Neuron, Vol. 45, 113–123, May 4, 2005 ©2005 Elsevier Inc. DOI 10.1016/j.neuron.2005.02.029

Functional Maturation of CA1 Synapses Involves Activity-Dependent Loss of Tonic Kainate Receptor-Mediated Inhibition of Glutamate Release

Sari E. Lauri,[1] Aino Vesikansa,[1] Mikael Segerstråle,[1] Graham L. Collingridge,[2] John T.R. Isaac,[2,3] and Tomi Taira[1,*]

[1] Neuroscience Center and Department of Bio- and Environmental Sciences
P.O. Box 65 (Viikinkaari 1)
00014 University of Helsinki
Finland

[2] Medical Research Council Centre for Synaptic Plasticity
Department of Anatomy
Medical School
University of Bristol
BS8 1TD Bristol
United Kingdom

[3] National Institute of Neurological Disorders and Stroke
National Institutes of Health
35 Convent Drive
Bethesda, Maryland 20892

Summary

Early in development, excitatory synapses transmit with low efficacy, one mechanism for which is a low probability of transmitter release (Pr). However, little is known about the developmental mechanisms that control activity-dependent maturation of the presynaptic release. Here, we show that during early development, transmission at CA3-CA1 synapses is regulated by a high-affinity, G protein-dependent kainate receptor (KAR), which is endogenously activated by ambient glutamate. By tonically depressing glutamate release, this mechanism sets the dynamic properties of neonatal inputs to favor transmission during high frequency bursts of activity, typical for developing neuronal networks. In response to induction of LTP, the tonic activation of KAR is rapidly down regulated, causing an increase in Pr and profoundly changing the dynamic properties of transmission. Early development of the glutamatergic connectivity thus involves an activity-dependent loss of presynaptic KAR function producing maturation in the mode of excitatory transmission from CA3 to CA1.

Introduction

Glutamatergic synaptic connectivity in the hippocampus develops during the first 2 weeks of life (Fiala et al., 1998; Hsia et al., 1998; Tyzio et al.,1999). In neonatal (e.g., P3–P6) area CA1, a large proportion of glutamatergic synapses are believed to be functionally silent because they do not respond to low-frequency activity at resting membrane potentials (e.g., Isaac et al. [1995] and Durand et al. [1996]). In addition, it has been shown that the AMPA receptor-mediated signaling at immature synapses is extremely labile and sensitive to presynaptic stimulation, leading to silencing of AMPA-R-mediated responses (Xiao et al., 2004). Concomitant presynaptic mechanisms exist that can maintain a low probability (Pr) of release (Gasparini et al., 2000) and that may result from a developmentally small size of the readily releasable pool of vesicles (Renger et al., 2001; Mozhayeva et al., 2002) or from a nonexpanding fusion pore mode of exocytosis (Choi et al., 2000). Low Pr synapses exhibit pronounced facilitation in response to high frequency afferent activation (K. Lamsa et al., 1999, Soc. Neurosci., abstract; Hanse and Gustafsson, 2001). However, this type of synaptic operation is lost within the first 2 weeks of life because it is not exhibited in juvenile (e.g., P14–P16) CA1. The mechanism by which this transformation occurs is not known.

Presynaptic kainate-type glutamate receptors (KARs) can regulate transmitter release at both glutamatergic and GABAergic synapses in many areas of the nervous system (Kullmann, 2001; Lerma, 2003; Isaac et al., 2004; Lauri et al., 2004). At most synapses, only pharmacological activation of the presynaptic receptors has been described, thus relatively little is known about the physiological role of these receptors. Synaptic activation of kainate receptors has, however, been shown to facilitate glutamate release at mossy fiber synapses in the hippocampus (Lauri et al., 2001a, 2001b; Schmitz et al., 2001; Contractor et al., 2001) and to inhibit glutamate release at thalamocortical synapses (Kidd et al., 2002). Both synaptic facilitation and synaptic depression mediated by presynaptic KARs have been identified at cerebellar synapses (Delaney and Jahr, 2002). These studies indicate that KARs can rapidly regulate release (within 10 ms) and thus can efficiently control short-term dynamics of synaptic transmission. Paradoxically no physiological function has been ascribed to the KAR that downregulates glutamate release at CA1 synapses despite these being the first presynaptic KAR to be described (e.g., Chittajallu et al. [1996], Vignes et al. [1998], Kamiya and Ozawa [1998], Frerking et al. [2001], and Clarke and Collingridge [2002]).

KAR subunit mRNA expression is high early in development in the hippocampus and declines thereafter to lower adult levels (Bahn et al., 1994, Ritter et al., 2002). Recently, we have found that in neonatal CA3, presynaptic KARs are activated by ambient glutamate, and this regulates the frequency of mEPSCs and spontaneous network activity (Lauri et al., 2005). Therefore, we wondered whether the presynaptic KAR that regulates glutamatergic transmission at CA1 synapses contributes specifically to the developmental properties of CA1 transmission. Our findings show that these KARs are indeed physiologically activated by ambient levels of glutamate in the neonatal hippocampus. By tonically inhibiting glutamate release, these receptors maintain a low probability of release at neonatal synapses, which produces a strong facilitation in transmission during high frequency activity. Short, high-frequency bursts of synchronous activity represent a characteristic mode of transmission in the immature neuronal networks and are spontaneously generated in the newborn hippocampus (Lamsa

*Correspondence: tomi.taira@helsinki.fi

et al., 2000; Palva et al., 2000). Therefore, it appears that the KARs in the immature CA1 tune the synapses to respond to high-frequency "natural type of stimuli" that occur in the neonatal CA3-CA1 network both in vitro and in vivo (Palva et al., 2000; Lahtinen et al., 2002).

We further show that induction of LTP at neonatal inputs causes a rapid loss of this presynaptic KAR function. This removes the inhibitory tone on glutamate release, thereby dramatically altering the dynamic properties of CA3-CA1 transmission. Our findings describe the first physiological role for KARs regulating glutamate release in the area CA1, in setting the dynamic properties of the neonatal inputs to favor transmission during high-frequency bursts of activity. Further, our data suggests a new activity-dependent mechanism for regulation of KAR function by rapid changes in affinity of the receptors.

Results

Tonic Activation of the Presynaptic Kainate Receptors at CA3-CA1 Synapses in the Neonate Hippocampus

Pharmacological activation of presynaptic KARs in area CA1 leads to depression of glutamatergic transmission (e.g., Chittajallu et al. [1996], Vignes et al. [1998], Kamiya and Ozawa [1998], Frerking et al. [2001], and Clarke and Collingridge [2002]). However, the physiological roles of these receptors are unknown, and their endogenous activation has not been demonstrated. KARs are prominently expressed early in development in the hippocampus; therefore, we investigated the functions of KARs at glutamatergic CA1 synapses in neonatal (P3–P6) hippocampal slices. To determine if these receptors could be activated physiologically by endogenous glutamate, we used the GluR5-selective KAR antagonist LY382884 (10 μM) (Bortolotto et al., 1999; Lauri et al., 2001b). Application of LY382884 reversibly increased evoked EPSC amplitude in seven out of 10 cells in P3–P6 slices, an effect that was highly significant on average of all 10 cells (EPSC amplitude 163% ± 9% of control, n = 10, $p < 0.005$) (Figures 1A and 1F). In a subset of these experiments, minimal stimulation was used, and in these cells, the effects of LY382884 was associated with a decrease in failure rate (from 24% ± 5% to 5% ± 2%, n = 5, $p < 0.05$). These findings indicate that KARs at immature CA1 synapses are tonically activated and depress transmission. To investigate this further, we next recorded action-potential independent, spontaneous AMPA-R-mediated miniature EPSCs (mEPSCs) in the presence of TTX (1 μM) in the neonate. To rule out any indirect effects via $GABA_B$ receptors (Frerking et al., 1999) we also included 1 μM CGP55845A (Davies et al., 1993) in the extracellular solution. LY382884 reversibly increased the frequency of mEPSCs (160% ± 12%, n = 9) (Figures 1B and 1F) with no effect on amplitude (98% ± 6%, n = 9). The increase in mEPSC frequency was significant within six out of nine cells and highly significant when all the data was pooled ($p < 0.005$).

An effect on failure rate and mEPSC frequency is indicative of a presynaptic effect but could also be due to the insertion of AMPA receptors at silent synapses. Therefore, we tested the effect of LY382884 on NMDA-receptor-mediated transmission in the presence of 50 μM GYKI53655. Antagonism of KARs by LY382884 caused a significant increase in the frequency of NMDA-R mediated mEPSCs (161% ± 14%, n = 6; $p < 0.05$; effect significant within four out of six cells) (Figures 1C and 1F) but had no effect on their amplitude (91% ± 8%). The effect of LY382884 on AMPA-R and NMDA-R mEPSCs was not significantly different, strongly suggesting a presynaptic effect on Pr. The increase in NMDA-R-mediated mEPSC frequency was also observed with another KAR antagonist, NBQX (20 μM; 153% ± 20%, n = 5; $p < 0.005$; significant within four out of five cells) (Figure 1F), providing further pharmacological evidence that mEPSC frequency is regulated by a presynaptic KAR-dependent mechanism.

The most likely explanation for the effects of KAR antagonists on mEPSCs is that the KAR-regulating Pr is located at the presynaptic terminal. However, it is also a possibility that the effect on Pr is mediated indirectly, via KARs that control action potential-independent release of a modulatory substance that regulates glutamate release. Such possibilities include acetylcholine binding to nicotinic receptors (Maggi et al., 2004), ATP/adenosine binding to purine and A1 receptors, respectively (Zhang et al., 2003, Safiulina et al., 2005), and glutamate acting on metabotropic receptors (e.g., Scanziani et al. [1997] and Zakharenko et al. [2002]). However, antagonism of these receptors had no effect on the action of KARs at neonatal CA1 (see Figure S1). Taken together, these findings demonstrate that a presynaptic KAR directly regulating glutamate release tonically inhibits transmission at neonatal synapses onto CA1 pyramidal neurons.

The tonic inhibitory effect of KARs on glutamate release has not been detected previously in experiments on more mature CA1 neurons (e.g., Chittajallu et al. [1996], Vignes et al. [1998], Kamiya and Ozawa [1998], Frerking et al. [2001], and Clarke and Collingridge [2002]). Therefore, we investigated whether the endogenous tonic activation is developmentally down regulated. To test this, we next studied the effect of LY382884 on EPSCs in CA1 from juvenile rats (P14–P16). LY382884 (10 μM) had no significant effect on either evoked EPSCs (92% ± 13%, n = 10) (Figures 1D and 1F) or the frequency or amplitude of mEPSCs (98% ± 17% and 93% ± 6%, respectively; n = 6) (Figure 1F) at this age. However, presynaptic KARs are still present at this developmental stage because application of KAR agonist ATPA (1 μM) caused a reversible depression of EPSC amplitude at P14–P16 (53% ± 6% of control, n = 7, $p < 0.005$) (Figure 1E), as reported previously (Vignes et al., 1998; Bortolotto et al., 1999; Clarke and Collingridge, 2002). We also tested the effects of ATPA in the neonate, and this also produced a depression as expected (EPSC amplitude 67% ± 5% of control, n = 15, $p < 0.005$) (Figure 1F). Therefore, the presynaptic KAR is present at P14–P16 and can be pharmacologically activated to depress glutamatergic transmission; however, these receptors are not tonically activated by endogenous glutamate.

