frequencies, except 0.2 Hz (MF-EPSC facilitation: 0.2 Hz, 212 ± 22%; 0.5 Hz, 342 ± 30%; 1 Hz, 373 ± 17%; 2 Hz, 411 ± 44%;  $n = 5-17$ ; KA-EPSCs: 0.2 Hz, 186 ± 10%; 0.5 Hz, 213 ± 11%; 1 Hz, 257 ± 38%; 2 Hz, 278 ± 23%;  $n = 3-9$ ;  $**p < 0.01$ ). Facilitation was measured by averaging amplitudes of eight EPSCs at the peak facilitation. Amplitudes were normalized to those during the control period preceding higher-frequency stimulation.

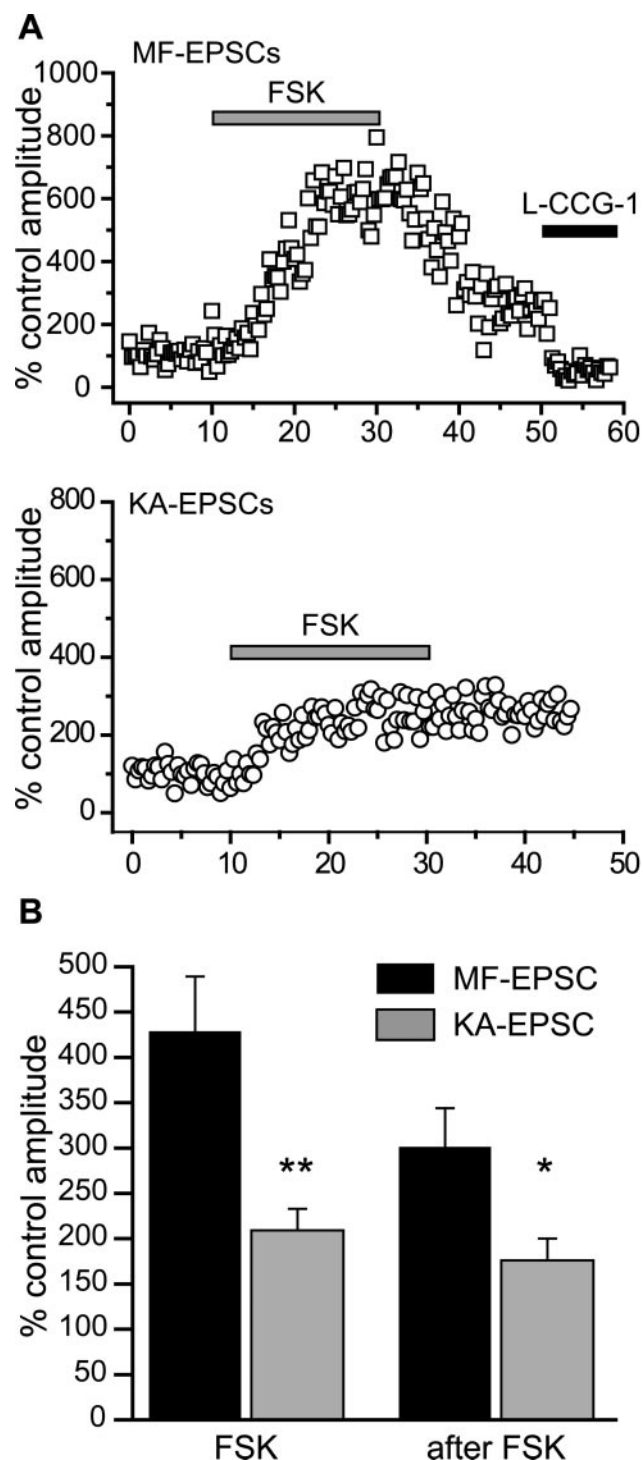
proved not to be the case. Instead, the apparent depression was found to result from rundown of the KA-EPSC amplitudes over the course of the experiments, as has been observed previously (Hirbec et al., 2003). In the absence of tetanic stimulation, the mean KA-EPSC amplitude was  $52 \pm 7\%$  of control after 30 min



