compact disk with Adaptec Toast 3.5.4. Copies of the updated DTT on a compact disk are available from the corresponding author. The updated DTT contained a preponderance of tunes played incorrectly (17/26), but our data suggest that whether a tune was played correctly or incorrectly did not significantly bias subjects' answers. Four tunes were played twice in the updated DTT; each was played correctly once and incorrectly once. Wrong answers on these eight questions were evenly distributed; 51% were correct melodies identified as incorrect, and 49% were incorrect melodies identified as correct. Two methods were used to determine the role of long-term memory and cultural experience in the performance on the updated DTT. First, subjects were asked whether they were familiar with each tune presented, and familiarity was positively correlated with correct answers. We also developed a second test, termed the International Tunes Test, that consisted of 18 short melodies chosen to be unfamiliar to all subjects and that used both Western and non-Western tonal systems. Subjects were presented with each melody played correctly twice in succession and were then asked whether a third rendition played immediately thereafter was the same as or different than the first two playings. Six of the International Tunes were played correctly the third time, whereas 12 were played incorrectly the third time. The distribution of scores on the International Test were indistinguishable from those on the updated DTT, with no significant differences between males and females. Individuals' scores on the DTT and the International Test were highly correlated (r = 0.71). These results suggest that performance on the updated DTT is not highly dependent on long-term musical memory.

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Presynaptic Kainate Receptor Mediation of Frequency Facilitation at Hippocampal Mossy Fiber Synapses

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Inhibition of transmitter release by presynaptic receptors is widespread in the central nervous system and is typically mediated via metabotropic receptors. In contrast, very little is known about facilitatory receptors, and synaptic activation of a facilitatory autoreceptor has not been established. Here we show that activation of presynaptic kainate receptors can facilitate transmitter release from hippocampal mossy fiber synapses. Synaptic activation of these presumed ionotropic kainate receptors is very fast (<10 ms) and lasts for seconds. Thus, these presynaptic kainate receptors contribute to the short-term plasticity characteristics of mossy fiber synapses, which were previously thought to be an intrinsic property of the synapse.

Neurotransmitter receptors are located on the presynaptic, as well as the postsynaptic, side of the synapse. In vertebrates these presynaptic receptors are typically metabotropic receptors and inhibit transmitter release (*1*, *2*), although in invertebrates facilitatory metabotropic actions have also been described (*3*, *4*). Ionotropic receptors are also present on presynaptic terminals (*5*), and their activation generally inhibits synaptic transmission (*6*–*11*). Although facilitation has been observed, there is no evidence that synaptically released transmitter could have access to these receptors (*5*). Kainate receptors (KARs) have recently been shown to exert presynaptic effects on glutamatergic (*10*, *12*) as well as GABAergic terminals (*9*, *13*). However, at both types of terminals activation of kainate receptors causes an inhibition of release. Here we report that activation of presynaptic kainate receptors by low levels of synaptically released glutamate enhances transmitter release at hippocampal mossy fiber synapses.

Previous studies found that application of kainate inhibits synaptic transmission at hippocampal mossy fiber synapses (*14*–*16*). However, kainate applied at concentrations considerably below those used in these previous studies strongly facilitates synaptic transmission. Kainate (50 nM) produced a robust and reversible enhancement of α-amino-3-hydroxy-5-methyl-4-isoxazolepropri-onic acid receptor (AMPAR) mediated excitatory postsynaptic currents (EPSCs) (Fig. 1A) (*17*), and this enhancement was associated with a decrease in paired pulse facilitation (PPF) (P2/P1: control, 2.95 ± 0.15; kainate, 1.9 ± 0.16) (Fig. 1A), suggesting that the enhancement is mediated by an increase in the probability of transmitter release (*18*). Kainate (50 nM) also produced a robust and reversible enhancement in the synaptic field potential responses (170 ± 9%), and this enhancement was also associated with a decrease in PPF (control, 2.7 ± 0.16; kainate, 1.9 ± 0.17) (*19*). To study the mechanism involved in this facilitation, AMPA and $GABA_A$ receptors were selectively blocked by GYKI 53655 and picrotoxin, respectively, and N-methyl-D-aspartate (NMDA) receptor (NMDAR)–mediated EPSCs were recorded at positive holding potentials. We used the non-NMDAR antagonists CNQX or NBQX, which block KARs, to identify effects of bath-applied kainate and synaptically released glutamate that are due to KAR activation. Low concentrations of kainate (50 nM) also reversibly enhanced NMDAR EPSCs, and this effect was completely blocked by CNQX (10 μM), indicating that the enhancement was mediated by high-affinity KARs (Fig. 1B). The enhancement of AMPAR- and NMDAR-mediated EPSCs occurred in the absence of any change in the rise time, indicating that no polysynaptic inputs were recruited (Fig. 1, A and B, insets). This same concentration of kainate also enhanced the size of the presynaptic fiber volley, and this effect was also blocked by CNQX (see Fig. 1E).