Tonically Active Presynaptic KARs Developmentally Regulate the Dynamic Properties of Glutamatergic Transmission in Neonatal CA1

Having established that KARs exert a tonic inhibitory action on glutamate release in the neonatal CA1, we went

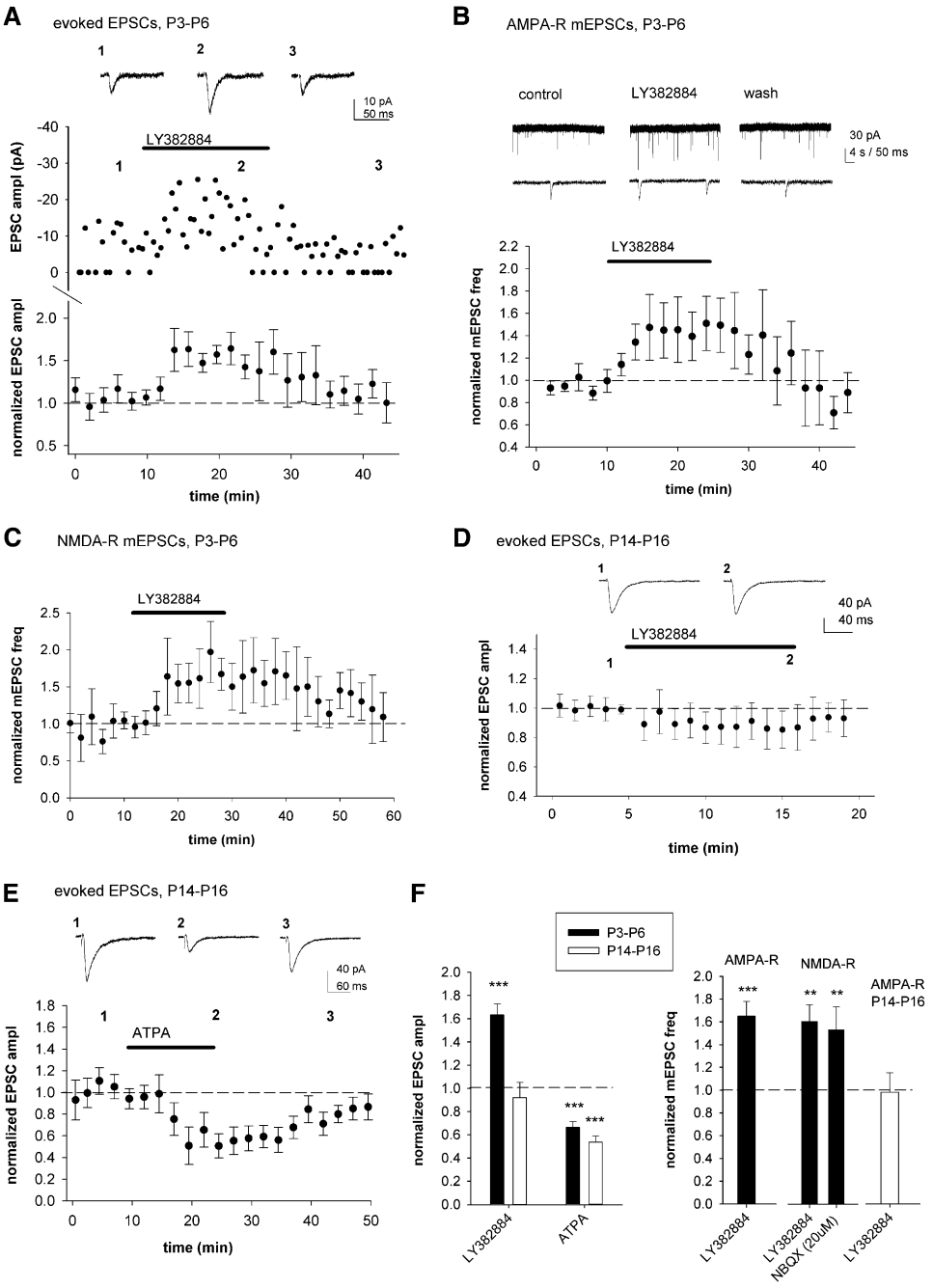

Figure 1. Endogenous Activation of KARs at CA3-CA1 Synapses during Early Postnatal Development

(A) LY382884 enhances EPSCs evoked by low-frequency (0.033 Hz) stimulation of Schaffer-collateral-commissural fibers. Single example (top) and pooled data (n = 10) showing the effect of LY382884 on EPSCs at P3–P6. The traces are averaged from five successive responses, obtained at the time points indicated (1–3). Error bars represent SEM.

(B) LY382884 causes a reversible increase in the frequency of mEPSCs in a CA1 pyramidal cell at P4. Example recordings (top) and pooled data calculated in 120 s bins and normalized to the baseline level, showing that LY382884 increases mEPSC frequency at P3–P6 (n = 9). Error bars represent SEM.

(C) LY382884 causes a reversible increase in the frequency of NMDA-receptor mediated mEPSCs at P3–P6 (n = 6). Error bars represent SEM.

(D) LY382884 has no effect on EPSC amplitude at P14–P16 (n = 10). Error bars represent SEM.

(E) ATPA depresses EPSC amplitude at P14–P16 (n = 7). Error bars represent SEM.

(F) Pooled data of the effects of KAR selective pharmacological agents on evoked and mEPSCs. Error bars represent SEM.

on to study the physiological consequences of this regulation. In particular, we were interested in studying their role in frequency dependent synaptic transmission because CA3 pyramidal neurons in the neonate predominantly fire in short, high-frequency bursts during spontaneous network events (Lamsa et al., 2000; Palva et al., 2000). Immature synapses in CA1 are heterogeneous in terms of their short-term dynamics. Although some

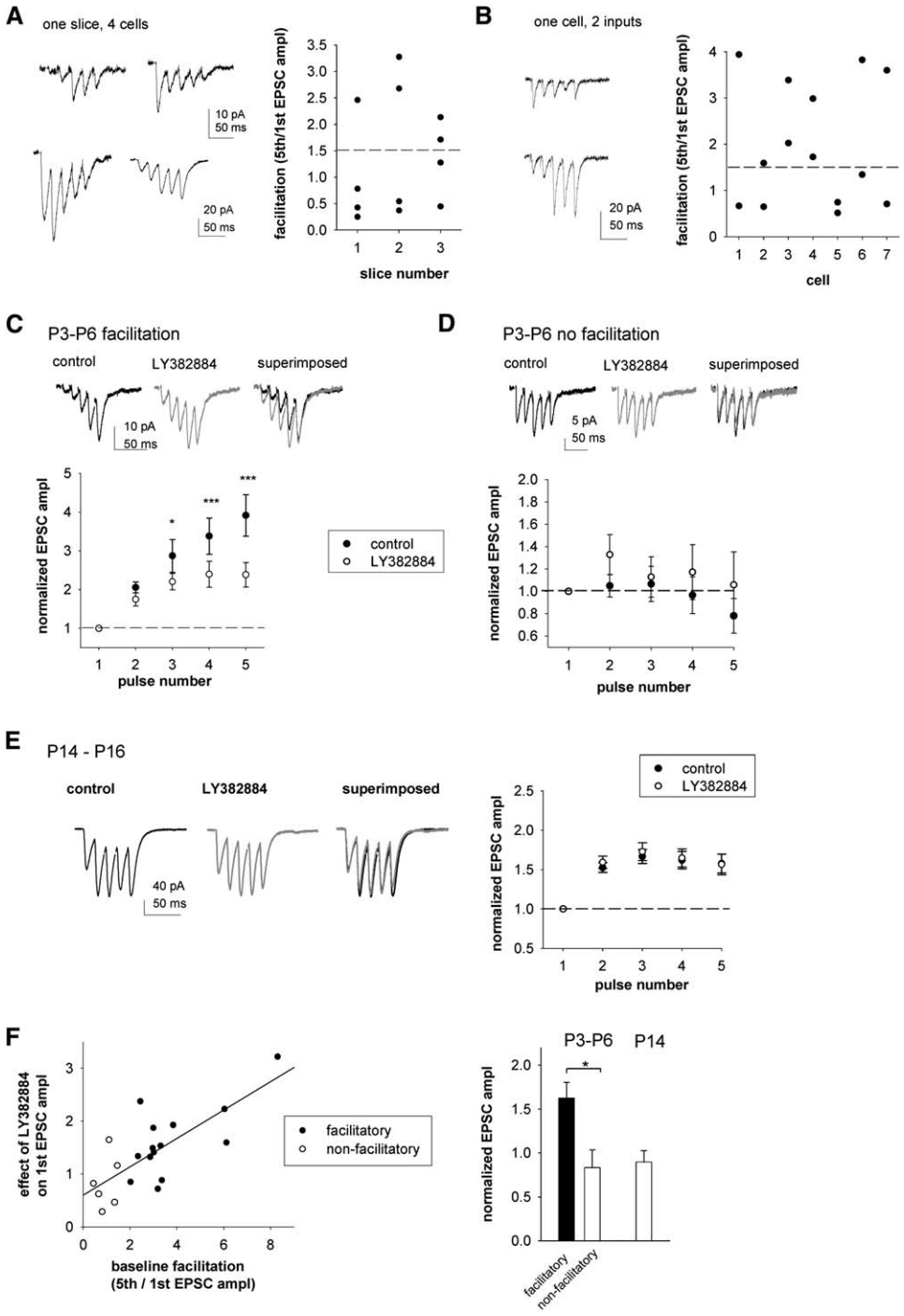

Figure 2. Tonically Active KARs Regulate Short-Term Facilitation at Developing Glutamatergic Synapses at CA1

(A) Input heterogeneity in the neonatal CA1. Example of recording from four different cells within the same slice, showing heterogenous response to 50 Hz stimulation of the same afferent fibers. The graph shows the facilitation ratio (fifth EPSC/first EPSC amplitude) of individual cells within slices.

(B) Example traces and a graph to show facilitatory and nonfacilitatory responses in the same cell, in response to stimulation of two different inputs in the neonate.

(C) LY382884 inhibits short-term plasticity in a population of synapses expressing facilitation in the neonatal CA1. Representative traces and pooled data (n = 14) showing the effect of LY382884 on EPSCs evoked by 50 Hz afferent stimulation. LY382884 inhibits facilitation of EPSCs by increasing the amplitude of the first EPSC in the train, whereas the amplitude of the 5th EPSC is on average not changed. The values in the graph show the amplitude of each EPSC in the train, normalized to the amplitude of the first EPSC under control conditions and in the presence of LY382884 (asterisk, $p > 0.05$; triple asterisk, $p < 0.005$). Error bars represent SEM.

(D) LY382884 has no significant effect on EPSCs in a population of cells in which no facilitation is present at P3–P6 (n = 6). Error bars represent SEM.

(E) LY382884 has no effect on EPSCs at P14–P16 (n = 10). Error bars represent SEM.

of the excitatory synapses have a high probability of release and are characterized by synaptic depression, others have a low probability of release and facilitate during repetitive activity (Hanse and Gustafsson, 2001; Palmer et al., 2004). Similar facilitation is a characteristic feature of the mossy fiber synapse in CA3, in which presynaptic KARs play an important role in regulating glutamate release (Lauri et al., 2001a, 2001b; Schmitz et al., 2001).

To investigate short-term frequency dynamics at CA1 synapses, we evoked EPSCs with five stimuli at 50 Hz. Based on the response to 50 Hz stimulation, inputs were divided into two groups: (1) facilitating (amplitude ratio of fifth/first EPSC > 1.5; 23 out of 40 recordings [58%]), and (2) not dynamic or depressing (42%). When the same population of inputs was stimulated, both facilitatory and nonfacilitatory responses could be evoked in different cells in the same slice (Figure 2A). Conversely, when two different inputs were stimulated onto the same cell, both types of responses could also be observed (Figure 2B). Thus, the heterogeneity in the short-term plasticity of inputs we observe in the neonate is not slice or cell dependent but rather represents a true heterogeneity in synaptic function at this developmental stage.