**Figure 3.** KA-EPSCs exhibit reduced LTP in response to high-frequency tetanic stimulation. *A*, Representative MF- and KA-EPSCs are shown before and 30 min after tetanic stimulation (3 trains of 100 Hz for 1 sec separated by 10 sec intervals) and during L-CCG-1 application (MF-EPSCs only). The dashed lines indicate the first EPSC amplitude before and after tetanus (MF-EPSCs). KA-EPSCs did not appear to exhibit LTP after this induction paradigm (but see Results). *B*, Mean normalized EPSC amplitudes for MF-EPSCs (squares) and KA-EPSCs (circles). The tetanus was delivered at time 0. The mean LTP for MF-EPSCs was  $218 \pm 28\%$ , whereas KA-EPSCs were lower than control amplitudes ( $83 \pm 7\%$ ). *C*, Cumulative probability histograms for mean LTP (25–30 min after tetanus) and PTP (first amplitude after tetanus) of MF- and KA-EPSCs. The differences in LTP and PTP between KA- and MF-EPSCs are highly significant ( $p < 0.05$ ). The dashed line indicates 100% (control amplitudes) in the LTP histogram.

( $n = 4$ ), revealing that KA-EPSCs were potentiated after tetanic stimulation (159% increase in the normalized mean amplitudes between rundown and LTP recordings) but not to the same degree as MF-EPSCs.

Chemically induced LTP of KA-EPSCs was also less than MF-EPSCs. The application of FSK, an activator of adenylyl cyclase, is known to increase release probability through a protein kinase A (PKA)-dependent pathway (Weisskopf et al., 1994). Representative experiments in which FSK (10  $\mu\text{M}$ ) was applied to slices while recording MF- and KA-EPSCs are shown in Figure 4*A*. After 20



**Figure 4.** FSK potentiates KA-EPSCs less than MF-EPSCs. *A*, Representative experiments in which MF- and KA-EPSCs were recorded while applying FSK ( $10 \mu\text{M}$ ). The black bar indicates the duration of FSK application. Amplitudes are normalized against the mean EPSC amplitude during the pre-FSK control period. *B*, Summary histogram of the effect of FSK application. MF- and KA-EPSCs were both potentiated in the presence of FSK [left columns;  $429 \pm 60\%$  ( $n = 7$ ) and  $209 \pm 24\%$  ( $n = 10$ ), respectively]. Fifteen minutes after removal of FSK, both types of EPSCs remained potentiated ( $301 \pm 43\%$  and  $176 \pm 24\%$ , respectively). MF-EPSCs were potentiated to a greater degree than KA-EPSCs both in the presence of (\*\* $p < 0.01$ ) and after (\* $p < 0.05$ ) FSK application.