The enhancing action of kainate, both on the NMDAR EPSC (Fig. 1, A and B) and on the fiber volley, was mimicked by the addition of 4 mM K^+ to the superfusing medium (Fig. 1C), suggesting that the effect of kainate was mediated by a depolarizing action on the terminal. Neither kainate nor K^+ had any effect on the holding current, making a postsynaptic site of action most unlikely. In addition, kainate (50 nM) had no effect on

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the responses to iontophoretically applied NMDA in *S. lucidum* (96 ± 6%, $n = 4$) (*19*). These findings, along with the finding that the AMPAR EPSP was enhanced to the same degree as the NMDAR EPSC and was associated with a reduction in PPF, imply that the enhancement is due to an increase in transmitter release.

Higher concentrations of kainate depress mossy fiber synaptic transmission (*14–16*), and Fig. 1D shows this biphasic effect in field potential recordings. As the kainate (500 nM) washes into the slice, the EPSP and fiber volley initially increase in size. As the concentration rises, the enhancement gives way to a depression of the EPSP, although the size of the fiber volley continues to increase. The dose-response characteristics of kainate were analyzed on the NMDAR EPSC (Fig. 1E) and confirmed the dose dependent bidirectional modification of synaptic transmission by exogenous kainate. These effects were accompanied by an increase in the size of the fiber volley, although at high concentrations of kainate the fiber volley was substantially depressed. The actions of kainate on the EPSC and the fiber volley were entirely mimicked by K^+ (Fig. 1E). A rather trivial explanation for the enhancement of synaptic responses is that kainate acts at the site of stimulation to recruit more fibers. Three observations rule out this possibility. First, it is possible to dissociate the enhancement of the NMDAR EPSC from the enhancement of the fiber volley by low concentrations of kainate (20 nM) and K^+ (2 mM) (Fig. 1E). Second, local application of kainate (50 to 200 nM) or K^+ (5 mM) to the recording site still enhanced synaptic transmission ($n = 4$ each) (*19*). Third, we previously reported that local application of CNQX to the site of stimulation has no effect on the kainate-induced enhancement of the fiber volley recorded in the CA3 region (*15*).

One might expect that if synaptically released glutamate activated these receptors, transmission would be facilitated. Repetitive stimulation at 25 Hz resulted in an envelope of outward current, which was largely blocked by D, L-2-amino-5-phosphonovaleric acid (APV), indicating that it was mediated by NMDAR activation (Fig. 2, A and B). Application of CNQX reversibly decreased the size of this envelope but, unlike APV, had no effect on the size of the first NMDAR EPSC of the train (Fig. 2, A and B). This action of CNQX cannot be accounted for by blockade of an underlying KAR-mediated EPSC (*20–22*), because compared with the NMDARs the contribution of KARs to the overall synaptic response in these experiments was less than 10% (Fig. 2A). Application of the group II mGluR agonist DCG-IV (2 μM) essentially abolished the response, indicating that it was generated by mossy fibers. Close examination indicates that CNQX reduced the second response during the train (Fig. 2A). NBQX (50 μM) had similar effects on the peak and second response ($n = 3$). Qualitatively similar results were observed when a brief 100-Hz tetanus was used (Fig. 2, C and D), indicating that the onset of the facilitation occurred within 10 ms ($n = 5$ for CNQX; $n = 3$ for NBQX). The results from five experiments with 25 Hz and five experiments with 100 Hz are summarized in Fig. 2E. It should be noted that the magnitude of the KAR induced facilitation of the NMDAR EPSC will be underestimated because of the