We next tested whether the heterogeneity might be related to KAR function at presynaptic terminals. At facilitating synapses, application of LY382884 inhibited facilitation of EPSCs during 50 Hz stimulation (facilitation in the presence of LY382884 62% ± 4% of control, n = 14, $p < 0.005$) (Figure 2C). This effect was associated with an increase in the amplitude of the first EPSC in the train, whereas the absolute amplitude of the fifth EPSC was not significantly changed (first EPSC 156% ± 19%, $p < 0.05$; fifth EPSC 90% ± 7%). In contrast, at synapses where no facilitation was seen, LY382884 had no significant effect on synaptic responses during the train (Figure 2D). At P14, there was little or no synaptic facilitation in response to 50 Hz stimulation (although some postsynaptic summation was observed), and LY382884 had no effect (102% ± 9% of control, n = 10) (Figure 2E). These findings suggest that the presynaptic KAR is endogenously activated only in the facilitating neonatal inputs and that this tonically keeps Pr low, allowing for the large facilitation during repetitive activity. In support of this, when the control level of synaptic facilitation in the individual experiments was plotted against the increase in the first EPSC amplitude in the presence of LY382884, there was a significant correlation ($r = 0.746$, Pearson's two-tailed correlation test, $p < 0.01$, n = 20) (Figure 2F). Thus, the effect of LY382884 on the first EPSC amplitude was significantly larger in facilitating versus nonfacilitating cells ($p < 0.01$) (Figure 2F).

Tonic Activation of the KAR Depends on Ambient Glutamate in the Extracellular Space

We have reported previously that KARs in the immature CA3 are activated by endogenous glutamate (Lauri et al., 2005). Therefore, one mechanism that might explain the heterogeneity and developmental differences in the short-term dynamics of transmission at CA1 synapses is differences in the extent of endogenous activation of the presynaptic KAR by ambient glutamate in the extracellular space. To investigate this, we first determined whether the presynaptic KAR in the neonate is sensitive to manipulations that alter ambient glutamate levels. In these experiments, in addition to the $GABA_B$ receptor antagonist, we included the broad-spectrum $mGluR_{1-8}$ antagonist LY341495 (100 μM) (Fitzjohn et al., 1998) to exclude any possible effect via mGlu receptors (e.g., Scanziani et al. [1997]). Blocking mGlu receptors alone had no effect on the actions of LY382884 on mEPSC frequency (153% ± 8%; n = 5; $p < 0.05$; and 160% ± 12%; n = 9; $p < 0.005$; with and without 100 μM LY341495, respectively).

In a first set of experiments, we used an enzymatic glutamate scavenger system (glutamic-pyruvic transaminase [GPT] + pyruvate) (Overstreet et al., 1997; Min et al., 1998) to reduce extracellular glutamate concentration. GPT or pyruvate alone had no effect on mEPSC frequency (98% ± 15%, n = 4 and 99% ± 11% of control, n = 4, respectively; not shown). In the neonate, the glutamate scavenger (GPT + pyruvate) increased mEPSC frequency to 232% ± 37% of control (n = 14, $p < 0.01$) (Figure 3A), and this fully occluded the effects of the subsequent application of LY382884 (mEPSC frequency 102% ± 8% of that in scavenger only; n = 9). The scavenger had no effect on the ability to pharmacologically activate the presynaptic KAR in the neonate: application of ATPA in the presence of the scavenger depressed mEPSC frequency (54% ± 5%, n = 9, $p < 0.01$) (Figure 3B). The glutamate scavenger also affected synaptic facilitation. Thus, at facilitating synapses, the scavenger inhibited facilitation of the fifth EPSC (57% ± 6%, n = 6, $p < 0.005$) (Figure 3C) and increased the amplitude of the first EPSC in the train (174% ± 27%, $p < 0.05$, n = 6). At nonfacilitating synapses, the scavenger had no significant effect (facilitation 85% ± 7% of control, first EPSC amplitude 96% ± 16% of control, n= 6) (Figure 3C). Moreover, the effect on facilitation correlated strongly with the effect of scavenger on the first EPSC amplitude ($r = 0.767$, $p < 0.005$, n = 12) (Figure 3C). In all respects, therefore, the scavenger mimicked the effects of LY382884 on synaptic facilitation. These data indicate that in the neonate, the presynaptic KAR at facilitating inputs is tonically activated by endogenous glutamate and that this regulates the dynamic properties of these synapses.

We also tested the effects of the glutamate scavenger in slices from P14–P16 animals. This had no effect on mEPSC frequency (93% ± 17%, n = 9) (Figure 3E) demonstrating that endogenous glutamate does not activate the presynaptic KAR in P14–P16 animals. To investigate whether this is due to a lower ambient glutamate concentration in the older animals, we used the glutamate transport inhibitor TBOA (50 μM) (Shimamoto et al., 1998), to experimentally increase the ambient glutamate concentration. Neither TBOA alone nor TBOA plus LY382884 had

(F) The level of synaptic facilitation correlates with the endogenous activation of KARs. Lefthand side, a plot of the baseline facilitation (fifth EPSC/first EPSC amplitude) versus the effect of LY382884 on the first EPSC amplitude in individual cells at P3–P6 (n = 20). Righthand side, pooled data of the effect of LY382884 on EPSC amplitude at facilitating (n = 14) versus nonfacilitating (n = 6) cells at P3–P6 and at P14–P16 (n = 10). Error bars represent SEM.

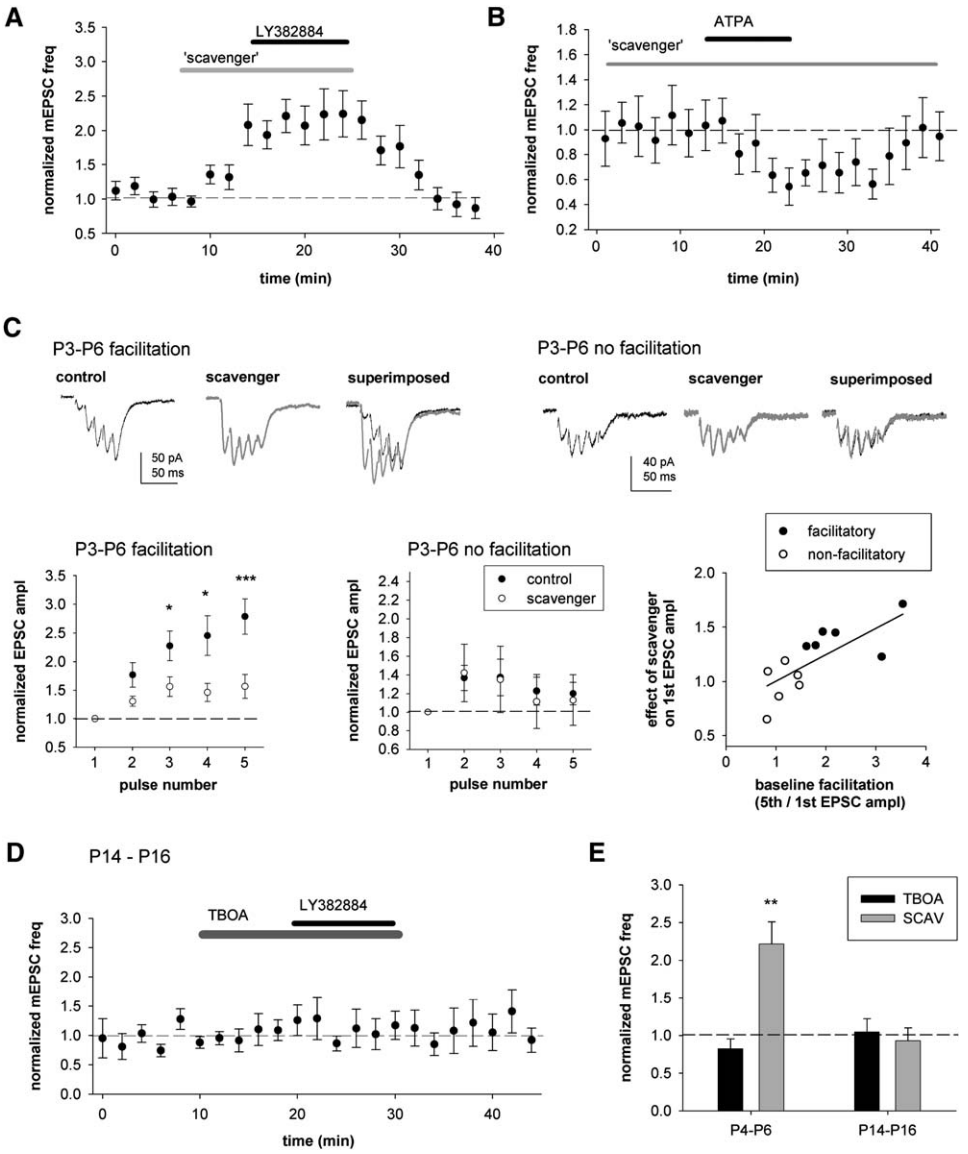

Figure 3. Ambient Levels of Glutamate Activate Presynaptic KARs in the Neonatal Hippocampus

(A) Pooled data (n = 9) to show that a glutamate scavenger increases mEPSC frequency and fully occludes the effect of LY382884 at P3–P6. Error bars represent SEM.

(B) Pooled data (n = 9) showing that that glutamate scavenger does not affect the ability to pharmacologically activate the presynaptic KAR because application of ATPA depresses mEPSC frequency. Error bars represent SEM.

(C) The effect of the glutamate scavenger on synaptic facilitation. The graphs plot the effects of the scavenger at facilitating and "nonfacilitating" synapses and a correlation between the effect of the scavenger on the first EPSC amplitude and the level of facilitation. Error bars represent SEM.

(D) The glutamate transport inhibitor TBOA (50 μM) applied alone or together with LY382884 had no effect on mEPSC frequency at P14–P16 (n = 7). Error bars represent SEM.

(E) Summary bar graphs showing that the scavenger, but not TBOA, has an age-dependent effect on mEPSC frequency. Error bars represent SEM.

any significant effect on mEPSCs (mEPSC frequency 99% ± 12% and 109% ± 15%, respectively, n = 7) (Figures 3D and 3E) or on evoked EPSCs (EPSC amplitude 89% ± 9% and 87% ± 4%, n = 6, not illustrated). In addition, TBOA had no significant effect on mEPSC frequency in the neonatal CA1 (82% ± 14%, n = 5) (Figure 3E). Thus, the developmental mechanism for endogenous activation of KARs cannot be recapitulated in 2 week old animals by increasing the ambient glutamate concentration. This indicates that during development, it is a change in the functional properties of the presynaptic KAR that prevents their activation by endogenous glutamate.