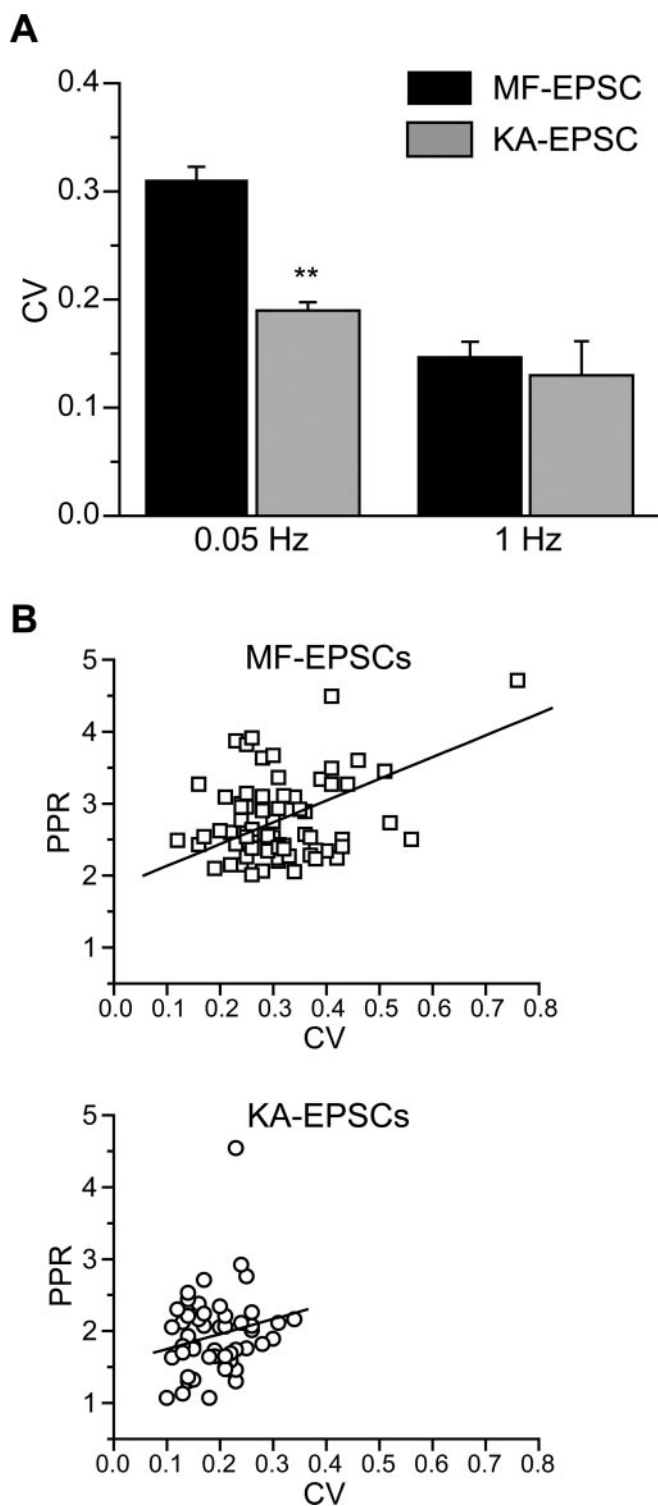
min in FSK, MF-EPSCs were potentiated by  $428 \pm 60\%$  ( $n = 10$ ); this increase diminished slightly after FSK was removed to a stable potentiation of  $301 \pm 43\%$  after 15 min without FSK (Fig. 4*B*). In contrast, KA-EPSCs were potentiated by  $209 \pm 24\%$  ( $n =$

10) in the presence of FSK and  $176 \pm 24\%$  after removal of the compound.

The attenuated sensitivity of KA-EPSCs to short- and long-term plasticity is consistent with the possibility that synaptic kainate receptors are preferentially located at synapses with higher basal release probabilities than synapses containing predominantly AMPA receptors. If this hypothesis is true, then KA-EPSC current amplitudes would be expected to exhibit a lower CV than MF-EPSCs. As shown in Figure 5*A*, analysis of CV values for all our basal (0.05 Hz) recordings revealed that the mean CV of KA-EPSCs ( $0.19 \pm 0.01$ ;  $n = 54$ ) indeed was significantly lower than the MF-EPSC mean CV value ( $0.31 \pm 0.01$ ;  $n = 74$ ;  $p < 0.01$ ). Consistent with the idea that a ceiling release probability is achieved during 1 Hz stimulation, CV values for MF- and KA-EPSCs were reduced to similar values during the higher-frequency stimulation ( $0.15 \pm 0.01$  and  $0.13 \pm 0.03$ ,  $n = 14$  and 10, respectively). Interestingly, there was a weak, but significant, relationship between basal PPR and CV values for MF-EPSCs ( $r = 0.54$ ;  $p < 0.01$ ), but not for KA-EPSCs ( $r = 0.21$ ;  $p = 0.13$ ) (Fig. 5*B*). This lack of a positive correlation between PPR and CV suggests that both presynaptic and postsynaptic factors could contribute to the attenuated short- and long-term facilitation of KA-EPSCs.

