GluR5 kainate receptor activation in interneurons increases tonic inhibition of pyramidal cells

Rosa Cossart, Monique Esclapez, June C. Hirsch, Christophe Bernard and Yehezkel Ben-Ari

Epilepsie et Ischémie Cérébrale, INSERM U29, Hôpital de Port Royal, 123 Bld de Port Royal, 75014 Paris, France.
Correspondence should be addressed to Y.B-A. (ben-ari@cochin.inserm.fr)

We studied the modulation of GABAergic inhibition by glutamate and kainate acting on GluR5-containing kainate receptors in the CA1 hippocampal region. Glutamate, kainate or ATPA, a selective agonist of GluR5-containing receptors, generates an inward current in inhibitory interneurons and cause repetitive action potential firing. This results in a massive increase of tonic GABAergic inhibition in the somata and apical dendrites of pyramidal neurons. These effects are prevented by the GluR5 antagonist LY 293558. Electrical stimulation of excitatory afferents generates kainate receptor-mediated excitatory postsynaptic currents (EPSCs) and action potentials in identified interneurons that project to the dendrites and somata of pyramidal neurons. Therefore glutamate acting on kainate receptors containing the GluR5 subunit may provide a protective mechanism against hyperexcitability.

In the cerebral cortex, extrinsic and intrinsic excitatory glutamatergic pathways terminate on principal glutamatergic neurons and local inhibitory GABAergic interneurons. Activation of these pathways leads to a sequence of excitatory and inhibitory responses in the principal neurons. The output of these neurons is thus tightly controlled by the interaction between excitatory and inhibitory synaptic inputs, both to the principal neurons themselves and to the local inhibitory interneurons. Fast glutamatergic neurotransmission is mediated by three classes of ionotropic receptors, namely NMDA, AMPA and kainate receptors. Fast GABAergic transmission is mediated by ionotropic $GABA_A$ receptors. Classically, the interplay between glutamatergic excitation and GABAergic inhibition during normal synaptic neurotransmission is believed to involve mainly AMPA, NMDA and $GABA_A$ receptors.

Although kainate receptors that include the GluR5 and GluR6 subunits are widely expressed throughout the brain in excitatory and inhibitory neurons at both the protein[1–3] and the mRNA[4,5] levels, their functional role remains elusive[6]. Activation of kainate receptors in the hippocampus generates a seizure and brain-damage syndrome that provides an animal model of human temporal-lobe epilepsy[7,8]. The CA3 region of the hippocampus has a particularly low threshold for the generation of seizures by kainate[7–11]. Studies done in hippocampal slices have reported two effects of exogenous application of kainate on pyramidal neurons that could contribute to kainate-induced epileptiform activity. First, kainate has a direct excitatory action on CA3 pyramidal cells[7–11], presumably due to the activation of GluR6-containing receptors at the mossy-fiber synapse[12–17]. Second, kainate causes a reduction in GABAergic inhibition (that is, disinhibition) in CA1 pyramidal neurons[18], presumably via the activation of GluR5-containing receptors on GABAergic terminals leading to a decrease of the probability of GABA release[19,20].

However the net effect of kainate receptor activation in the hippocampal network is difficult to predict. Inhibitory interneurons exert a central role in modulating the excitability of pyramidal cells and hence the behaviour of the hippocampal network[21]. They also express a high level of kainate receptor protein[1] and mRNA[4], especially that of the GluR5 subunit[4]. It is therefore essential to determine the direct consequence of kainate receptor activation on inhibitory interneurons. It can be proposed that the activation of kainate receptors on interneurons may excite them, leading to a paradoxical enhanced release of GABA onto pyramidal neurons. This could affect the integration of excitatory and inhibitory signals in pyramidal cells and reduce or overcome the disinhibitory effects[18–20].

To test the hypothesis that glutamate activation of kainate receptors on interneurons increases GABAergic inhibition in pyramidal cells, we made patch-clamp recordings from interneurons and from the somata and dendrites of pyramidal neurons in the CA1 area of the hippocampus. We report that glutamate, kainate or ATPA, a selective agonist of GluR5-containing receptors[20], produces a massive and sustained excitation of identified interneurons, resulting in a large increase of the tonic inhibitory input to the somata and dendrites of CA1 pyramidal neurons. Moreover, electrical stimulation of excitatory afferents generates an EPSP in interneurons that is mediated by kainate receptors containing the GluR5 subunit; this represents the first evidence for kainate receptor-mediated synaptic responses in interneurons. We suggest that in the hippocampus, there are at least two kainate receptor-mediated systems. The first involves GluR6-containing synapses on CA3 pyramidal neurons and mediates the epileptogenic effects of kainate[7,8]. The second system involves GluR5-containing kainate receptors on inhibitory interneurons, and activation of