The Tonically Activated Presynaptic KAR in the Neonate Is Metabotropic

Previous work has shown that the inhibition of synaptic transmission between CA3 and CA1 neurons, by pharmacological activation of KARs, involves a G protein-dependent mechanism (Frerking et al., 2001). Having established that early in development there is a

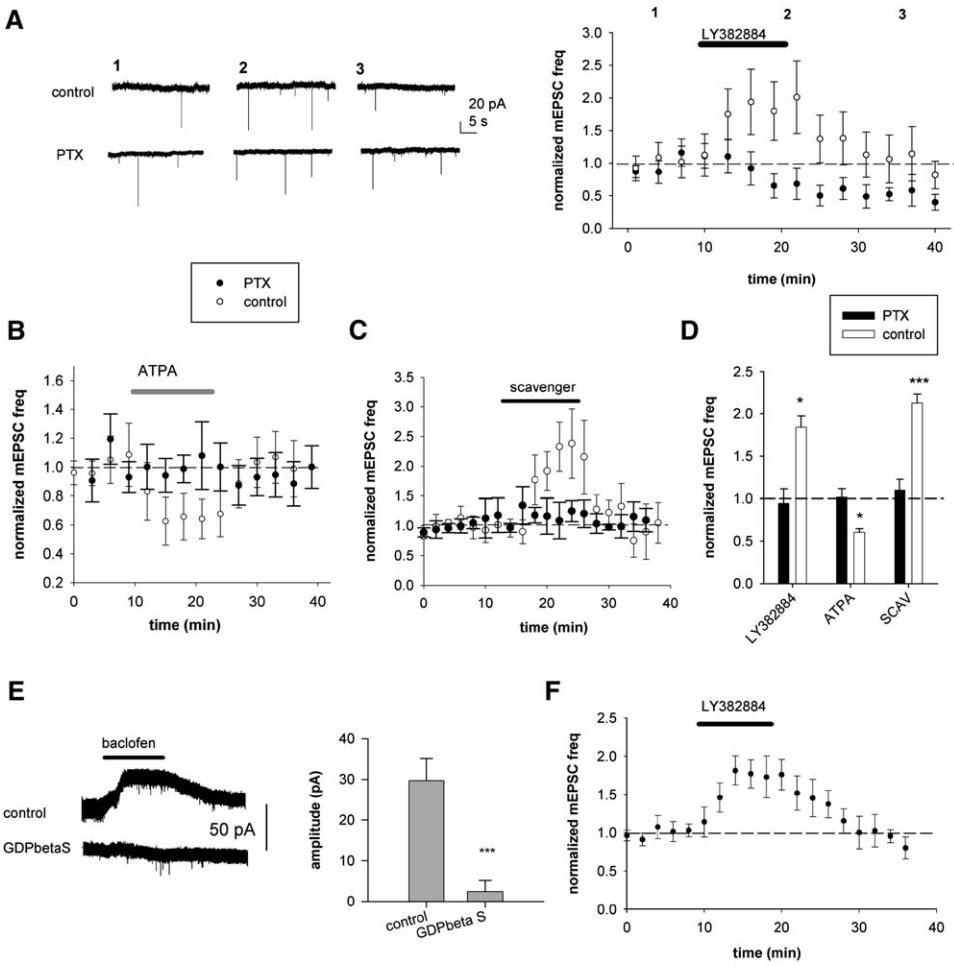

Figure 4. Endogenous Activation of Presynaptic KARs Occurs via a G Protein-Mediated Mechanism

(A) Single example and pooled data showing that LY382884 increases the frequency of mEPSCs in control slices (n = 7) but not in pertussis toxin (PTX)-treated slices (n = 9). mEPSCs were calculated in 180 s bins and normalized to the baseline level before application of LY382884. Error bars represent SEM.

(B) Corresponding data showing the lack of effect at ATPA on mEPSC frequency after PTX pretreatment (control, n = 6; ATPA, n = 6). Error bars represent SEM.

(C) The effect of the glutamate scavenger on mEPSC frequency is dependent on G proteins. Pooled data of the effect of scavenger on mEPSC frequency from slices preincubated with PTX (n = 6) or under control conditions (n = 5). Error bars represent SEM.

(D) Pooled data showing the effects of LY382884, ATPA, and the L-glutamate scavenger on mEPSC frequency in PTX-preincubated versus control slices. Error bars represent SEM.

(E) GDPβS (0.3 mM) in the electrode-filling solution was able to fully inhibit baclofen (20 μM)-induced outward current in CA1 pyramidal neurons (control 29 ± 5 pA, n = 5; GDPβS 2 ± 3 pA, n = 5). Error bars represent SEM.

(F) GDPβS did not prevent the facilitation of mEPSC frequency in response to LY382884 (n = 6). Error bars represent SEM.

physiological activation of KARs in area CA1, we next explored whether this effect involves G proteins. Neonatal slices were maintained overnight with or without pertussis toxin (PTX; 5 μg/ml). This treatment with PTX fully blocked the depression of IPSCs in response to application of the $GABA_B$ receptor agonist baclofen (Lauri et al., 2005), indicating that PTX was effective in blocking G protein-mediated signaling under these conditions. In slices incubated overnight under control conditions, LY382884 increased mEPSC frequency, and this was of similar magnitude to that observed in acute slices (177% ± 10%; n = 7; $p < 0.005$) (Figures 4A and 4D). In PTX-treated slices, however, LY382884 did not increase mEPSC frequency but rather produced a slowly developing decrease (Figure 4A). PTX treatment also completely inhibited the effect of ATPA, which under control conditions decreases mEPSC frequency (control 61% ± 9%, n = 6, $p < 0.05$; PTX 101% ± 10%, n = 6) (Figures 4B and 4D). In addition, the effect of the glutamate scavenger on mEPSC frequency was also dependent on G proteins because the scavenger had no effect in PTX-treated slices (scavenger in control 212% ± 45%, n = 5, $p < 0.005$; scavenger + PTX 109% ± 13%, n = 6) (Figures 4C and 4D).

Pertussis toxin pretreatment inhibits both pre- and postsynaptic G proteins. Given that the KARs act via a presynaptic mechanism, selective inhibition of postsynaptic G proteins should not interfere with their action. To test this, we included GDPβS in the electrode-filling solution (0.3 mM). This concentration was able to fully inhibit outward currents evoked in CA1 pyramidal neurons by 20 μM baclofen (control 29 ± 5 pA, n = 5;

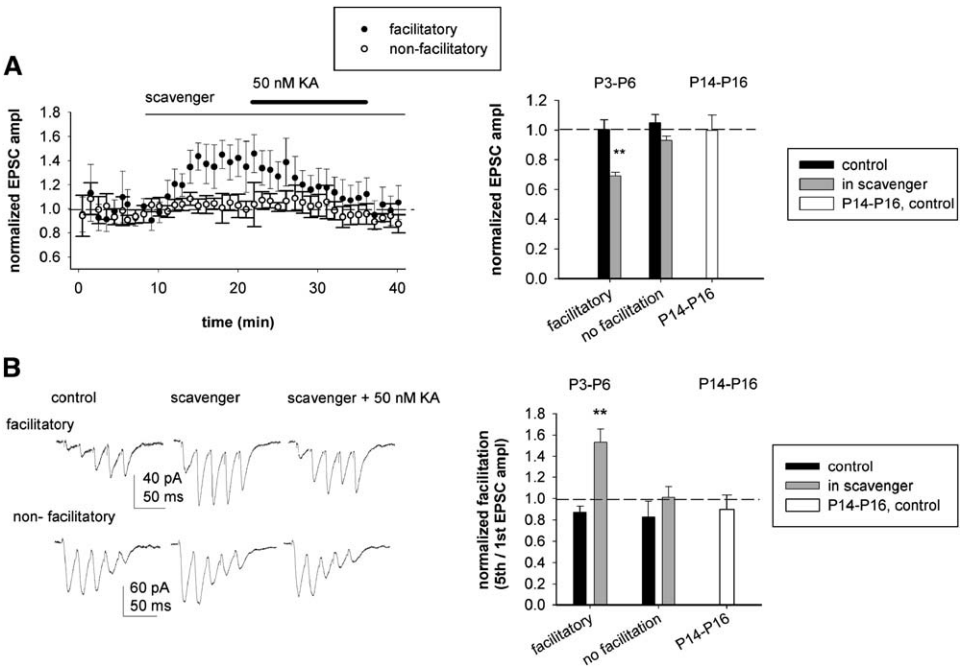

Figure 5. A Tonically Activated High-Affinity KAR Regulates Synaptic Transmission at Facilitating Inputs

(A) Effect of 50 nM KA on EPSC amplitudes under control conditions and in the presence of the glutamate scavenger. A graph shows the effects of the scavenger and 50 nM KA on EPSC amplitude on responses that were originally facilitatory (n = 13) and nonfacilitatory (n = 7). The bar graph on the right depicts the effects of 50 nM KA on EPSC amplitude, normalized to the level before KA application, under control conditions (P3–P6, n = 6 [nonfacilitatory], n = 10 [facilitatory]; P14–P16, n = 6) and in the presence of scavenger. Error bars represent SEM.

(B) Effect of 50 nM KA on EPSCs evoked at 50 Hz. Example traces showing the effect of the scavenger and then the addition of 50 nM KA on facilitatory and nonfacilitatory responses, evoked by 50 Hz stimulation at the neonatal CA1. Pooled data shows the level of facilitation (fifth/first EPSC amplitude) in the presence 50 nM KA, normalized to value just before KA application, under control conditions (P4–P6, n = 6 [nonfacilitatory], n = 6 [facilitatory], P14–P16, n = 6) and in the presence of scavenger (n = 7 [nonfacilitatory], n = 10 [facilitatory]). Error bars represent SEM.

GDPβS 2 ± 3 pA, n = 5) (Figure 4E). However, postsynaptic GDPβS did not prevent the facilitation of mEPSC frequency in response to LY382884 application (161% ± 10% n = 6, p < 0.05) (Figure 4F). Together, these findings demonstrate that, similar to mature CA1 (Frerking et al., 2001), the presynaptic KARs inhibiting glutamate release in neonatal CA1 act via a G protein-dependent mechanism. Our experiments reveal, however, that this G protein-coupled KAR is tonically activated by endogenous glutamate in the neonate.

High-Affinity KARs Are Preferentially Expressed at Neonatal Facilitating Synapses

Our data demonstrate that the presynaptic KAR regulating glutamate release is present in the neonate and also at P14–P16 synapses. However, the presynaptic KAR is only activated by endogenous glutamate at facilitating synapses in the neonate. One possible explanation for this is that the affinity of KARs at facilitating synapses is higher than at nonfacilitating inputs. To investigate this, we used 50 nM KA, which selectively activates high-affinity KARs (Lauri et al., 2001a; Schmitz et al., 2001). 50 nM KA had no effect on EPSCs under control conditions, either at P3–P6 or at P14–P16 (EPSC amplitude in the presence of 50 nM KA 103% ± 3%, n = 16 and 99% ± 10%, n = 6, respectively) (Figure 5A). However, when tonic activation of the receptors in the neonate was removed by the addition of the glutamate scavenger, 50 nM KA depressed EPSC amplitude at facilitatory synapses (EPSC amplitude 69% ± 2%, n = 13, p < 0.005) (Figure 5A) but had no effect on nonfacilitating synapses (93% ± 3%, n = 7) (Figure 5A). The depression by 50 nM KA in the presence of the scavenger was also associated with an increase in facilitation of EPSCs during a 50 Hz train (facilitatory inputs: 153% ± 12% of facilitation in scavenger alone, n = 10, p < 0.01; nonfacilitatory: 101% ± 10%, n = 7) (Figure 5B). In interleaved controls, EPSC amplitude remained potentiated at facilitatory inputs during long-term application of scavenger (EPSC amplitude 40 min after scavenger application 170% ± 14% of control, n = 5, p < 0.01). Further, the differences in the sensitivity of facilitatory and nonfacilitatory inputs to 50 nM KA persisted if the release probability was experimentally increased by altering the divalent cation concentrations (Figure S2). These findings strongly suggest that the facilitatory synapses in the neonate selectively express a high-affinity KAR that is tonically activated by ambient glutamate. Further, this indicates that the affinity difference of the presynaptic KAR is responsible for generating the heterogeneity in the dynamic properties of neonatal glutamatergic synapses onto CA1 pyramidal neurons.