Kainate receptors are known to be located within mossy fiber synapses because they respond to quantal release of glutamate (Cossart et al., 2002), suggesting that their attenuated responses to increases in release probability might result from (1) a heterogeneous population of synapses, some which preferentially contain synaptic kainate receptors and have a relatively limited capacity for presynaptic increases in strength, or (2) intrinsic properties of the postsynaptic kainate receptors themselves (e.g., distinct sensitivities for glutamate, desensitization properties, or kinetic behavior). Little evidence for any of these possibilities exists (with the exception of different AMPA and kainate receptor desensitization kinetics), and thus elucidating the synaptic or cellular mechanisms that underlie the slow kinetics of KA-EPSCs has been problematic.

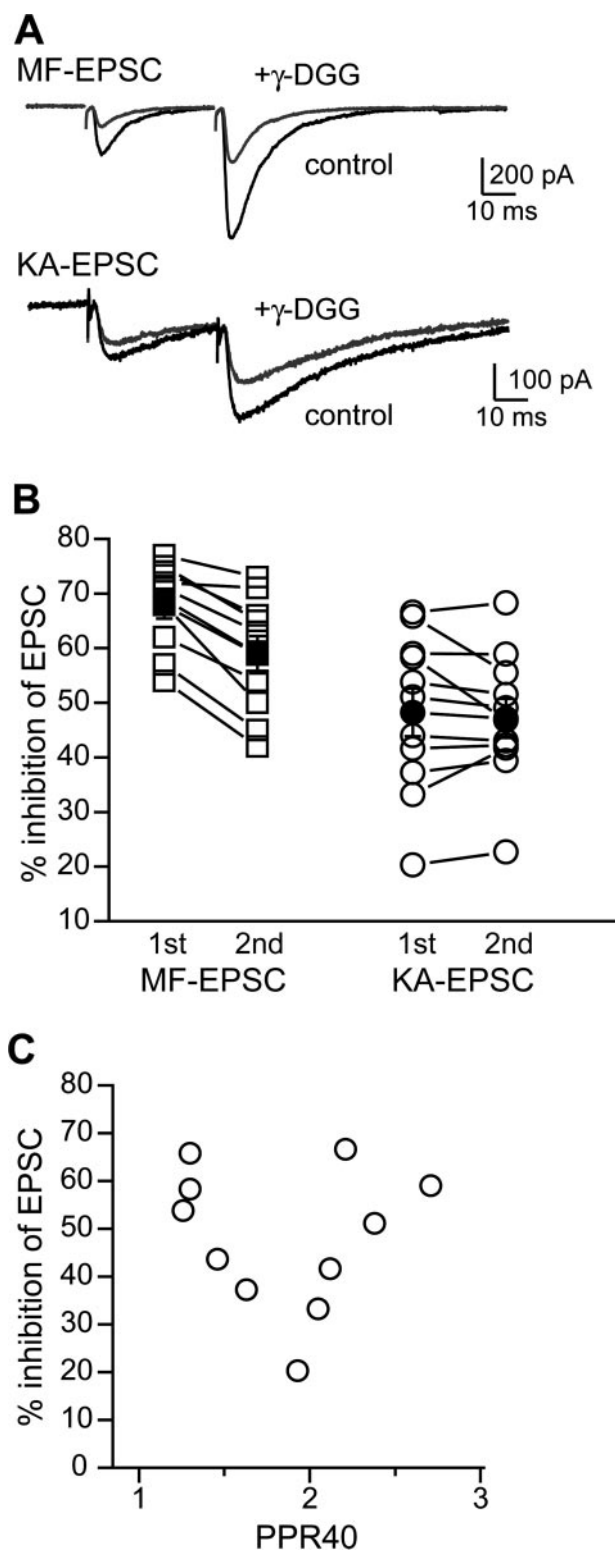
To address some of the uncertainties associated with mossy fiber kainate receptor function, we tested whether heterogeneity in synaptic kainate receptor function was detectable on stimulation of mossy fibers by comparing inhibition of MF- and KA-EPSCs with the low-affinity, rapidly equilibrating antagonist  $\gamma$ -DGG. The degree of inhibition of synaptic EPSCs by a rapidly equilibrating competitive antagonist is dependent primarily on the  $EC_{50}$  value of glutamate for the receptors, the  $IC_{50}$  value of the antagonist, and the glutamate concentration in the synapse (Liu et al., 1999). Representative MF- and KA-EPSCs are shown in Figure 6*A* before and during application of 1 mM  $\gamma$ -DGG to the slice preparations. MF-EPSCs were inhibited to relatively equivalent degrees between recordings ( $68 \pm 2\%$  inhibition; range, 54–77%;  $n = 10$ ). Surprisingly, KA-EPSCs were both less sensitive and more variable in their inhibition by the antagonist ( $48 \pm 4\%$  inhibition; range, 20–67%;  $n = 11$ ) (Fig. 6*B*). Furthermore, inhibition of MF-EPSCs was consistently less for the second EPSC during paired stimuli ( $p < 0.05$ ; paired  $t$  test), suggesting that the synaptic glutamate concentration was higher during the second stimulation. In contrast, second KA-EPSCs in the pairs did not show equivalent reductions in  $\gamma$ -DGG inhibition. In addition, the degree of inhibition of KA-EPSCs by  $\gamma$ -DGG did not correlate with the basal PPR (Fig. 6*C*). These data strongly suggest that heterogeneity in either postsynaptic or presynaptic function exists at mossy fiber synapses containing kainate receptors.