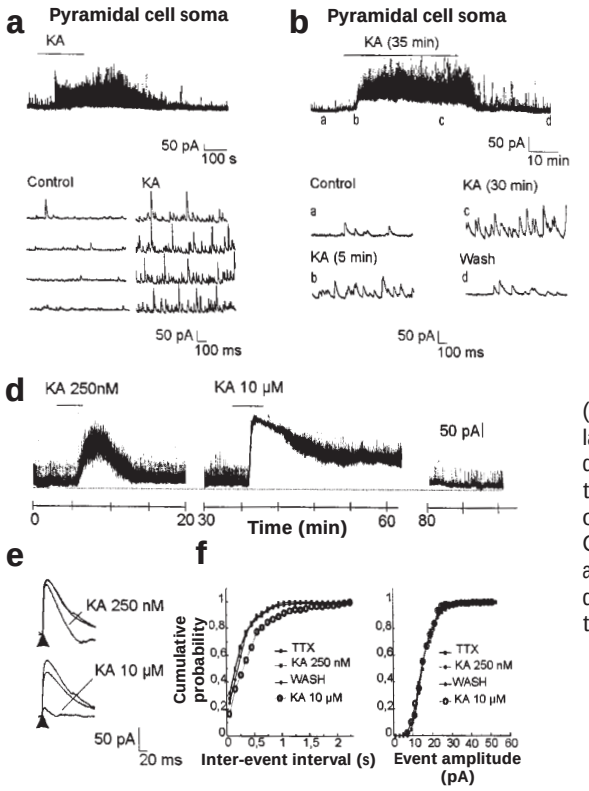


Fig. 1. Kainate increases tonic inhibition in CA1 pyramidal neurons. **(a–d)**. Bath application of kainate in the presence of GYKI 53655 (30 µM) and D-APV (50 µM) reversibly increased the frequency of spontaneous IPSCs (Vh +10 mV); traces below illustrate sIPSCs on an expanded time base. (a) A brief application of kainate (250 nM) increased the frequency of sIPSCs. (b) The increase persisted during a long application (35 min). (c) Kainate (250 nM) also increased the frequency of sIPSCs in apical dendrites. (d) Increasing the concentration of kainate from 250 nM to 10 µM caused a larger shift of the baseline current due to the summation of the high frequency sIPSCs. **(e)** In the same cell, 10 µM kainate but not 250 nM reduced the amplitude of the evoked monosynaptic IPSCs (superimposed averages of five responses recorded with, without and after wash-out of kainate). **(f)** Cumulative probability plots of miniature (TTX-insensitive) IPSCs intervals and amplitudes. Kainate at 10 µM but not 250 nM caused a significant decrease of the inter-event distribution; neither dose modified the amplitude of the mIPSCs.

these receptors enhances tonic inhibition. The latter system may act as an endogenous regulatory mechanism to prevent epileptogenesis.

Results

KAINATE INCREASES TONIC INHIBITION IN PYRAMIDAL CELLS

We investigated the effect of exogenous kainate on currents recorded in pyramidal neurons of area CA1. In most subsequent experiments, we used GYKI 53655 (30 µM) and D-APV (50 µM) to block AMPA and NMDA receptors respectively. In the presence of these inhibitors, application of kainate (250 nM) dramatically increased the frequency of spontaneous inhibitory postsynaptic currents (sIPSCs) recorded in the soma of CA1 pyramidal neurons (from 4.74 ± 0.91 Hz to 25.87 ± 4.34 Hz, Fig. 1a). The increase was observed with either brief (3–5 min, Fig. 1a) or long (20–40 min, Fig. 1b) applications of kainate (711 ± 95%, $p < 0.0001$, $n = 10$ and 795 ± 88%, $p < 0.0001$, $n = 4$, respectively). The effect persisted throughout the application of kainate and was fully reversed after the drug was washed out (Fig. 1a and b). The frequency values are underestimates, because it was impossible to resolve events separated by intervals of less than 5 ms. The total charge crossing the membrane also increased during the application of kainate (725 ± 133% of control, $p < 0.0001$, $n = 10$). Comparable observations were made in the apical dendrites ($n = 8$, *post hoc* identification of the recording sites located 268 ± 54 µm from the soma). The frequency of sIPSCs in the dendrites (2.35 ± 0.65 Hz, $n = 8$) was dramatically increased by brief (816 ± 216% of control, $p < 0.0001$, $n = 5$, Fig. 1c) or long ($n = 3$, not shown) applications of kainate.