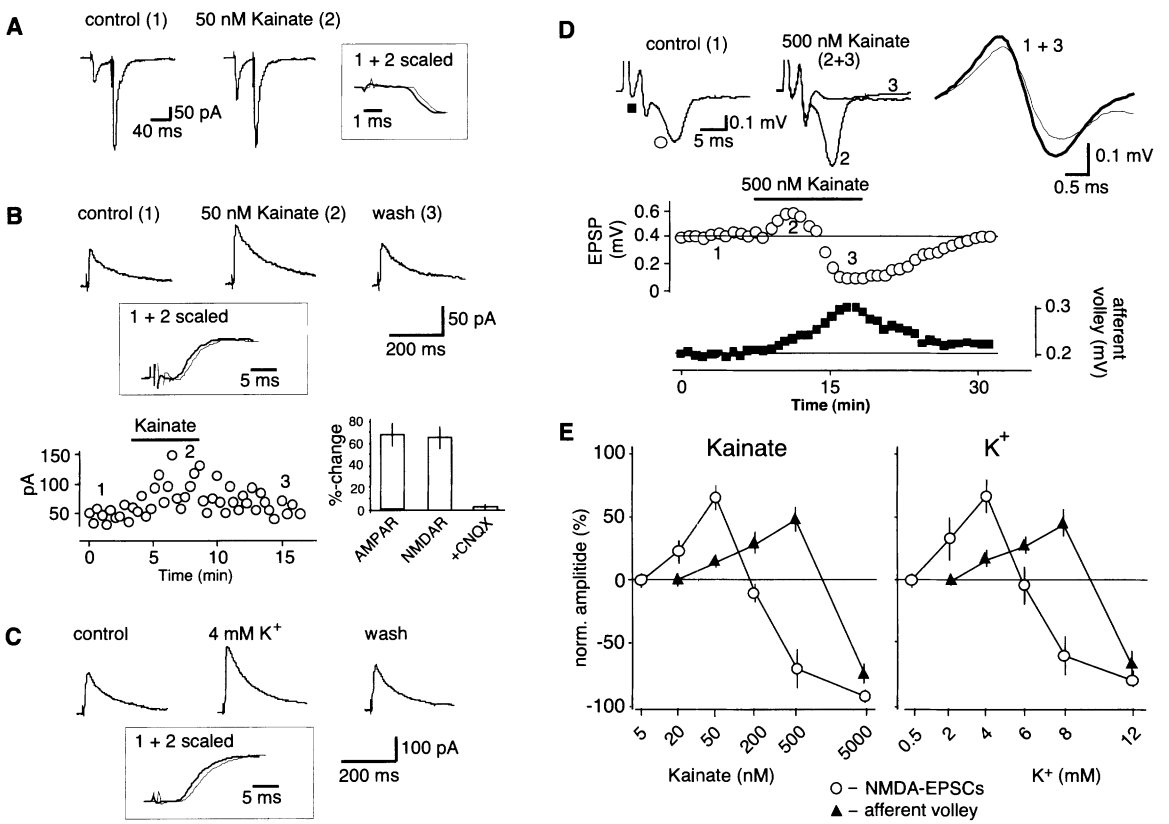

Fig. 1. Bidirectional control of synaptic transmission by kainate and presynaptic membrane potential. (**A**) Averaged traces of AMPAR-EPSCs recorded at −70 mV holding potential in the presence of picrotoxin (100 μM). 50 nM kainate increases the amplitude of the first synaptic current, whereas the second is unchanged, thereby changing PPF. Note that the increase is not associated with a change in the rising phase of the EPSC. (**B**) Averaged traces of NMDAR-EPSCs recorded at +30 mV holding potential in the presence of GYKI 53655 (20 μM) and picrotoxin (100 μM). 50 nM kainate reversibly increases the amplitude of the synaptic current. The increase is not associated with a change in kinetics of the EPSC. (Bottom) Time course of this experiment and summarizing bar graphs for six experiments with 50 nM kainate on AMPAR-mediated EPSCs, seven experiments with 50 nM kainate on NMDAR-mediated EPSCs, and five experiments with 50 nM kainate and 10 μM CNQX on NMDAR-mediated EPSCs. (**C**) Extracellular potassium mimics the kainate effects. Addition of 4 mM K^+ facilitates NMDAR EPSCs to a similar extent as 50 nM kainate. Note again that the time course of the EPSCs is not changed. (**D**) (Top) 500 nM kainate causes an initial increase in field EPSP (fEPSP) (open circles) amplitude (trace 2) followed by a decrease (trace 3). (Bottom) During the same experiment, the fiber volley (filled squares) only increases. (**E**) Concentration dependency of the effects of kainate and K^+ additions on NMDAR EPSCs and afferent volley size. Note that 20 nM kainate and 2 mM K^+ significantly increase the amplitude of the NMDAR EPSC, whereas the fiber volley is not affected. Note also that 500 nM kainate and 8 mM K^+ cause an enhancement of the afferent volley, whereas synaptic transmission is strongly suppressed. ($n \geq 5$ for each experiment.)