**Figure 5.** The CV is lower for KA-EPSCs. *A*, MF- and KA-EPSC CV values are significantly different at the basal frequency of stimulation ( $0.31 \pm 0.01$  and  $0.19 \pm 0.01$ ,  $n = 74$  and  $54$ , respectively;  $**p < 0.01$ ) but not during 1 Hz stimulation ( $0.14 \pm 0.01$  and  $0.13 \pm 0.03$ , respectively). CV were calculated as the SD/mean amplitude for 8–10 EPSCs from each recording. *B*, MF-EPSCs show a weak, but significant, correlation between PPR and CV ( $r = 0.54$ ;  $p < 0.01$ ), whereas KA-EPSCs do not ( $r = 0.21$ ;  $p = 0.13$ ).

### Discussion

We show here that synaptic currents arising from kainate and AMPA receptors exhibit markedly different sensitivities to changes in mossy fiber release probability. Our initial observation



**Figure 6.** Inhibition by  $\gamma$ -DGG reveals synaptic heterogeneity. *A*, Sample MF- and KA-EPSCs before (control; black traces) and during (gray traces) bath application of  $\gamma$ -DGG (1 mM). *B*, Scatter graph demonstrating that MF-EPSCs (squares) were consistently inhibited by  $\gamma$ -DGG ( $68 \pm 2\%$ ;  $n = 10$ ), whereas KA-EPSCs (circles) show a lower mean inhibition ( $48 \pm 4\%$ ) and significant variability in sensitivity to the antagonist.  $\gamma$ -DGG inhibition of the second MF-EPSC in a pair ( $59 \pm 3\%$ ) was significantly lower than inhibition of the first MF-EPSC ( $p < 0.05$ ), but an equivalent depression in inhibition was not observed for KA-EPSCs ( $47 \pm 4\%$  inhibition of second EPSC). Open symbols are individual experiments shown as a scatter plot; filled symbols are mean  $\pm$  SEM. *C*, Plot of the PPR versus percentage inhibition for KA-EPSCs. No correlation between the two parameters is apparent.

that the PPR of KA-EPSCs was lower than that of MF-EPSCs, which are predominantly mediated by AMPA receptor activation, led us to compare potentiation of EPSCs during other well characterized forms of short- and long-term synaptic plasticity. In each case, KA-EPSCs responded with lower increases in amplitude compared with MF-EPSCs. In addition, we obtained evidence for heterogeneity in the synaptic function of KA-EPSCs consistent with different receptor populations or synaptic glutamate concentrations. These results suggest that the contribution of AMPA and kainate receptor activation to mossy fiber transmission will vary in a frequency-dependent manner during physiological firing patterns of mossy fibers, which can occur in short high-frequency bursts (Henze et al., 2002).