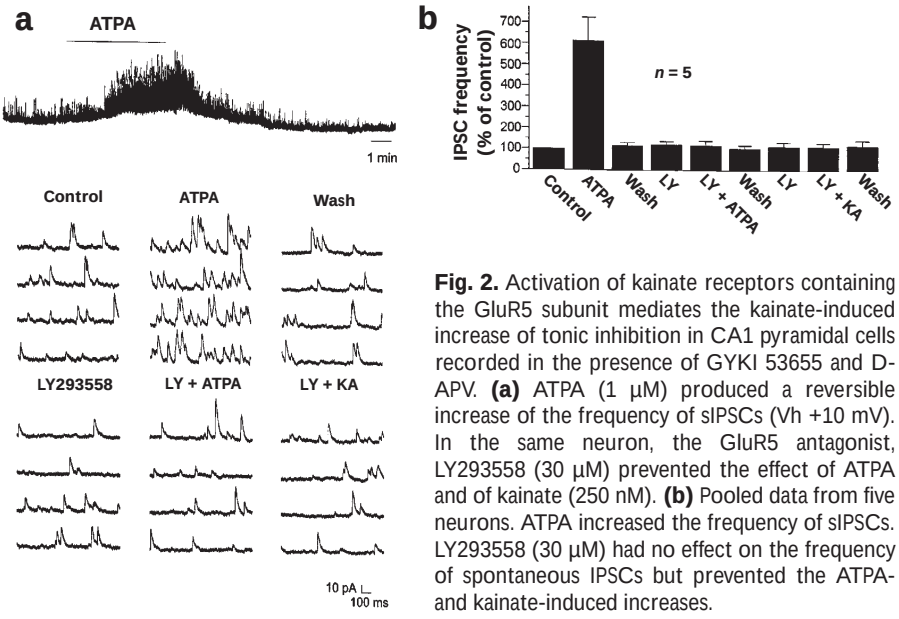


Fig. 2. Activation of kainate receptors containing the GluR5 subunit mediates the kainate-induced increase of tonic inhibition in CA1 pyramidal cells recorded in the presence of GYKI 53655 and D-APV. **(a)** ATPA (1 µM) produced a reversible increase of the frequency of sIPSCs (Vh +10 mV). In the same neuron, the GluR5 antagonist, LY293558 (30 µM) prevented the effect of ATPA and of kainate (250 nM). **(b)** Pooled data from five neurons. ATPA increased the frequency of sIPSCs. LY293558 (30 µM) had no effect on the frequency of spontaneous IPSCs but prevented the ATPA- and kainate-induced increases.