Induction of LTP at Facilitating Neonatal Inputs Rapidly Alters Tonic KAR Activation

The tight correlation between the tonic activation of KARs and the dynamic properties of glutamatergic CA1 synapses suggests that a developmental mechanism

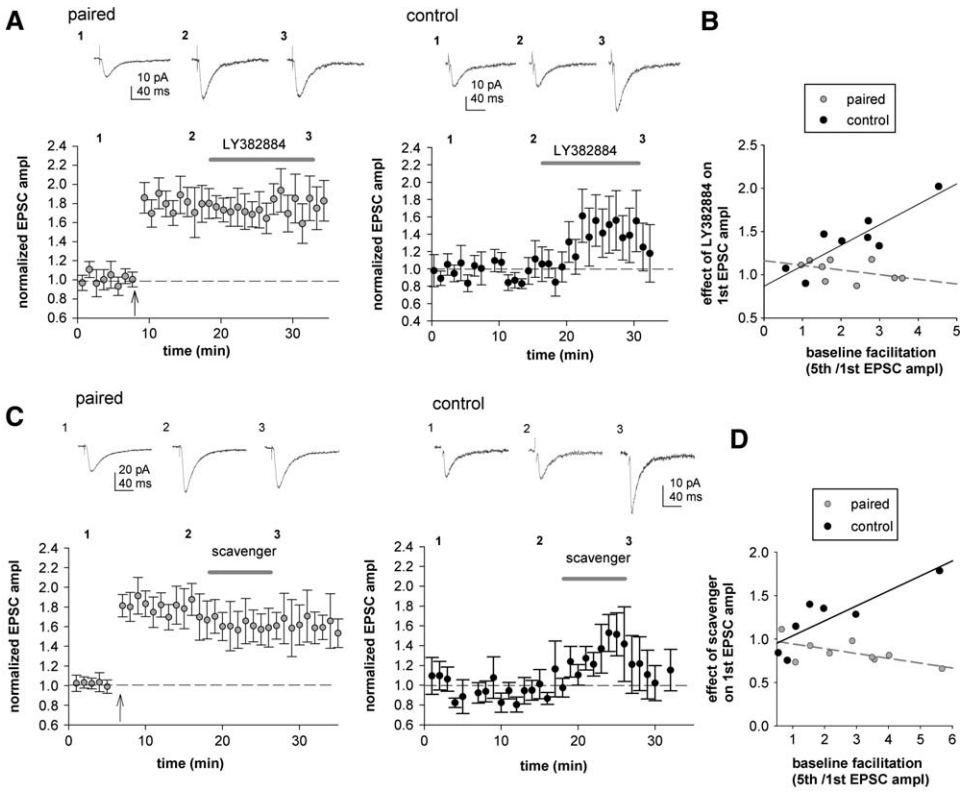

Figure 6. LTP Induction in the Neonate Occludes the Tonic Inhibitory Effect of Presynaptic KARs

(A) Data from two-pathway experiments showing that induction of LTP prevents the effect of LY382884 on evoked EPSC amplitude. The level of facilitation in response to 50 Hz stimulation was measured at the beginning of the experiment, and only facilitatory responses are included in the time course plots. LY382884 had no effect on EPSC amplitude after pairing (n = 6), whereas in the control pathway (middle), LY382884 still has the typical facilitatory effect on EPSC amplitude (n = 6, $p < 0.05$). Error bars represent SEM.

(B) The correlation between the effect of LY382884 on first EPSC amplitude and the baseline (before pairing) level of facilitation was lost after LTP induction (control, n = 8; paired n = 9).

(C) Induction of LTP prevents the effect of the glutamate scavenger on evoked EPSC amplitude. The level of facilitation in response to 50 Hz stimulation was measured during baseline and only facilitatory responses are included in the time course plots. The scavenger had no effect on EPSC amplitude after pairing (n = 7), whereas in the control pathway, the scavenger increased the amplitude of EPSCs (n = 4, $p < 0.05$). Error bars represent SEM.

(D) The correlation between the effect of the scavenger on first EPSC amplitude and the baseline level of facilitation was lost after LTP induction (paired, n = 9; control, n = 7).

exists to alter presynaptic KAR function to produce the mature nonfacilitating type of inputs. LTP is thought to be critically important for the development of neural circuits; therefore, we investigated whether this form of plasticity also regulates presynaptic KAR function. To address this issue, we studied the effects of LTP induction on the tonic activation of KARs and synaptic facilitation in neonatal CA1 pyramidal neurons. In these experiments, two pathways were stimulated and perforated patch recordings were used to prevent intracellular dialysis.

Application of a pairing protocol produced a robust pathway specific LTP (183% ± 6%, n = 33, $p < 0.005$) in P3–P6 neurons. In the control, unpotentiated pathways LY382884 (10 μM) increased the EPSC amplitude to 150% ± 11% of baseline (n = 8; $p < 0.05$) (Figure 6A), as expected; however, in the same cells, LY382884 had no effect on EPSC amplitude in the LTP pathway (105% ± 4%, n = 9) Figure 6A). In these experiments, we measured the amount of synaptic facilitation in both pathways during baseline prior to LTP induction. This demonstrated that LTP could be reliably induced in both facilitating and nonfacilitating inputs; however, the level of potentiation in the facilitating inputs was significantly higher than in the nonfacilitating inputs (Figure S3). A correlation analysis shows that consistent with the previous data, LY382884 increases EPSC amplitude only at facilitating inputs in the control pathways. However, in the LTP pathway after LTP induction, LY382884 has no effect on EPSC amplitude regardless of whether the input was initially facilitatory or nonfacilitatory during baseline (Figure 6B). We also tested whether prior application of LY382884 prevented LTP induction. In these experiments, application of LY382884 caused an increase in EPSC amplitude in facilitatory inputs as expected; however, LTP could still be induced, yet the level of potentiation was lower than in control facilitatory inputs but comparable to nonfacilitatory inputs (Figures S3D and S3E).

We also found that LTP induction in the neonate occluded the effects of the glutamate scavenger: in the LTP path, the glutamate scavenger did not cause a facilitation of EPSC amplitude (EPSC amplitude in scavenger percent of preceding amplitude = 84% ± 5%, n = 9)

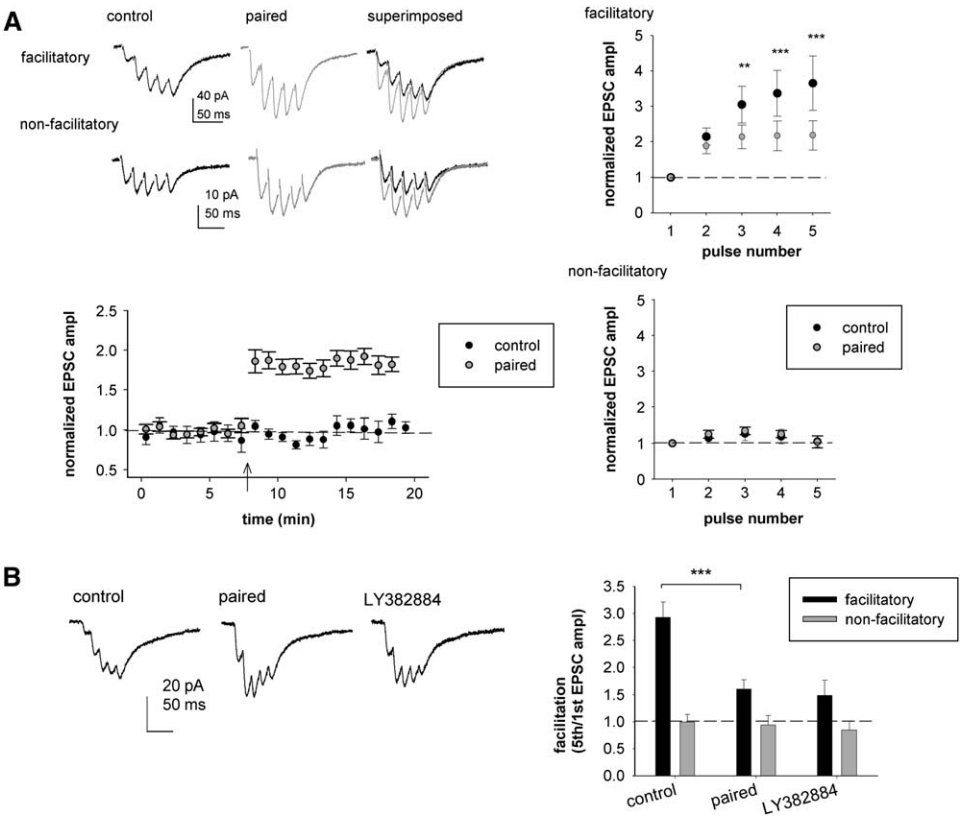

Figure 7. LTP Causes a Change in the Dynamic Properties of Neonatal Inputs

(A) Representative traces and pooled data showing the effect of pairing-induced LTP on EPSCs evoked by 50 Hz afferent stimulation. After LTP induction, facilitation ratio was reduced to 60% ± 5% of the level before pairing at facilitatory responses (n = 11, $p < 0.005$), whereas there was no significant effect at nonfacilitatory responses (n = 7). The values in the graphs (top, right) show the amplitude of each EPSC in the train, normalized to the amplitude of the first EPSC under control conditions and after pairing (asterisk, = $p > 0.05$; triple asterisk, = $p < 0.005$). Error bars represent SEM.

(B) Example traces and pooled data showing that LY382884 had no effect on the synaptic facilitation after LTP induction, independent of the dynamic properties of the response during baseline (facilitatory n = 6; nonfacilitatory, n = 3). Error bars represent SEM.

(Figure 6C), whereas in control facilitatory pathways, the scavenger caused an increase in EPSC amplitude, as expected (149% ± 10%, n = 4, $p < 0.05$) (Figure 6C). Similar to the LY382884 data, correlation analysis of initial baseline facilitation with effects of the scavenger after LTP showed that the scavenger had no effect on inputs in the LTP path regardless of whether they were facilitatory or nonfacilitatory during baseline (Figure 6D). In contrast, induction of LTP had no effect on the ability of adenosine receptor agonist N6-cyclopentyladenosine (CPA) to depress EPSC amplitude (Figure S4).

Induction of LTP at Facilitating Neonatal Inputs Rapidly Alters Short-Term Dynamics of Synapses

Because neonatal LTP is associated with a rapid change in the properties of the presynaptic KAR such that it is no longer tonically activated by ambient glutamate, LTP should also alter the dynamic properties of neonatal inputs. To test this directly, we measured the amount of synaptic facilitation before and after LTP induction in two pathway experiments. At facilitatory synapses, LTP induction was associated with a decrease in the facilitation (facilitation of fifth EPSC 60% ± 5% of baseline level, n = 11, $p < 0.005$) (Figure 7A), whereas the level of facilitation was not changed at synapses that were initially nonfacilitatory (93% ± 10%, n = 7) (Figure 7A). No changes in facilitation were observed in the control pathways. We further investigated this by testing the effect of LY382884 application on the dynamic properties of inputs after LTP induction. In control pathways that were facilitatory, LY382884 caused a reduction in facilitation consistent with our previous findings. However, in facilitatory pathways after LTP induction, facilitation was greatly reduced, and LY382884 had no further effect on this (facilitation of fifth EPSC in LY382884 expressed as percent facilitation prior to LY382884 application = 98% ± 8%, n = 6) (Figure 7B). In nonfacilitating inputs, LTP and LY382884 had no effect on the level of facilitation as expected. Therefore, LTP occludes not only the increase in EPSC amplitude caused by LY382884 but also occludes the effects of LY382884 on the dynamic properties of facilitatory synapses.