In our initial experiments, we observed a marked lower PPR for KA-EPSCs than for MF-EPSCs (PPR40 of 1.9 vs 2.8, respectively). Indeed, the mean PPR40 for KA-EPSCs is lower than that typically used as one criterion for identification of mossy fiber inputs to CA3 neurons. However, KA-EPSCs are found only on mossy fiber synapses in CA3 neurons (Castillo et al., 1997; Vignes and Collingridge, 1997; Mulle et al., 1998), obviating any concerns of potential contamination by other types of synaptic inputs. The difference in paired-pulse facilitation was dependent on the interstimulus interval (Fig. 1C,D); KA-EPSCs facilitated significantly less than MF-EPSCs at all intervals, except the shortest pairing of 20 msec.

The lower KA-EPSC PPR and frequency facilitation that we observed was surprising because it was reported previously that KA- and AMPA-EPSCs at a number of synapses, including mossy fiber–CA3 pyramidal cells, CA1 interneurons, and thalamocortical synapses, exhibited similar short-term plasticity (Castillo et al., 1997; Frerking et al., 1998; Kidd and Isaac, 2001). In contrast, the PPR of KA-EPSCs was lower than that of AMPA-EPSCs at climbing fiber–Purkinje cell synapses (Huang et al., 2004), and KA-EPSCs were reduced to a greater degree when the release probability was lowered, suggesting that kainate receptors had a lower receptor occupancy than AMPA receptors at these cerebellar synapses. In our study, stimulation of mossy fibers at 0.2 Hz facilitated both types of synaptic currents to approximately equivalent degrees (~190%), consistent with a previous report (Castillo et al., 1997); however, potentiation of AMPA-EPSCs was significantly larger than KA-EPSCs at higher frequencies. Thus, facilitation of KA-EPSCs increased in relatively small increments to a maximum of <300% at 2 Hz stimulation, whereas MF-EPSCs were potentiated by ~400% at the same frequency. The facilitation at 2 Hz was only slightly lower than the ~350% PTP of KA-EPSC after 100 Hz stimulation (Fig. 4), suggesting that the gain in frequency-dependent scaling of KA-EPSCs is markedly compressed relative to AMPA receptors, which facilitate to ~600% during PTP in our experiments.

Electrically and chemically induced LTP was attenuated for mossy fiber KA-EPSCs as well. Thirty minutes after a strong tetanic stimulation, the mean KA-EPSC amplitude was lower than the control level (83%), in contrast to MF-EPSCs, which exhibited potentiation to 218% of control amplitudes. At least a portion of the reduction in KA-EPSC amplitude is accounted for by a progressive rundown independent of tetanic stimuli (52% of control amplitude after 30 min), similar to a previous study that observed a somewhat more modest degree of rundown in the mossy fiber KA-EPSC (Hirbec et al., 2003). Adjustment of KA-EPSC relative to the mean rundown in amplitudes reveals a small degree of LTP, consistent with the observation that KA-EPSCs are capable of facilitating, to some degree, in response to increased release probability. Chemically induced potentiation

through the activation of FSK also produced significant differences between KA-EPSCs and MF-EPSCs (Fig. 5). Whereas adenylyl cyclase and PKA activity are thought to primarily increase release probability (Weisskopf et al., 1994), we observed that potentiation by FSK was significantly larger than that produced by tetanic stimulation, suggesting that FSK either increased the release probability to a greater degree than tetanic stimulation or that PKA activation had postsynaptic effects to reduce rundown of the KA-EPSC.