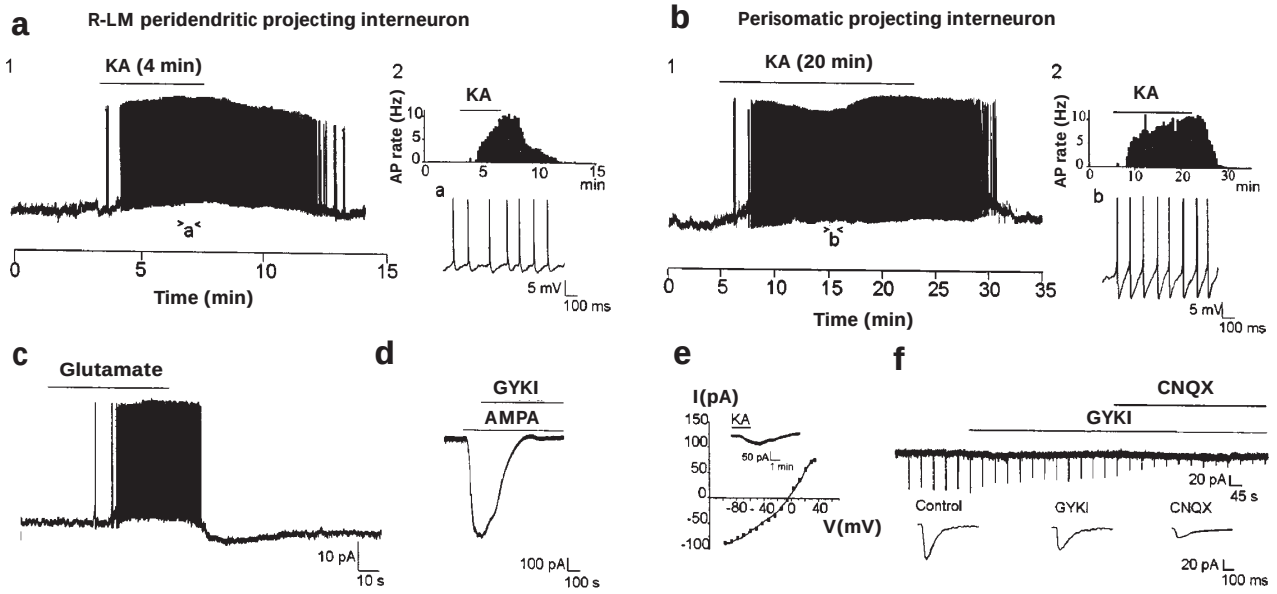


Fig. 3. Kainate and glutamate applied in the presence of GYKI and D-APV induce high frequency firing in multiple types of interneurons. **(a)** 1, Kainate (250 nM) caused a reversible depolarization of the resting membrane potential and repetitive action potential firing. 2, Upper trace: frequency versus time plot of action potentials (bin width, 10 s). Lower trace shows on an expanded time scale the continuous discharge of action potentials during the application of kainate. Vm, -64 mV. **(b)** 1, The membrane potential remained depolarized and the interneuron fired repetitively as long as kainate was applied (20 min). 2, Upper trace: frequency versus time plot of action potentials (bin width 10 s). Lower trace shows the action potentials on an expanded time scale. In (a) and (b), the amplitude of the action potentials is attenuated because of the slow sampling frequency (3 kHz). **(c)** Glutamate (100 µM) applied in the presence of D-APV and GYKI caused a reversible depolarization of the resting membrane potential and repetitive action potential firing. **(d)** AMPA (10 µM) applied in the presence of TTX, and D-APV evoked a large inward current (Vh, –60 mV) that was fully suppressed by GYKI. **(e)** Linear current–voltage curve induced by kainate (250 nM). Voltage-clamp recordings in the presence of TTX, GYKI and D-APV. The inset shows the kainate-induced current at a holding potential of –60 mV. **(f)** Puff application of 3 mM glutamate (100 kPa for 10 ms) to the surface of s. radiatum about 80 µm away from the soma of the recorded interneuron. In the presence of TTX and D-APV, glutamate evokes an inward current that is partially blocked by GYKI and further reduced by CNQX (100 µM). Averages of five consecutive responses are shown at a faster time scale below the continuous record. (a) Schaffer-associated interneuron illustrated in Fig. 6e. (b) Putative axo-axonic cell illustrated in Fig. 6c. (c) S. radiatum interneuron. (d) Bistratified interneuron in s. oriens. (e, f) Schaffer-associated interneurons.

These effects were not caused by activation of AMPA receptors, because the increase of sIPSCs frequency was the same with or without GYKI 53655 and D-APV (645 ± 112%, $p < 0.0001$, $n = 5$, not shown). The small current induced by 250 nM kainate (Fig. 1a and d) was entirely due to the summation of sIPSCs occurring at high frequency, as this current was suppressed by bicuculline applied in the presence of TTX (1 µM, not shown).

The increased frequency of sIPSCs in the presence of kainate could be physiologically relevant, because it is mimicked by glutamate. When glutamate (100 µM) was applied in the presence of GYKI and APV, it too increased the frequency of sIPSCs (369 ± 22% of control, $p < 0.0001$, $n = 4$, not shown). Therefore, the activation by glutamate of kainate receptors located on GABAergic interneurons enhances tonic inhibition in both the apical dendrites and the somata of CA1 pyramidal cells.

We then determined whether kainate (250 nM) could have other pre- or postsynaptic effects on $GABA_A$-receptor mediated synaptic currents in pyramidal neurons. Kainate did not affect the amplitude of monosynaptically evoked IPSCs (95 ± 8% of control, $p > 0.05$, $n = 10$, Fig. 1e), the frequency (2.34 ± 0.43 Hz before and 2.31 ± 0.33 Hz after kainate, 95 ± 9% of control, $p > 0.05$, $n = 6$, Fig. 1f) or the amplitude (Fig. 1f) of miniature IPSCs recorded in the presence of TTX (1 µM). Because it has been shown previously that kainate does not interact with postsynaptic $GABA_A$ receptors[18], we conclude that at low concentrations (250 nM), kainate increases spontaneous GABAergic inhibition without affecting other features of $GABA_A$ receptor-mediated inhibition (but see ref. 22 for effects on $GABA_B$ receptor-mediated inhibition).