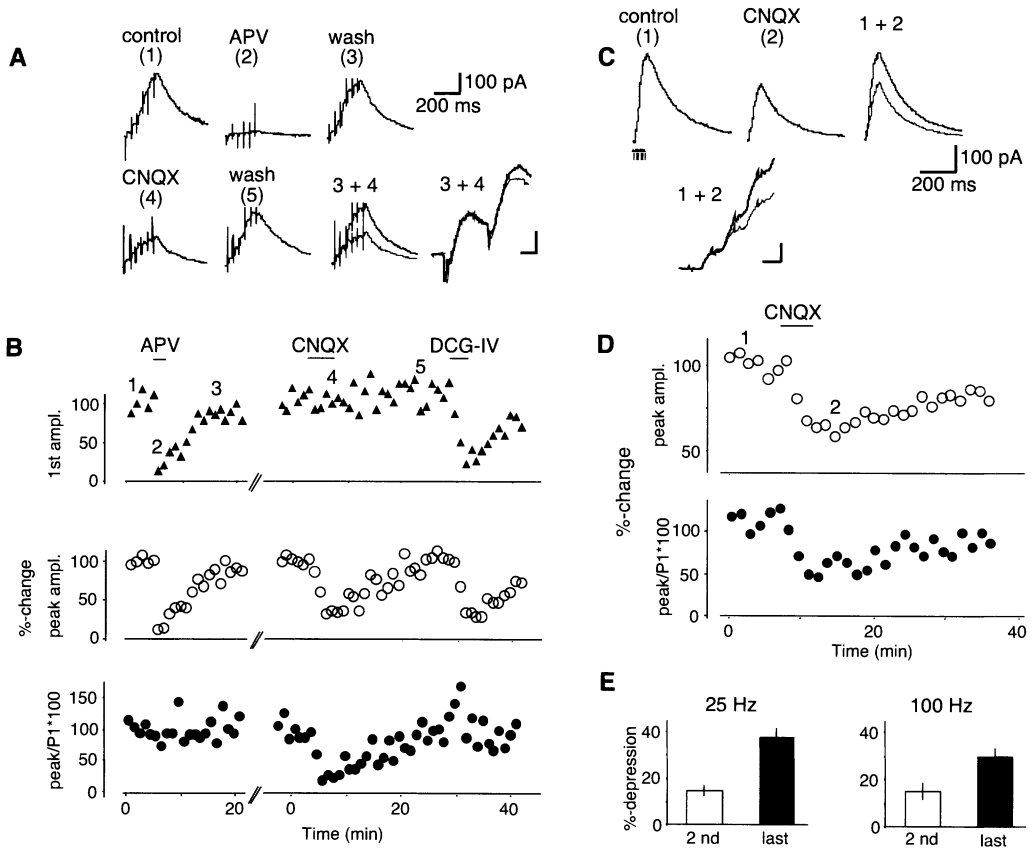

Fig. 2. Presynaptic kainate receptors are involved in homosynaptic facilitation. (**A** and **B**) A short train of six pulses at 25 Hz to mossy fibers elicited a long-lasting, NMDAR-mediated synaptic current in a CA3 pyramidal neuron held at +40 mV. CNQX (10 μM) reduces the peak response of the synaptic current (A). Note on the expanded time and amplitude scale that the second pulse is reduced by CNQX, whereas the first one is not. Bar, 10 ms and 10 pA. (B) Time course for the same experiment. APV (20 μM) blocks the first response (filled triangles) as well as the peak response (open circles) to the same extent, thereby leaving the ratio (filled circle) of both unaffected. CNQX leaves the first response unaffected, whereas the peak response of the current is strongly reduced, thereby depressing the ratio of both. (**C** and **D**) Higher stimulation-frequencies (five pulses at 100 Hz) revealed that kainate receptors are activated within 10 ms [see the first three responses during the train on the expanded time scale in (C)]. Bar, 10 ms and 20 pA. (D) Time course of the same experiment, where the open circles show the peak response and the closed circles the ratio of the peak response and the first response. (**E**) Summary bar graphs of the effects of CNQX shown in (A) through (D), showing the ratios of the second and the peak response versus the first response for 25 Hz and 100 Hz stimulation (n = 5 for each).