Most of our observations (the different PPRs, CV, frequency facilitation, and LTP) could be explained by heterogeneity in presynaptic mossy fiber function. Kainate receptors might be preferentially located in synapses that have a higher release probability, on average, than synapses predominantly expressing AMPA receptors. Alternatively, postsynaptic kainate receptors might be predominantly localized to synapses that lack facilitatory autoreceptors, such as presynaptic kainate receptors, which have been shown to contribute to frequency facilitation and LTP (Contractor et al., 2001, 2003; Lauri et al., 2001b; Schmitz et al., 2001). Although the average facilitation of KA-EPSCs at each stimulation frequency was lower than MF-EPSCs, in several individual recordings, the KA-EPSCs exhibited robust frequency facilitation. For example, facilitation at 1 Hz ranged from 101% (i.e., no facilitation) to 486% (greater than the mean MF-EPSC facilitation). Evidence for unequal distribution of receptors and presynaptic function has not been reported for mossy fiber synapses, and indeed a previous report that described quantal KA-EPSCs concluded that most mossy fiber synapses contained both AMPA and kainate receptors (Cossart et al., 2002). However, this analysis did not exclude the possibility that unequal distributions of AMPA and kainate receptors occur in synapses with different release probabilities. Interestingly, in a recent quantal analysis of mossy fiber transmission, Lawrence et al. (2004) found that although most mossy fiber–CA3 synaptic connections had a linear variance–mean current relationship, consistent with a low release probability, one EPSC recording had a relationship described by a parabola, from which a significantly higher release probability of 0.6 was derived. These data suggest that a subset of mossy fiber terminals might have relatively high release probabilities, and it is possible that in finding synaptic connections with detectable KA-EPSCs evoked by single stimulations, which constitute <50% of whole-cell recordings, we bias our recordings toward these putative high-probability synapses. This possibility is also consistent with the lower CV values for KA-EPSCs compared with MF-EPSCs. Interestingly, both PPRs and CV during 1 Hz stimulation were depressed to the same level for KA-EPSCs and MF-EPSCs, despite the higher initial PPR and CV of MF-EPSCs. This suggests that the maximal release probability is equivalent for synapses with AMPA and kainate receptors.

Presynaptic heterogeneity is not the sole hypothesis that could account for the difference in plasticity between MF- and KA-EPSCs. Perhaps the most obvious possibility, that synaptic kainate receptors are located outside the postsynaptic density and therefore are exposed to a lower concentration of glutamate, is considered least likely because synaptic kainate receptors respond to quantal release of glutamate (Cossart et al., 2002), KA-EPSC kinetics are not altered by increasing extrasynaptic glutamate spillover (Castillo et al., 1997; Vignes and Collingridge, 1997), and immunoreactivity for kainate receptor subunits is located within postsynaptic densities (Petralia et al., 1994; Darstein et al., 2003). The slow kinetics are at least partially an intrinsic property of the receptor, because gene-targeted mice lacking the KA2 subunit have more rapid KA-EPSC decays (Contractor et al.,