Earlier studies using higher concentrations of kainate (3–20 µM) reported that kainate reduced the frequency of miniature IPSCs[19] and decreased the amplitude of evoked monosynaptic IPSPs[18–20], suggesting that kainate may cause disinhibition of pyramidal neurons. Consistent with these studies, we observed that kainate (10 µM) produced a small reduction of the frequency (but not of the amplitude) of miniature IPSCs (77 ± 8% of control, $p < 0.001$, $n = 8$; Fig. 1f) and a larger decrease of the amplitude of monosynaptic IPSCs (29 ± 9% of control, $p < 0.0001$, $n = 6$, Fig. 1e). The large sustained current, recorded at the reversal potential of glutamatergic currents (around +10 mV, see Methods) was associated with a 30-fold increase of the total charge crossing the membrane (2967 ± 967% of control, $p < 0.0001$, $n = 6$, Fig. 1d). This is entirely due to the activation of $GABA_A$ receptors because it was blocked by the addition of bicuculline in the presence of TTX (3 ± 3 pA, $n = 4$, not shown). Thus despite its presynaptic effects, the net effect of 10 µM kainate was a massive increase of tonic inhibition.

Therefore in contrast to earlier conclusions[18–20], our obser-

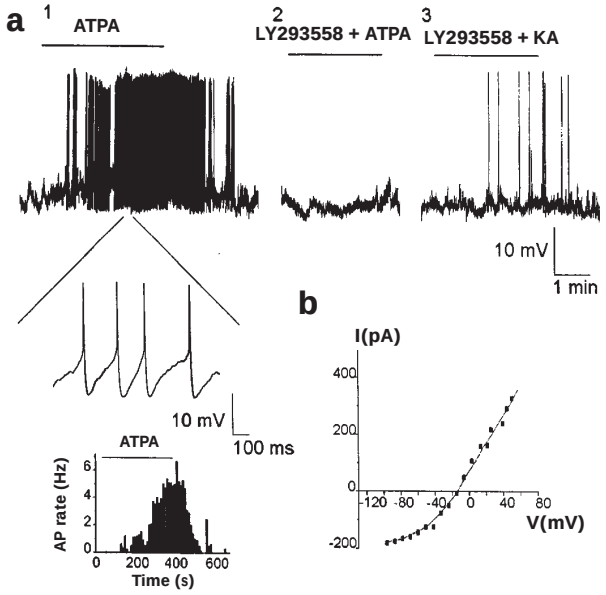


Fig. 4. The excitatory effects of kainate on interneurons are mediated primarily by GluR5-containing receptors. **(a)** Current clamp recording (GYKI, D-APV). 1, Upper trace, ATPA (1 μM) depolarized the membrane potential and induced repetitive action potential firing. Middle trace, action potentials on an expanded time scale (their amplitude is attenuated due to the low sampling frequency, 3 kHz). Lower trace, frequency versus time plot of action potentials (bin width, 10s). 2, LY 293558 (30 μM) prevented the ATPA-induced depolarization. 3, Kainate (250 nM) in the presence of LY293558 caused only small depolarizing shifts of the membrane potential and occasional firing. Vm, –57 mV. **(b)** Linear current–voltage curve of the ATPA-induced current. Voltage-clamp recording in the presence of TTX, GYKI and D-APV. (a) Bistratified interneuron illustrated in Fig. 6a2. (b) O-LM interneuron illustrated in Fig. 6a3.

vations suggest that the overall effect of high concentrations of kainate is to enhance the inhibitory drive on principal neurons, probably through the sustained excitation of CA1 interneurons. Interneurons in the stratum (s.) oriens of CA1 express high levels of glutamate receptors containing the GluR5 subunit[4]; we therefore tested the effect of selectively activating GluR5-containing receptors on the inhibitory drive on CA1 pyramidal cells.