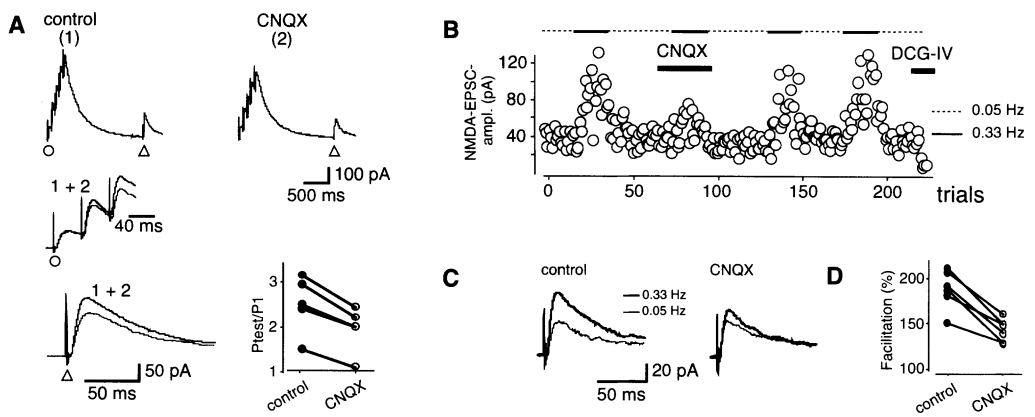

Fig. 3. Kainate receptors contribute to low-frequency facilitation. (**A**) After a conditioning train of five stimuli at 25 Hz, a test pulse to the same fibers was given 1.5 s after the last stimulus within the train. The test response is enhanced in comparison to the first response within the train, but more importantly this enhancement is reduced by CNQX (10 μM). The first three responses from the conditioning train are shown enlarged below. The first response is unaffected by CNQX. (Bottom) Test responses on an expanded time and amplitude scale. Five experiments are summarized in the scatter plot (ratio of the test response to the first train response) on the right. (**B** and **C**) Changing the frequency of stimulation from 0.05 to 0.33 Hz results in a facilitation of the NMDAR EPSC, which is depressed by CNQX (10 μM). This is demonstrated in both the trial-by-trial plot (B) and the example traces below (C). (**D**) Graph showing the results from six such experiments.

slow unbinding of glutamate from the NMDARs and NMDAR desensitization.

Given the very high affinity for the presynaptic KAR, one might expect the facilitation to persist long after the tetanus. We examined this possibility by applying a test stimulus after the initial conditioning train (Fig. 3A). Application of CNQX reduced the size of the test EPSC, but had no effect on the size of the first EPSC in the tetanus. The long duration of the KAR-mediated enhancement suggests that these receptors may contribute to the unusually strong frequency facilitation observed at mossy fiber synapses (*23*, *24*). When the frequency of stimulation was increased from 0.05 to 0.33 Hz, the size of the NMDAR EPSCs increased (Fig. 3, B through D). Application of CNQX had no effect on the baseline responses but reversibly reduced the frequency facilitation. Thus, it appears that presynaptic KARs are primarily responsible for frequency facilitation at mossy fiber synapses.

The results thus far show that synaptically released glutamate can activate a presynaptic facilitatory autoreceptor. However, it is not clear whether the release of glutamate from one mossy fiber synapse can also gain access to neighboring presynaptic kainate receptors. To test this, the stimulus intensity of the conditioning train was initially the same as for the test