LTP Involves an Alteration in the Affinity of KARs Regulating Glutamate Release

The rapid loss of tonic presynaptic KAR activity with LTP could be due to a complete loss of the presynaptic KAR function, for example, by rapid internalization of the

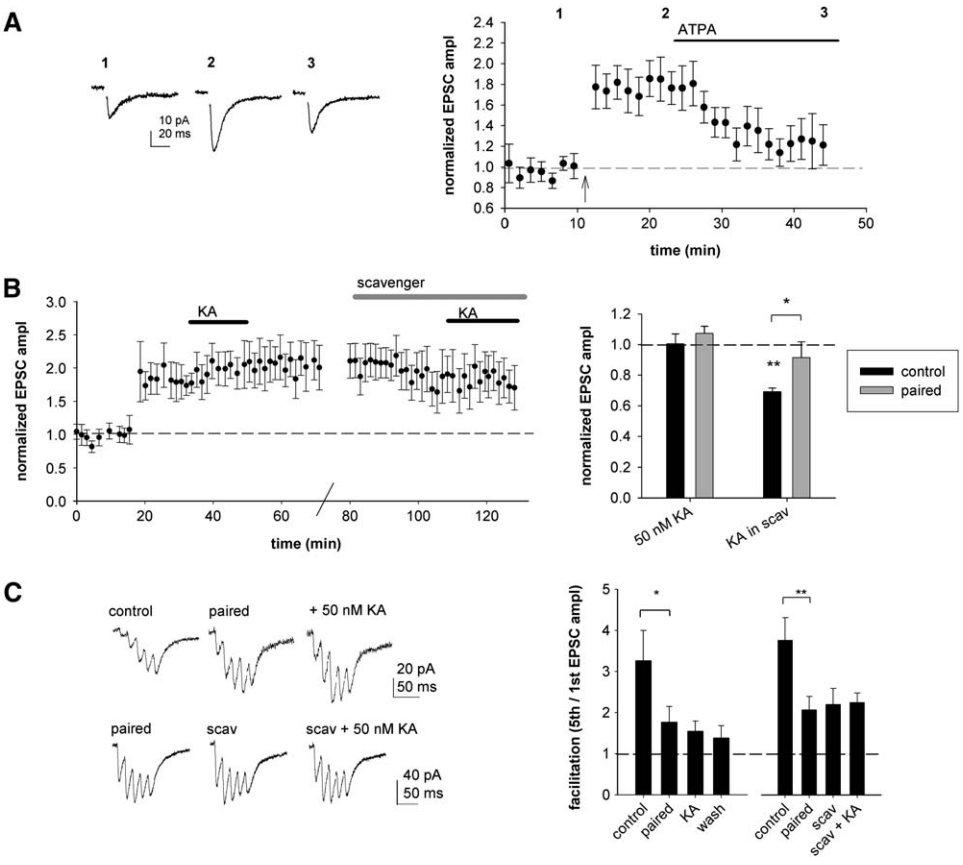

Figure 8. LTP Induction in the Neonate Results in a Loss of the High-Affinity KAR

(A) ATPA induces significant depression of EPSC amplitude after pairing-induced LTP (59% ± 6%, n = 9, $p < 0.05$). Error bars represent SEM.
(B) Effect of 50 nM KA after LTP induction. 50 nM KA has no effect on EPSC amplitude after pairing, in the absence or the presence of glutamate scavenger, in synapses that were initially facilitatory (KA, n = 7; KA in scavenger, n = 8). Error bars represent SEM.
(C) 50 nM KA has no effect on facilitation after LTP induction in the absence or the presence of the glutamate scavenger, in synapses that were initially facilitatory (KA, n = 7; KA in scavenger, n = 8; same cells as in [B]). Error bars represent SEM.

receptor complement. Alternatively, there might be an alteration in the properties of the KAR. To test if a functional KAR remains at inputs after LTP induction, we applied the KAR agonist ATPA, which depresses transmission in neonatal and P14 inputs (see Figure 1F). After induction of LTP, ATPA still caused a reliable and robust depression of EPSC amplitude (EPSC amplitude in ATPA percent of preceding baseline = 59% ± 6%, n = 9, $p < 0.05$) (Figure 8A), thus showing that the presynaptic receptor was still present and functional after LTP induction.

Because our data indicates that the tonic activation of the presynaptic KAR relies upon it having high affinity, we next investigated whether the affinity of KARs is rapidly altered during LTP. To test this, we applied 50 nM KA after induction of LTP, in pathways that were originally facilitatory and so were initially expressing the presynaptic KAR sensitive to low concentrations of KA (cf. Figure 5). 50 nM KA had no effect on EPSC amplitude (107% ± 5%, n = 7) or synaptic facilitation (97% ± 9%, n = 7) after LTP induction (Figure 8B). Our previous data show that the actions of 50 nM KA are only observed when the tonic activation of the high affinity receptor by ambient glutamate is removed by the addition of the glutamate scavenger. Therefore, in the second part of the experiment, we applied the scavenger and reapplied 50 nM KA. Under these conditions, no effect of 50 nM KA was observed in the LTP path (EPSC amplitude in 50 nM KA = 90% ± 9%, n = 8) (Figure 8B). However, KA still induced a reliable depression in the control path (66% ± 2% of control, n = 4).

Finally, we also investigated the effects of 50 nM KA on synaptic facilitation in these experiments. LTP caused a reduction in the amount of facilitation and subsequent application of 50 nM KA alone, or scavenger plus 50 nM KA had no further effect on synaptic facilitation (Figure 8C). Therefore, these data suggest that LTP causes a rapid decrease in the affinity of the presynaptic KAR at facilitating inputs. Importantly, it suggests that this process is involved in the expression mechanism for neonatal LTP at these inputs because the loss of the tonic activity of the presynaptic KAR potentiates transmission and alters the dynamic properties of the synapses.

Discussion

One of the most distinct features of the immature CA3-CA1 transmission is its fragility and highly dynamic nature when compared to the adult (e.g., K. Lamsa et al., 1999, Soc. Neurosci., abstract and Hanse and Gustafsson

[2001]). As shown in the present study, synapses exhibiting either prominent facilitation or depression can be found in the same cell. This heterogeneity is no longer observed in the adult and is most likely attributed to developmental changes in Pr (e.g., Bolshakov and Siegelbaum [1995] and Wasling et al. [2004]). However, little is known concerning the mechanisms for this regulation. We now show that a presynaptic KAR that directly downregulates release at immature CA1 synapses is tonically activated by endogenous glutamate and is responsible for determining short-term plasticity at this input. During development, and rapidly after LTP induction, the function of this autoreceptor is altered so that it is no longer tonically activated, leading to the observed developmental alteration in the short-term dynamics of the CA1 input.

Endogenous Activation of the Presynaptic KAR at Immature CA1 Synapses

Although the existence of presynaptic KARs at CA3-CA1 excitatory synapses is well documented (Chittajallu et al., 1996; Vignes et al., 1998; Kamiya and Ozawa, 1998; Frerking et al., 2001; Clarke and Collingridge, 2002), there has hitherto been no evidence that these receptors can be activated endogenously or what their physiological role is. KARs are highly expressed in the developing hippocampus and in particular, the GluR5 subunit shows a pronounced peak in the mRNA expression during the first week of life with a rapid decay thereafter (Bahn et al., 1994; Ritter et al., 2002). Our data shows that early in development, GluR5 subunit-containing KARs in area CA1 are tonically activated by endogenous glutamate and act to inhibit glutamate release via a direct, action-potential-independent mechanism.

An intriguing feature of some kainate receptors is that they possess both ionotropic and metabotropic activity (e.g., Rodriguez-Moreno and Lerma [1998], Frerking et al. [2001], Melyan et al. [2002], Rozas et al. [2003], and Lauri et al. [2005]). The tonic regulation of glutamate release by KARs in the neonate CA1 as well as in CA3 appears to be G protein dependent because both the effects of KAR agonism and antagonism were fully blocked by pertussis toxin. These data suggest that the physiological role of G protein linked KARs at Schaffer collaterals might be specifically related to regulation of transmission during maturation of the glutamatergic synapses.

During early development, glutamate transport mechanisms are less efficient (Danbolt, 2001; Diamond 2005), and the diffusional barriers in the extracellular space are not yet fully developed (Sykova et al., 2000). Our data indicates that ambient glutamate in the neonate critically influences synaptic glutamate release. Although ambient glutamate might act via several receptor mechanisms, it is of particular interest to note that in neonatal slices the effects of the scavenger are similar to, and fully occluded, the actions of LY382884. This suggests that KARs play a major role in restricting glutamate release, while glutamate transport processes and the extracellular matrix are developing.

Functional Maturation of CA1 Glutamatergic Synapses

Our data revealed dramatic differencies in the dynamic properties of the newborn (P3–P6) CA1 synapses, accounted for by different level of activation of the presynaptic KARs. Specifically, we found that tonic activation of KARs keeps the Pr low in a subset of synapses, and causes prominent short-term facilitation of transmission in response to high-frequency input. Intriguingly, another population of synapses expressed no facilitation, and they lack the endogenously active presynaptic KAR. These synapses resemble mature CA3-CA1 inputs, thus suggesting that the loss of the tonic activation of presynaptic KARs is linked to the functional maturation of these synapses.

The developmental loss of the endogenous activation of the presynaptic KAR could be due to alterations in the levels of ambient glutamate or its access to the receptor and/or may involve changes in the localization or affinity of the presynaptic KAR itself. Our data strongly suggests that the latter mechanism contributes to the developmental loss of the tonic inhibitory action of KARs on glutamate release. Namely, nonfacilitatory inputs were not sensitive to the low agonist concentrations that selectively activate high-affinity KARs (e.g., Lauri et al. [2001a], and Ruiz et al. [2005]) under conditions where significant depression was induced in the neonatal facilitatory inputs. Importantly, these differences persisted if the release probability was altered by an independent mechanism (e.g., changing the divalent cation concentrations), thus excluding the possibility that the pharmacological heterogeneity was due to baseline variation in the glutamate release probability. Therefore, our data supports the conclusion that the presynaptic KARs can rapidly and activity-dependently change from high to low affinity, thus being no longer activated by ambient glutamate under physiological conditions. The pattern of expression of high-affinity KARs results in heterogeneity in Pr and, consequently, short-term plasticity at the neonatal synapses, thus explaining the apparently contrasting reports on the dynamic properties of immature synapses (e.g., Bolshakov and Siegelbaum [1995], Gasparini et al. [2000], and Wasling et al. [2004]). Further, during development, the high-affinity presynaptic KARs are gradually lost because of activity-dependent maturation of the circuitry. Thus, at P14, the presynaptic KARs cannot be activated by ambient glutamate levels, although they can still be activated by a strong agonist to depress glutamate release.