GluR5-mediated increase of tonic inhibition

When AMPA and NMDA receptors were blocked by GYKI and APV respectively, the GluR5 agonist ATPA (1 μM) increased the frequency of sIPSCs in pyramidal neurons (613 ± 112% of control, $p < 0.0001$, $n = 5$, Fig. 2). The effect persisted during long application (20 minutes) of ATPA ($n = 4$, not shown) and was reversible (114 ± 13% of control, $p > 0.05$, $n = 5$, Fig. 2). The amplitude of the evoked monosynaptic IPSC was not affected by ATPA (101 ± 13% of control, $p > 0.05$, $n = 5$, not shown). The GluR5 antagonist LY293558 (30 μM) did not alter the baseline frequency of sIPSCs (121 ± 16% of control, $p > 0.05$, $n = 5$, Fig. 2), but fully prevented the ATPA-induced increase in frequency (117 ± 18% of control, $p > 0.05$, $n = 5$, Fig. 2). LY 293558 also blocked the increase of tonic inhibition by kainate (250 nM) (120 ± 18% of control, $p > 0.05$, $n = 5$, Fig. 2). Therefore, the increase of the inhibitory drive in pyramidal neurons is mediated by the direct activation of kainate receptors containing the GluR5 subunit.

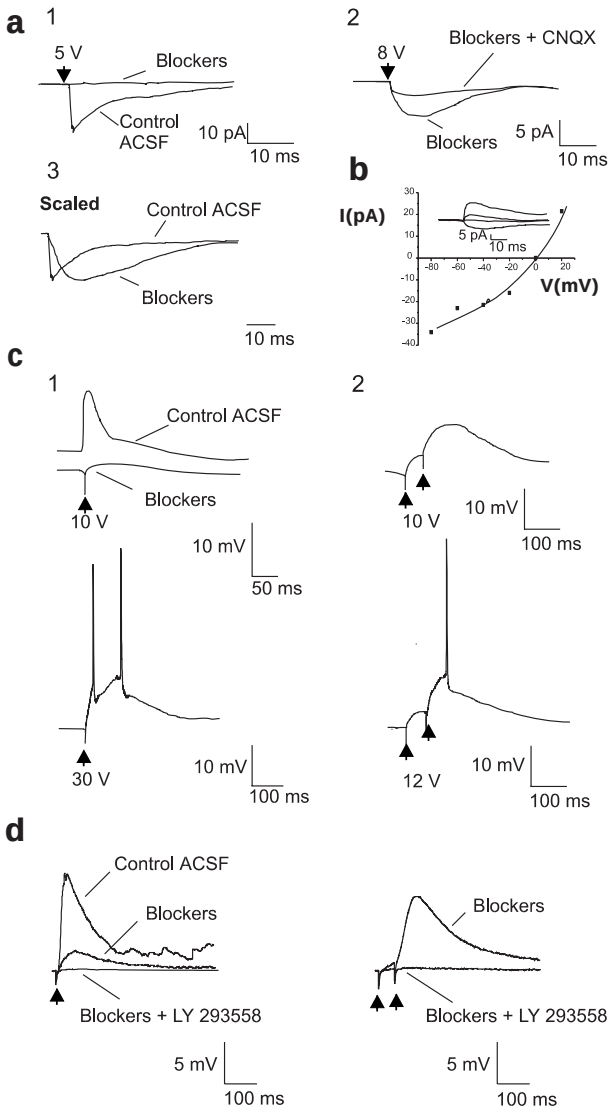
Excitatory effects of kainate on interneurons

To test the hypothesis that these receptors are located on interneurons, we recorded from morphologically identified interneurons in the presence of AMPA and NMDA receptor blockers. Under these conditions, the application of kainate (250 nM) induced a depolarization (11.6 ± 3.4 mV, $n = 14$, $p < 0.0001$, Fig. 3a) and a regular firing of action potentials (frequency: 8.2 ± 2.4 Hz, $n = 14$, Fig. 3a, Table 1). The effect persisted with little change during long-lasting applications (20 min, $n = 3$, Fig. 3b). Similar depolarization and action potential firing patterns were generated by 10 μM kainate (14.6 ± 6.4 mV, $n = 3$, not shown) and by glutamate (100 μM; $n = 4$, Fig. 3c). The effects of kainate on interneurons and on pyramidal cells showed similar time courses (Figs. 1 and 3). Kainate (250 nM) applied in the presence of TTX (1 μM) generated an inward current (51.8 ± 7.9 pA, Vh –60 mV, $n = 8$, Fig. 3e, inset), which reversed at –14.3 ± 3.9 mV ($n = 8$, Fig. 3e).