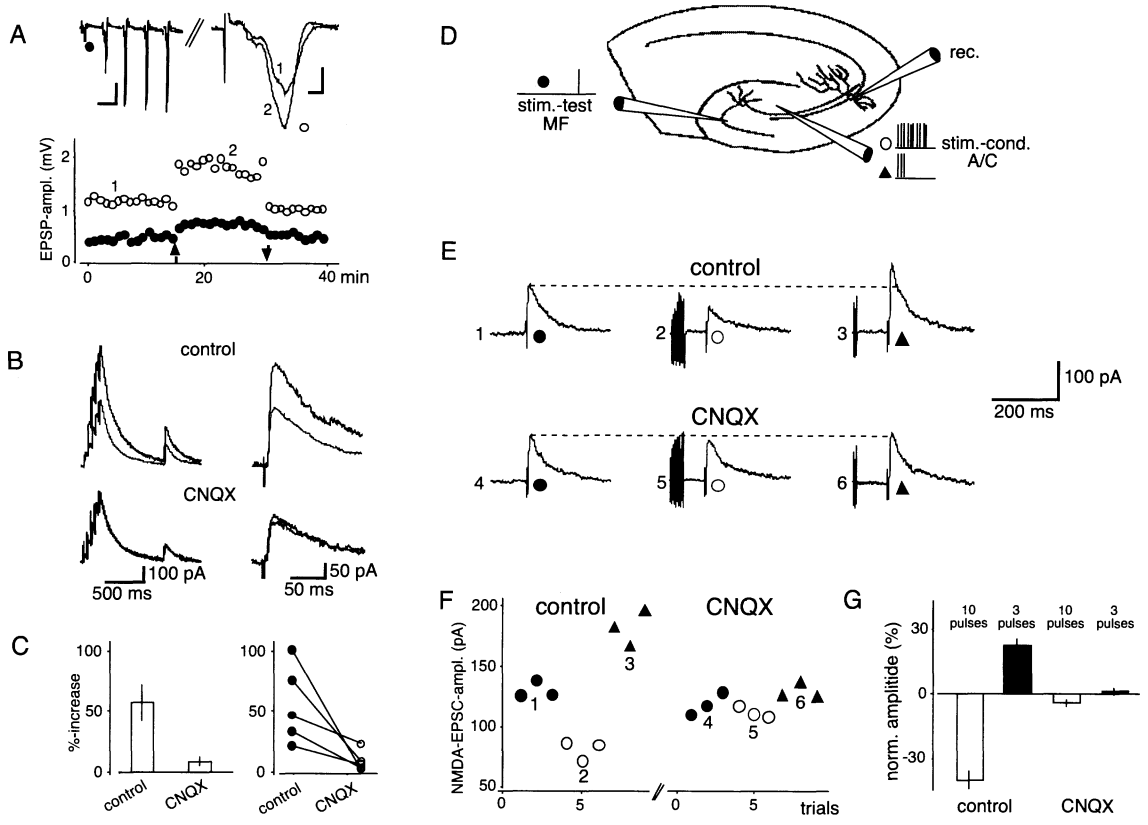

Fig. 4. Kainate receptor activation causes heterosynaptic facilitation. (**A** through **C**) Kainate receptors are responsible for mossy fiber heterosynaptic facilitation. (A) Increasing the stimulation intensity (arrows) of a conditioning train (five pulses, 25 Hz) given to the granule cell layer causes an increase in the test fEPSP response given 1 s after the conditioning train. The test stimulus intensity is kept constant. The amplitudes of the first fEPSP in the conditioning train (filled circles) and test fEPSP (open circles) are shown in the graph. Bar, left: 1 mV and 50 ms. Bar, right: 0.5 mV and 2 ms. (B) The same protocol causes an increase in facilitation of NMDA responses during the train (top), which is suppressed by CNQX (10 μM) (bottom). Enlarged traces shown on the right. Bar graph and scatter plot show the same test pulse data from five experiments (C). (**D** through **F**) Synaptic activation of kainate receptors bidirectionally modifies mossy fiber synaptic strength. (D) Diagram of the stimulation protocol used. (E) Test responses of mossy fiber NMDAR EPSCs are depressed by a long train (10 pulses, 200 Hz) and facilitated by a short train (three pulses, 200 Hz) of stimuli to A/C fibers. The A/C NMDAR response was previously selectively blocked by MK-801 (50 μM) application (*15*, *30*). The bidirectional modification is blocked by application of CNQX (10 μM). A trial-by-trial plot of the experiment is shown below (F). (**G**) Bar graph (n = 3). SCH50911 (20 μM) and MCPG (500 μM) were present in all whole-cell experiments.

stimulus, but was then increased approximately twofold to recruit more mossy fibers, while the test stimulus intensity was kept constant. Field potential recordings (Fig. 4A) clearly showed that when more fibers were recruited with the conditioning tetanus, the test response was enhanced. To characterize the receptor mechanisms involved in this heterosynaptic facilitation, we repeated the experiments using the NMDAR EPSC (Fig. 4, B and C). As expected for the involvement of kainate receptors, the facilitation was largely prevented by CNQX. Results from five different cells are summarized in Fig. 4C. In the presence of CNQX, the size of the response to the conditioning train at high stimulus strength was 26 ± 10% (n = 5) larger than the size for the lower stimulus strength. This experiment indicates that as more mossy fiber synapses are recruited glutamate can act heterosynaptically on presynaptic KARs.