Mechanism of Expression of Neonatal CA1 LTP

There is recent evidence showing that in a subset of newborn hippocampal CA1 synapses, the induction of LTP is associated with an increase in success rate and decrease in paired-pulse facilitation, thus suggesting increased Pr as an underlying mechanism (Palmer et al., 2004). This type of LTP is only seen during the first postnatal week, and thereafter, LTP in CA1 pyramidal cells is mainly accounted for by postsynaptic mechanisms. Consistently, we found that at facilitatory (e.g., low Pr) synapses, LTP induction was associated with a strong reduction in short-term plasticity, indicating an increase in Pr. In contrast, at nonfacilitatory synapses (e.g., high Pr), no changes in synaptic facilitation were seen in response to LTP induction. Thus, the pre- and postsynaptic mechanisms for expression of LTP coexist in the neonatal CA1, the first being restricted to the inputs with a low baseline Pr.

Our data suggest that a rapid change in the function of the presynaptic KAR is an important mechanism underlying the expression of the presynaptic forms of LTP at the neonatal CA1 synapses. This accounts for the loss of the tonic activation of the receptor after LTP induction. Because the tonic activation of the presynaptic KAR keeps Pr low in the naive neonatal input, the relief from this tonic suppression of transmission can also, at least in part, explain the potentiation in transmission caused by LTP. The molecular changes underlying this expression mechanism remain to be elucidated. A rapid reduction in affinity could be due to a direct modification of the receptor, such as phosphorylation.

Interestingly, in adolescent mossy fiber CA3 synapses, LTP induction results in downregulation of presynaptic KAR activity (Lauri et al., 2001a), and a developmental change in the functional role of KARs has been shown also at thalamocortical synapses (Kidd et al., 2002) and nociceptors (Lee et al., 2001). Considered together with the present study, this suggests that there is a critical role for KARs in the maturation of the synaptic connectivity. However, it should be noted that the KARs described in these previous studies are dynamically activated, their activation requiring either high-frequency afferent stimulation or pharmacological activation. In contrast, the tonically active KAR characterized in the present study represents a previously uncharacterized type of presynaptic KAR with different physiological implications.

Role of the Neonatal High-Affinity Presynaptic KAR in Information Transfer from CA3 to CA1

In the developing hippocampus, the spontaneous synchronous activity in area CA3 is transferred to the area CA1 where it is seen as short high-frequency (>20 Hz) barrages of EPSPs and IPSPs (Lamsa et al., 2000; Palva et al., 2000). This type of activity is the major mode of CA3-CA1 transmission during hippocampal development and is thought to be important for stabilization of the glutamatergic synaptic contacts (see Lauri et al. [2003]). Moreover, brief activity bursts, and their short-term synaptic dynamics, may critically determine the information processing power in the hippocampus (Lisman, 1997). The presynaptic KAR described here can tune the CA1 pyramidal neurons to respond to these "natural type" patterns of activity generated in CA3. It should be noted that in the newborn hippocampus, highly synchronous CA3 bursts at greater than 100Hz can occur as often as three to four times a minute, each lasting 200–500 ms (Lamsa et al., 2000; Palva et al., 2000). One of the physiological roles of the inhibitory presynaptic KAR characterized here may thus be to filter the continuous high-frequency glutamatergic bombardment to CA1 and provide a mechanism for the selection of postsynaptic target cells. In the absence of hyperpolarizing $GABA_A$R-mediated inhibition early in development, presynaptic KARs regulating glutamate release could thus provide effective spatiotemporal control for changes in synaptic weights; the task later in development carried out mainly by GABAergic inputs (Paulsen and Moser, 1998). The developmental loss of this KAR mechanism, driven by activity, would thus switch the properties of the CA3-CA1 glutamatergic input to favor a mature linear mode of transmission.

In summary, we have described a previously uncharacterized mechanism for synaptic maturation in the hippocampus, namely rapid activity-dependent alteration in endogenous activation of presynaptic KARs that regulate glutamatergic transmission in the neonatal CA1. This mechanism confers important, developmentally regulated, properties on transmission in the hippocampus. These properties are likely to be critical for information transfer from CA3-CA1 during early development and for the appropriate development of hippocampal circuitry.

Experimental Procedures

Acute hippocampal slices were prepared from the brain of neonatal (P3–P6) or young (P14–P16) rats by standard methods. Briefly, the brain was quickly dissected into ice-cold solution containing (mM): NaCl, 124; KCl, 3; $NaH2PO_4$, 1.25; $MgSO_4$, 10; $NaHCO_3$, 26; D-glucose, 10–15; $CaCl_2$, 1; saturated with 5% CO_2/95% O_2. A tissue block containing the hippocampi was dissected and glued into the stage of a vibratome (Vibratome Co., St. Louis, MO). 400–600 μm thick slices were cut transversally or sagittally in the above solution and stored at room temperature in a solution containing (mM): NaCl, 124; KCl, 3; $NaH2PO_4$, 1.25; $MgSO_4$, 4; $NaHCO_3$, 26; D-glucose, 10–15; $CaCl_2$, 2; 5% CO_2/95% O_2. The slices were used 1–4 hr after cutting, except for the experiments where pertussis toxin was used. For these experiments, after 30 min of recovery, the slices (400 μm; P3–P6) were washed with 1 ml of a modified slice culture medium, consisting of 75% minimal essential medium supplemented with HEPES and bicarbonate (Gibco Laboratories, Grand Island, NY), 25% Eagles balanced salt solution (Gibco), 2 mM L-glutamine, and 6.5 mg/ml glucose. The slices were then placed into Millicell-CM membranes (Millipore, Bedford) in 6-well culture trays with 1 ml of the above medium with of without pertussis toxin (5 mg/ml) and transferred into a CO_2 incubator (35ºC under 5% CO_2 in air) for 12–15 hr.

For electrophysiological recordings, the slices were placed in a submerged recording chamber and perfused with extracellular solution containing (mM): NaCl, 124; KCl, 3; $NaH2PO_4$, 1.25; $MgSO_4$, 1; $NaHCO_3$, 26; D-glucose, 10–15; $CaCl_2$, 2; bubbled with 5% CO_2/95% O_2, at room temperature (evoked EPSCs) or at 32ºC (all recordings of mEPSCs). Whole-cell recordings were made from CA1 pyramidal cells, with patch electrodes (2–5 MΩ) filled with a solution containing (in mM): $CsMeSO_4$, 130; HEPES, 10; EGTA, 0.5; Mg-ATP, 4; Na-GTP, 0.3; QX-314, 5; NaCl, 8; 285 mOsm (pH 7.2). Guanosine 5′-[β-thio]diphosphate (GDPbetaS, Sigma) was included in the filling solution at concentration of 0.3 mM. $GABA_B$-mediated currents were recorded with a potassium-based filling solution. For recording of NMDA-R-mediated responses, 10 mM BAPTA was included in the electrode solution, and the cells were voltage clamped at +40 mV. Perforated patch clamp recordings were made with high-resistance electrodes (12–14 MΩ) to prevent spontaneous rupture of the gigaseal. The tip of the electrodes were filled with a solution containing (in mM) K-gluconate, 135; HEPES, 10; EGTA, 5; Mg-ATP, 4; Na-GTP, 0.5; KCl, 2; $Ca(OH)_2$, 2; and 290 mOsm (pH 7.2) and then backfilled with the same solution without ATP and GTP but containing amphotericin (500 μg/ml, Sigma) or gramicidin (100 μg/ml, Sigma). Recordings were started when an access resistance lower than 150 MΩ was reached.

A bipolar electrode was used for afferent stimulation. AMPA-R-mediated EPSCs were evoked by stimulation of Schaffer collateral-commissural fibers and recorded from CA1 pyramidal neurons in the presence of antagonists of $GABA_A$ and NMDA receptors (picrotoxin [PiTX] [100 μM] and D-AP5 [50 μM], respectively). NMDA-R-mediated responses were pharmacologically isolated with PiTX and GYKI53655 (50 μM). Baseline stimulation frequency was 1/30 s, except for collection of the trains at 50 Hz, when the baseline stimulation frequency was 1/60 s. For train stimulation, at least ten trials were averaged. LTP was induced by pairing postsynaptic depolarization (−10 mV) to five to ten brief high-frequency trains (five pulses at 50 Hz) of afferent activation in the presence of PiTX. In the LTP experiments, two pathways were stimulated, one input was paired, and the other was used as a control pathway. If stimulation of only

one input evoked synaptic responses, that input was used for pairing. Experiments in which no LTP was induced were excluded from the analysis. Control pathway was excluded if heterosynaptic depression or potentiation was induced. LTP230D (Anderson and Collingridge, 2001) (http://www.ltp-program.com/) and WinEDR 2.3.3 (Strathclyde Electrophysiology Software, UK) software were used for data acquisition. All compounds were from Tocris Cookson (Bristol, UK), except for pertussis toxin and glutamic-pyruvic transaminase that were from Sigma and LY382884 that was a gift from Eli Lilly, Co.

The amplitude of the evoked synaptic responses was measured as the peak relative to the average baseline level 2–8 ms before the stimulation. A response was considered a failure if the amplitude of the current was less than the baseline noise level (2–3 pA) and was verified visually. Spontaneous miniature EPSCs were detected and analyzed with the Mini Analysis Program 5.6.6. (Synaptosoft). The amplitude detection threshold was set between 4–6 pA (two times the baseline RMS noise level), and the detected events were verified visually. The number of spontaneous events was calculated in 120 or 180 s bins and normalized to the average baseline value before drug application. In addition, the average number of events/min was calculated from a 5–10 min period before, during, and after washout of the drug. Neurons that exhibited a very low frequency of mEPSCs (less than one event/2 min; about 50% of recorded cells at P4) were excluded from the analysis. Data are expressed as percent control (i.e., 100% = no change). All the pooled data are given as mean ± SEM for the number of cells indicated. For statistical analysis, Student's two-tailed t test and Pearson's correlation test were used. $p < 0.05$ was considered as statistically significant.

Supplemental Data

The Supplemental Data for this article can be found online at http://www.neuron.org/cgi/content/full/50/3/415/DC1/.

Acknowledgments

Supported by Academy of Finland (T.T. and S.E.L.), The Sigrid Juselius Foundation (T.T. and S.E.L.), The University of Helsinki research funds (S.E.L.), the Medical Research Council (G.L.C.), National Institute of Neurological Disorders and Stroke Intramural Program (J.T.R.I.), and The Wellcome Trust (J.T.R.I.). We thank Eli Lilly & Co. for providing LY382884.

Received: April 14, 2005
Revised: November 30, 2005
Accepted: March 13, 2006
Published: May 3, 2006

References

Anderson, W.W., and Collingridge, G.L. (2001). The LTP Program: a data acquisition program for on-line analysis of long-term potentiation and other synaptic events. J. Neurosci. Methods *108*, 71–83.

Bahn, S., Volk, B., and Wisden, W. (1994). Kainate receptor gene expression in the developing rat brain. J. Neurosci. *14*, 5525–5547.

Bolshakov, V.Y., and Siegelbaum, S.A. (1995). Regulation of hippocampal transmitter release during development and long-term potentiation. Science *269*, 1730–1734.

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.

Chittajallu, R., Vignes, M., Dev, K.K., Barnes, J.M., Collingridge, G.L., and Henley, J.M. (1996). Regulation of glutamate release by presynaptic kainate receptors in the hippocampus. Nature *379*, 78–81.

Choi, S., Klingauf, J., and Tsien, R.W. (2000). Postfusional regulation of cleft glutamate concentration during LTP at 'silent synapses'. Nat. Neurosci. *3*, 330–336.

Clarke, V.R.J., and Collingridge, G.L. (2002). Characterisation of the effects of ATPA, a GLUK5 receptor selective agonist, on excitatory synaptic transmission in area CA1 of rat hippocampal slices. Neuropharmacology *42*, 889–902.