Table 1. The different types of interneurons tested for their kainate sensitivity

	Interneuron type	Depolarization with 250 nM KA	Depolarization with 1 μM ATPA	Synaptically activated kainate receptors
	Perisomatic projecting I. ($n = 5$)	3/3	n.a.	1/2
	O-LM I. ($n = 7$)	3/4	1/1	2/3
S . oriens	O-O I. ($n = 3$)	1/1	3/3	n.a.
interneurons	Bistratified I. ($n = 4$)	1/1	2/2	1/1
	Unclassified I. ($n = 8$)	2/2	3/3	2/3
	Subtotal ($n = 27$)	10/11	9/9	6/9
	Perisomatic projecting I. ($n = 4$)	2/2	n.a.	2/2
	Schaffer associated I. ($n = 13$)	4/8	0/4	1/4
S. radiatum and	Perforant path associated I. ($n = 4$)	4/4	n.a.	n.a.
LM interneurons	Unclassified I. ($n = 36$)	18/19	2/6	5/11
	Subtotal ($n = 57$)	28/33	2/10	8/17
	All interneurons ($n = 84$)	38/44	11/19	14/26

n.a., not applicable.



Blockers, D-APV, GYKI, CGP 55845A and bicuculline

Fig. 5. Characterization of a GYKI-resistant synaptic response in CA1 interneurons. Averages of five individual responses to stimulation of s. radiatum (arrowheads). **(a)** 1, The EPSC evoked in control ACSF (5 V) was abolished by the application of a cocktail of blockers (GYKI 53655 (30 µM), D-APV (50 µM), bicuculline (20 µM) and CGP 55845 (100 µM)). 2, Increasing the stimulus revealed a synaptic response that was partially abolished by CNQX (50 µM). Note the different current scales in 1 and 2. 3, The GYKI-resistant component has been scaled to the peak of the AMPA receptor component to illustrate the different kinetics of these two synaptic responses. Vm, –70 mV. **(b)** The EPSC evoked in the presence of GYKI, reversed at 0 mV. The I–V curve displayed a slightly outward rectification. The inset shows averaged synaptic responses recorded at different holding potentials (from top to bottom, +20, +10, 0 and –20 mV). Vm, –58 mV. **(c)** 1, Increasing the stimulus from 10 to 30 V triggered a burst of action potentials on top of the GYKI-resistant response. 2, Paired-pulse facilitation of the GYKI-resistant EPSP (interval 50 ms). Increasing the stimulation from 10 to 12 V triggered an action potential on the second EPSP**. (d)** LY293558 suppressed the GYKI-resistant EPSP and the large paired-pulse facilitation. Vm, –61 mV. (a) S. radiatum interneuron. (b) Putative basket cell illustrated in Fig. 6a1. (c) S. radiatum interneuron. (d) S. oriens interneuron.

The following additional experiments were performed to examine the pharmacological properties of kainate receptors on interneurons, and to determine whether these receptors could be activated by glutamate. First we confirmed that GYKI 53655 blocked AMPA receptors in interneurons. The presence of TTX (1 µM), the application of GYKI suppressed the inward current generated by AMPA (1 µM, $n = 4$, Fig. 3d) but had no effect on the inward current induced by kainate (250 nM, $n = 5$, data not shown). Thus in CA1 interneurons, GYKI 53655 selectively antagonizes AMPA receptors, whereas kainate (250 nM) does not activate AMPA receptors. Bath application of glutamate (100 µM) in the presence of TTX, AMPA- and NMDA-receptor antagonists induced an inward current (98 ± 13 pA, Vh –60 mV, $n = 6$, not shown). Puff application of glutamate (3 mM) in the presence of TTX close to the soma of the recorded interneuron evoked an inward current that was reduced to 37 ± 10% of control by GYKI 53655 and D-APV ($n = 4$, $p < 0.0001$, Fig. 3f) and was further reduced by the addition of the mixed AMPA/kainate receptor antagonist CNQX (100 µM; 5 ± 2% of control, $n = 4$, Fig. 3f). Therefore selective activation of kainate receptors by either glutamate or kainate excites interneurons.

GluR5-mediated excitation of interneurons

The GluR5 agonist ATPA (1 µM) produced similar effects on 8 out of the 16 interneurons tested, including membrane depolarization (10.6 mV ± 3.6 mV, $p < 0.0001$, $n = 8$, Fig. 4a1) and a regular firing of action potentials (6.85 ± 2.15 Hz, $n = 8$, Fig. 4a1). The time course of these events was similar to that of the APTA-induced increase in IPSC frequency in pyramidal cells (compare Figs 4a1 and 2a). The ATPA-induced ($n = 8$, Fig. 4a) and kainate-induced ($n = 7$, not shown) depolarizations were blocked by the selective GluR5 antagonist LY 293558 (30 µM). The current induced by ATPA in the presence of TTX reversed at -16.0 ± 1.0 mV ($n = 3$, Fig. 4b). These results suggest that receptors with apparent GluR5 pharmacology exist in interneurons.