We have previously shown that activation of presynaptic KARs on mossy fibers by synaptically released glutamate can inhibit transmitter release from mossy fibers (*15*). On the basis of the dose-dependent bidirectional action of kainate (Fig. 1E), one might expect that the amount of synaptically released glutamate would determine whether a facilitation or inhibition is generated. We tested this hypothesis by comparing the effect of a weak and strong tetanus to the neighboring associational/commissural synapses (A/C). When a brief conditioning tetanus was applied to the A/C fibers, the mossy fiber test response was enhanced (filled triangles in Fig. 4E). However, when the conditioning tetanus was prolonged, the test response was depressed (open circles in Fig. 4E). This bidirectional control of synaptic transmission was observed in five experiments, and, importantly, both the enhancement and depression were almost completely blocked by CNQX in the three cells tested (Fig. 4G), indicating that the two actions were mediated by KAR activation.

The exact mechanism involved in the presynaptic facilitation described here is unclear. The finding that the facilitation begins and is maximal within 10 ms makes a metabotropic action unlikely. The fact that low concentrations of K^+ mimic this facilitation suggests that KAR activation may exert its effects by depolarizing the terminal via its ionotropic action. For instance, if depolarization were to inactivate some of the K^+ channels involved in action potential repolarization, the action potential would be prolonged (*25*). This would enhance Ca^{2+} entry and transmitter release (*25*). The facilitation could also be explained if the presynaptic KARs were permeable to Ca^{2+} (*26*–*28*). The above scenarios imply that action potential–dependent release of transmitter from mossy fiber synapses is remarkably sensitive to changes in the membrane potential of the terminal. An alternative explanation is that the mimicry with K^+ is coincidental and that the action of KARs on release is independent of presynaptic membrane potential.

In summary, contrary to the long-held assumption that frequency facilitation is an intrinsic property of synapses due to Ca^{2+} channel activation and the cumulative effects of intracellular Ca^{2+} on release (*29*), the present results indicate that at mossy fiber synapses this short-term plasticity is mediated, at least in part, by the long-lasting activation of a kainate autoreceptor.