2003). Interaction with cytoplasmic proteins has been shown to alter GluR6-containing kainate receptor kinetics (Garcia et al., 1998; Bowie et al., 2003), although not to a degree matching that of KA-EPSC decay kinetics. Alternatively, it is possible that kainate receptors underlying KA-EPSCs might be activated by much lower concentrations of glutamate than recombinant kainate receptors expressed in heterologous systems, most of which are approximately equivalent to AMPA receptors in their sensitivity to glutamate (Schiffer et al., 1997; Dingledine et al., 1999). Indeed, NMDA-EPSCs at mossy fiber synapses have a lower PPR than AMPA-EPSCs (Mori-Kawakami et al., 2003), which could arise from receptor saturation because of the relatively high sensitivity of NMDA receptors for glutamate. Higher occupancy of kainate receptors than AMPA receptors would be predicted to produce less facilitation, as we observed in these experiments, and could account for the weaker inhibition of KA-EPSCs by  $\gamma$ -DGG in a subset of recordings. There is, however, no experimental evidence for a mechanism that could shift the glutamate concentration–response relationship for synaptic kainate receptors so profoundly from that observed with recombinant receptors or kainate receptor whole-cell currents in cultured neurons (Wilding and Huettner, 1997; Paternain et al., 1998). Furthermore, a previous examination of the role of glutamate diffusion in shaping mossy fiber EPSCs found that tetanus-induced KA-EPSCs increased in amplitude in the presence of dextran, which increased viscosity in the extracellular space, suggesting that synaptic KARs were not saturated (Min et al., 1998). Finally, the slow kinetics and reduced potentiation might, in part, be caused by a slower rate of recovery from desensitization of kainate receptors compared with AMPA receptors. Recombinant kainate receptors have a significantly slower reactivation time course than AMPA receptors (Lomeli et al., 1994; Heckmann et al., 1996; Swanson and Heinemann, 1998; Bowie and Lange, 2002), and this may play a role in shaping mossy fiber KA-EPSCs because kainate receptors with intermediate desensitized states have slowed kinetics (Bowie and Lange, 2002). Although we did not observe the >80% reduction in KA-EPSC amplitudes during 1 Hz stimulation predicted by this model, a clear attenuation in amplitudes was apparent during the higher-frequency trains, such that the potentiation at the end of the train stimuli was 141% (compared with 294% at the peak of facilitation) (Fig. 2). This modest reduction might arise if the synaptic receptors recover at a faster rate than homomeric GluR6 receptors, which were used for the previous analysis of glutamate receptor desensitization (Bowie and Lange, 2002). We attempted to test this hypothesis directly by altering the rate of desensitization (or decay) of synaptic kainate receptors with concanavalin A (con A), but application of 0.3 mg/ml of the plant lectin for up to 30 min did not alter KA-EPSC amplitudes or kinetics (data not shown). These data, while suggestive, do not exclude the possibility of a role for desensitization, because we could not be certain that the con A had penetrated into synaptic sites in the slice preparations. Thus, we cannot yet say definitively which mechanisms, presynaptic or postsynaptic, contribute to the distinct behavior of kainate receptor at mossy fiber synapses.

The variable inhibition of KA-EPSCs by  $\gamma$ -DGG was intriguing because it supports the idea of synaptic heterogeneity, but as with other observations, the  $\gamma$ -DGG data do not exclude postsynaptic differences in receptor composition.  $\gamma$ -DGG bound to non-NMDA receptors is rapidly replaced by glutamate on vesicular release, and the relative inhibition was used previously to estimate synaptic glutamate concentrations (Wadiche and Jahr, 2001). The degree of inhibition by the fast-dissociating inhibitor

depends on kainate receptor sensitivities to glutamate and  $\gamma$ -DGG as well as the synaptic glutamate concentration. It is possible that the composition of individual kainate receptors vary at mossy fiber synapses and that these receptors have different sensitivities to  $\gamma$ -DGG (or glutamate), resulting in different levels of inhibition by the antagonist. It is clear that all postsynaptic kainate receptors contain GluR6 subunits (Mulle et al., 1998) and the KA2 subunit is present in some (perhaps all) of the synaptic receptors (Contractor et al., 2003; Darstein et al., 2003). The KA1 subunit also is expressed in CA3 pyramidal neurons (Bahn et al., 1994) and is located at a subset of mossy fiber postsynaptic densities (Darstein et al., 2003). The cellular machinery that targets kainate receptors selectively to mossy fiber synapses has not been identified, and there is no evidence for (or against) targeting of receptors with different subunit composition to distinct postsynaptic sites.

In summary, these results are consistent with the hypothesis that AMPA-EPSCs have a greater dynamic range than KA-EPSCs in response to short- and long-term increases in release probability. Although the slow time course of the KA-EPSC supports a role in frequency-dependent synaptic integration (Frerking and Ohliger-Frerking, 2002), our data suggest that mechanisms exist to limit the activation of synaptic kainate receptors in the presence of profound activity-dependent increases in presynaptic strength at mossy fiber terminals. This limitation could act as a critical brake that prevents pathological activation of mossy fiber kainate receptors, which potentially induce synchronized firing of the recurrent CA3 network.

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