Contractor, A., Swanson, G., and Heinemann, S.F. (2001). Kainate receptors are involved in short- and long-term plasticity at mossy fibre synapses in the hippocampus. Neuron *29*, 209–216.

Danbolt, N.C. (2001). Glutamate uptake. Prog. Neurobiol. *65*, 1–105.

Davies, C.H., Pozza, M.F., and Collingridge, G.L. (1993). CGP 55845A: a potent antagonist of GABAB receptors in the CA1 region of rat hippocampus. Neuropharmacology *32*, 1071–1073.

Delaney, A.J., and Jahr, C.E. (2002). Kainate receptors differentially regulate release at two parallel fiber synapses. Neuron *36*, 475–482.

Diamond, J.S. (2005). Deriving the glutamate clearance time course from transporter currents in CA1 hippocampal astrocytes: transmitter uptake gets faster during development. J. Neurosci. *25*, 2906–2916.

Durand, G.M., Kovalchuk, Y., and Konnerth, A. (1996). Long-term potentiation and functional synapse induction in developing hippocampus. Nature *381*, 71–75.

Fiala, J.C., Feinberg, M., Popov, V., and Harris, K.M. (1998). Synaptogenesis via dendritic filopodia in developing hippocampal area CA1. J. Neurosci. *18*, 8900–8911.

Fitzjohn, S.M., Bortolotto, Z.A., Palmer, M.J., Doherty, A.J., Ornstein, P.L., Schoepp, D.D., Kingston, A.E., Lodge, D., and Collingridge, G.L. (1998). The potent mGlu receptor antagonist LY341495 identifies roles for both cloned and novel mGlu receptors in hippocampal synaptic plasticity. Neuropharmacology *37*, 1445–1458.

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.

Frerking, M., Schmitz, D., Zhou, Q., Johansen, J., and Nicoll, R.A. (2001). Kainate receptors depress excitatory synaptic transmission at CA3-CA1 synapses in the hippocampus via a direct presynaptic action. J. Neurosci. *21*, 2958–2966.

Gasparini, S., Saviane, C., Voronin, L.L., and Cherubini, E. (2000). Silent synapses in the developing hippocampus: lack of functional AMPA receptors or low probability of glutamate release? Proc. Natl. Acad. Sci. USA *97*, 9741–9746.

Hanse, E., and Gustafsson, B. (2001). Factors explaining heterogenity in short-term synaptic dynamics of hippocampal glutamatergic synapses in the neonatal rat. J. Physiol. *537*, 141–149.

Hsia, A.Y., Malenka, R.C., and Nicoll, R.A. (1998). Development of excitatory circuitry in the hippocampus. J. Neurophysiol. *79*, 2013–2024.

Isaac, J., Nicoll, R.A., and Malenka, R. (1995). Evidence for silent synapses: Implications for the expression of LTP. Neuron *1*, 427–434.

Isaac, J.T.R., Mellor, J., Hurtado, D., and Roche, K.W. (2004). Kainate receptor trafficking: physiological roles and molecular mechanisms. Pharmacol. Ther. *104*, 163–172.

Kamiya, H., and Ozawa, S. (1998). Kainate receptor-mediated inhibition of presynaptic Ca^{2+} influx and EPSP in area CA1 of the rat hippocampus. J. Physiol. *509*, 833–845.

Kidd, F.L., Coumis, U., Collingridge, G.L., Crabtree, J.W., and Isaac, J.T.R. (2002). A presynaptic kainate receptor is involved in regulating the dynamic properties of thalamocortical synapses during development. Neuron *34*, 635–646.

Kullmann, D.M. (2001). Presynaptic kainate receptors in the hippocampus: slowly emerging from obscurity. Neuron *32*, 561–564.

Lahtinen, H., Palva, J.M., Sumanen, S., Voipio, J., Kaila, K., and Taira, T. (2002). Postnatal development of rat hippocampal gamma rhythm in vivo. J. Neurophysiol. *88*, 1469–1474.

Lamsa, K., Palva, J.M., Ruusuvuori, E., Kaila, K., and Taira, T. (2000). Synaptic GABA(A) activation inhibits AMPA-kainate receptor-mediated bursting in the newborn (P0–P2) rat hippocampus. J. Neurophysiol. *83*, 359–366.

Lauri, S.E., Bortolotto, Z.A., Bleakman, D., Ornstein, P.L., Lodge, D., Isaac, J.T.R., and Collingridge, G.L. (2001a). A critical role of a facilitatory presynaptic kainate receptor in mossy fiber LTP. Neuron *32*, 697–709.

Lauri, S.E., Delany, C., Clarke, V.R.J., Bortolotto, Z.A., Ornstein, P.L., Isaac, J.T.R., and Collingridge, G.L. (2001b). Synaptic activation of

a presynaptic kainate receptor facilitates AMPA receptor-mediated synaptic transmission at hippocampal mossy fibre synapses. Neuropharmacology *41*, 907–915.

Lauri, S.E., Lamsa, K., Pavlov, I., Riekki, R., Johnson, B., Molnar, E., Rauvala, H., and Taira, T. (2003). Activity blockade induces formation of functional synapses in the newborn rat hippocampus. Mol. Cell. Neurosci. *22*, 107–117.

Lauri, S.E., Clarke, V.R.J., and Collingridge, G.L. (2004). Delineation of the physiological role of kainate receptors by the use of subtype selective ligands. In Molecular Neuropharmacology: Strategies and Methods, A. Schousboe and H. Bräuner-Osborne, eds. (Totowa, NJ: The Humana Press, Inc.), pp. 27–46.

Lauri, S.E., Segerstråle, M., Vesikansa, A., Maingret, F., Collingridge, G.L., Isaac, J.T.R., and Taira, T. (2005). Endogenous activation of kainate receptors inhibits glutamatergic transmission and modulates network activity in the developing hippocampus. J. Neurosci., in press.

Lee, C.J., Kong, H., Manzini, M.C., Albuquerque, C., Chao, M.V., and MacDermott, A.B. (2001). Kainate receptors expressed by a subpopulation of developing nociceptors rapidly switch from high to low Ca^{2+} permeability. J. Neurosci. *21*, 4572–4581.

Lerma, J. (2003). Roles and rules of kainate receptors in synaptic transmission. Nat. Rev. Neurosci. *4*, 481–495.

Lisman, J.E. (1997). Bursts as a unit of neural information: making unreliable synapses reliable. Trends Neurosci. *20*, 38–43.

Maggi, L., Sola, E., Minneci, F., Le Magueresse, C., Changeux, J.P., and Cherubini, E. (2004). Persistent decrease in synaptic efficacy induced by nicotine at Schaffer collateral-CA1 synapses in the immature rat hippocampus. J. Physiol. *559*, 863–874.

Melyan, Z., Wheal, H.V., and Lancaster, B. (2002). Metabotropic-mediated kainate receptor regulation of IsAHP and excitability in pyramidal cells. Neuron *34*, 107–114.

Min, M.-Y., Rusakov, D.A., and Kullmann, D.M. (1998). Activation of AMPA, kainite, and metabotropic receptors at hippocampal mossy fibre synapses: role of glutamate diffusion. Neuron *21*, 561–570.

Mozhayeva, M.G., Sara, Y., Liu, X., and Kavalali, E.T. (2002). Development of vesicle pools during maturation of hippocampal synapses. J. Neurosci. *22*, 654–665.

Overstreet, L.S., Pasternak, J.F., Colley, P.A., Slater, N.T., and Trommer, B.L. (1997). Metabotropic glutamate receptor mediated long-term depression in developing hippocampus. Neuropharmacology *36*, 831–844.

Palmer, M., Isaac, J.T.R., and Collingridge, G. (2004). Multiple, developmentally regulated expression mechanisms of long-term potentiation at CA1 synapses. J. Neurosci. *24*, 4903–4911.

Palva, J.M., Lamsa, K., Lauri, S.E., Rauvala, H., Kaila, K., and Taira, T. (2000). Fast network oscillations in the newborn rat hippocampus in vitro. J. Neurosci. *20*, 1170–1178.

Paulsen, O., and Moser, E.I. (1998). A model of hippocampal memory encoding and retrieval: GABAergic control of synaptic plasticity. Trends Neurosci. *21*, 273–278.

Renger, J.J., Egles, C., and Liu, G. (2001). A developmental switch in neurotransmitter flux enhances synaptic efficacy by affecting AMPA receptor activation. Neuron *29*, 469–484.

Ritter, L.M., Vazquez, D.M., and Meador-Woodruff, J.H. (2002). Ontogeny of ionotropic glutamate receptor subunit expression in the rat hippocampus. Brain Res. Dev. Brain Res. *139*, 227–236.

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

Rozas, J.L., Paternain, A.V., and Lerma, J. (2003). Noncanonical signaling by ionotropic kainate receptors. Neuron *39*, 543–553.

Ruiz, A., Sachidhanandam, S., Utvik, J.K., Coussen, F., and Mulle, C. (2005). Distinct subunits in heteromeric kainate receptors mediate ionotropic and metabotropic function at hippocampal mossy fiber synapses. J. Neurosci. *25*, 11710–11718.

Safiulina, V.F., Kasyanov, A.M., Giniatullin, R., and Cherubini, E. (2005). Adenosine down-regulates giant depolarizing potentials in the developing rat hippocampus by exerting a negative control on glutamatergic inputs. J. Neurophysiol. *94*, 2797–2804.

Scanziani, M., Salin, P.A., Vogt, K.E., Malenka, R.C., and Nicoll, R.A. (1997). Use-dependent increases in glutamate concentration activate presynaptic metabotropic glutamate receptors. Nature *385*, 630–634.

Schmitz, D., Mellor, J., and Nicoll, R.A. (2001). Presynaptic kainate receptor mediation of frequency facilitation at hippocampal mossy fiber synapses. Science *291*, 1972–1976.

Shimamoto, K., Lebrun, B., Yasuda-Kamatani, Y., Sakaitani, M., Shigeri, Y., Yumoto, N., and Nakajima, T. (1998). DL-threo-beta-benzyloxyaspartate, a potent blocker of excitatory amino acid transporters. Mol. Pharmacol. *53*, 195–201.

Sykova, E., Mazel, T., Vargova, L., Vorisek, I., and Prokopova-Kubinova, S. (2000). Extracellular space diffusion and pathological states. Prog. Brain Res. *125*, 155–178.

Tyzio, R., Represa, A., Jorquera, I., Ben-Ari, Y., Gozlan, H., and Aniksztejn, L. (1999). The establishment of GABAergic and glutamatergic synapses on CA1 pyramidal neurons is sequential and correlates with the development of the apical dendrite. J. Neurosci. *19*, 10372–10382.

Vignes, M., Clarke, V.R.J., 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.

Wasling, P., Hanse, E., and Gustafsson, B. (2004). Developmental changes in release properties of the CA3-CA1 glutamate dynapse in rat hippocampus. J. Neurophysiol. *92*, 2714–2724.

Xiao, M.-Y., Wasling, P., Hanse, E., and Gustafsson, B. (2004). Creation of AMPA-silent synapses in the neonatal hippocampus. Nat. Neurosci. *7*, 236–243.

Zakharenko, S.S., Zablow, L., and Siegelbaum, S.A. (2002). Altered presynaptic vesicle release and cycling during mGluR-dependent LTD. Neuron *35*, 1099–1110.

Zhang, J., Wang, H., Ye, C., Ge, W., Chen, Y., Jinag, Z., Wu, C., Poo, M., and Duan, S. (2003). ATP released by astrocytes mediates glutamatergic activity-dependent heterosynaptic suppression. Neuron *40*, 971–982.