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17. Hippocampal slices were prepared from young adult [postnatal day 20 to 29 (p20-29)] Sprague-Dawley rats. In brief, animals were deeply anesthetized with halothane, decapitated, and the brains removed. Tissue blocks containing the subicular area and hippocampus were mounted on a Vibratome in a chamber filled with cold (~4°C) artificial cerebrospinal fluid (ACSF) containing the following : 119 mM NaCl; 26 mM $NaHCO_3$; 2.5 mM KCl; 1 mM NaH_2PO_4; 2.5 mM $CaCl_2$; 1.3 mM $MgSO_4$; 10 mM glucose, saturated with 95% O_2–5 % CO_2, pH 7.4. Transverse slices were cut at 300-μm thickness and were stored in a submerged chamber for 1 to 7 hours until transferred to the recording chamber, where they were perfused at a high rate of 3 to 4 ml/min. The recording chamber was mounted on an Olympus microscope equipped for infra red–differential interference contrast (IR-DIC) microscopy. At least 1 hour elapsed between slice preparation and recording. For all recordings, $CaCl_2$ and $MgSO_4$ were increased to 4 mM to minimize polysynaptic activation. For whole-cell voltage clamp recordings, the internal solution contained the following: 117 mM Cs-gluconate, 5 mM CsCl, 10 mM TEA-Cl, 8 mM NaCl, 10 mM Hepes, 5 mM EGTA, 4 mM MgATP, 0.3 mM Na_3GTP, 5 mM Hepes, and 5 mM QX-314, pH adjusted to 7.3 with CsOH. Electrode resistances ranged from 1.5 to 5 megohms. Access resistances ranged between 5 and 20 megohms and were continuously checked during the recording. No series resistance compensation was used. Field potential recordings were performed with low-resistance patch pipettes filled with Hepes-buffered external solution placed in stratum lucidum in CA3. Iontophoresis of NMDA (1 M, adjusted to a pH of 9) was accomplished with electrodes pulled from borosilicate glass and having resistances of 70 to 100 megohms. NMDA was ejected using a constant current iontophoresis instrument (WPI, Sarasota, FL) with pulses ranging from 5 to 20 ms. Bipolar tungsten electrodes were placed in the granule cell layer or in the hilus region to stimulate mossy fibers. The NMDARs on the A/C pathway (Fig. 4, D through G) were selectively blocked by using the irreversible, use-dependent antagonist MK-801. Average values are expressed as mean ± SEM. Drugs used were as follows: Kainate (0.002 to 10 μM), GYKI 53655 (20 μM), D-AP5 (20 μM), MCPG (0.5 mM), SCH50911 (20 μM), MK-801 (50 μM), picrotoxin (100 μM), DCG-IV (2 μM), CNQX (10 μM), and NBQX (50 μM).
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22. CNQX (10 μM) and NBQX (50 μM) caused a depression of the NMDA EPSC in approximately 35% of the cells, which were not included in the analysis. There are a number of possible reasons for this effect of CNQX. (i) There is a large underlying KAR-mediated EPSC that is removed. This is unlikely because APV inhibited the EPSC in all cells tested by at least 90%. Furthermore, the EPSC kinetics did not change on addition of CNQX. (ii) CNQX has a direct inhibitory effect on NMDA receptors. This is also unlikely because we see the same depression using NBQX (50 μM), which does not block NMDAR. Moreover, CNQX at 10 μM and NBQX at 50 μM had no effect on NMDAR responses evoked by iontophoresis (n = 4 each). (iii) There is an underlying tonic facilitation of presynaptic release mediated by KAR. Given the high affinity of these KARs (see also Fig. 1D), it is possible that the basal glutamate levels in the slice are sufficient to generate tonic activity in some cases. At present, this is the most likely possibility because we also observe an inhibition of the fiber volley amplitude with addition of CNQX in about 30% of recordings.
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Binding of DCC by Netrin-1 to Mediate Axon Guidance Independent of Adenosine A2B Receptor Activation

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Netrins stimulate and orient axon growth through a mechanism requiring receptors of the DCC family. It has been unclear, however, whether DCC proteins are involved directly in signaling or are mere accessory proteins in a receptor complex. Further, although netrins bind cells expressing DCC, direct binding to DCC has not been demonstrated. Here we show that netrin-1 binds DCC and that the DCC cytoplasmic domain fused to a heterologous receptor ectodomain can mediate guidance through a mechanism involving derepression of cytoplasmic domain multimerization. Activation of the adenosine A2B receptor, proposed to contribute to netrin effects on axons, is not required for rat commissural axon outgrowth or *Xenopus* spinal axon attraction to netrin-1. Thus, DCC plays a central role in netrin signaling of axon growth and guidance independent of A2B receptor activation.

Netrin-1 binds transfected cells expressing DCC, and antibodies to DCC block netrin-1–stimulated axon outgrowth and orientation in vitro (*1*–*5*), suggesting that DCC is a receptor or a necessary component of a receptor complex required for the actions of netrin-1 on axons. This possibility was supported by the finding that DCC and netrin-1 knockout mice have similar defects in axon guidance and by a comparable coincidence of phenotypes of mutants in DCC family receptors and their respective netrin ligands in invertebrates (*5*–*9*). Because the results in all species were based on loss of function of DCC family proteins, it was still possible that a coreceptor(s) was required for netrin function. These studies also did not establish whether DCC proteins function in signaling or are merely ligand-binding partners in a receptor complex. Recently, the importance of DCC for netrin signaling has been challenged (*10*, *11*). First, no binding of a soluble DCC ectodomain to netrin-1 was observed in vitro, raising the possibility that DCC does not even bind netrin directly but rather confers binding to transfected cells by complexing with some cellular cofactor (*10*). Second, the report that netrin-1 can activate the adenosine A2B receptor and stimulate cyclic adenosine 3′,5′-monophosphate production by binding its extracellular portion, while a cytoplasmic portion of A2B simultaneously binds the DCC cytoplasmic domain, led to the proposal that A2B is the central mediator of netrin signaling. In support, A2B protein was detected immunohistochemically on netrin-responsive commissural axons in collagen gels, and inhibitors of A2B function blocked commissural axon outgrowth in response to netrin-1 (*11*).

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