The following observations indicate the presence of other functional subunits of kainate receptors. In eight interneurons, ATPA did not affect the membrane potential (not shown). In four of these eight interneurons, the subsequent application of kainate depolarized the membrane (6.5 mV ± 1.6 mV, $n = 4$, not shown), resulting in repetitive regular firing of action potentials at a frequency of 3.08 ± 2.1 Hz ($n = 4$, not shown). Furthermore, in one interneuron, kainate applied in the presence of the GluR5 antagonist LY 293558 caused the firing of low frequency action potentials (Fig. 4a3).

In summary, kainate causes high frequency firing in a subpopulation of interneurons (see below) via the activation of GluR5-containing receptors, leading to the enhanced GABAergic tonic drive recorded in pyramidal neurons.

Kainate receptor-mediated EPSCs in interneurons

We then asked whether kainate receptor-mediated responses could be evoked by synaptic activation in interneurons. Single electrical stimulation in s. radiatum, in the presence of GYKI 53655 (30 µM), D-APV (50 µM), bicuculline (20 µM) and CGP 55845A (100 µM) to block AMPA, NMDA, $GABA_A$ and

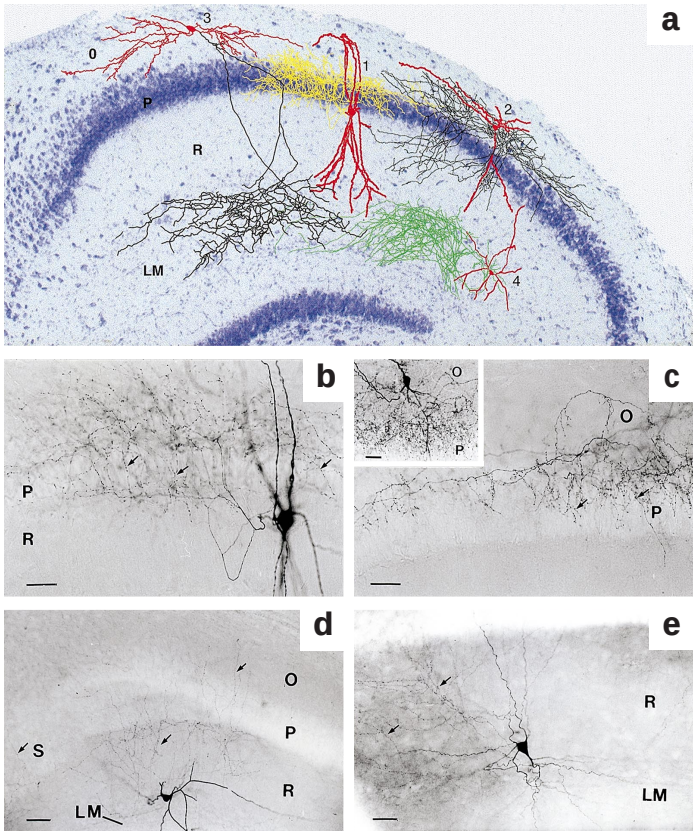


Fig. 6. Different types of interneurons depolarized by bath application of kainate and filled with biocytin in the CA1 region. **(a)** Montage showing the reconstructions with the neurolucida system of four different types of interneurons superimposed on a coronal section stained with cresyl violet. 1, Putative basket cell. 2, Bistratified cell. 3, O-LM cell. 4, Perforant-path associated interneuron. The cell bodies and dendritic trees are in red; the axons are in black (2,3) yellow (1) or green (4). Photomicrographs of interneurons. **(b)** High magnification of the putative basket cell illustrated in (a)**.** The soma was in s. radiatum (R) and its axon (arrows) arborized in s. pyramidale. **(c)** Putative axo-axonic cell with the soma in s. oriens (insert) and the axon confined to s. pyramidale and proximal oriens showing vertically running rows of boutons (arrows). **(d** and **e)** Schaffer-associated interneurons, with a soma at the s. radiatum–lacunosum moleculare border and an axon arbor that extended predominently in s. radiatum and s. oriens (arrows). The neuron illustrated in (d) displayed also some axonal collaterals entering the subiculum. O, s. oriens; P, s. pyramidale; R, s. radiatum; L-M, s. lacunosum moleculare. Scale bars (b–e), 100 µm.

$GABA_B$ receptors respectively, evoked an EPSC (n = 6/10, Fig. 5a) that was almost completely blocked by the mixed AMPA/kainate receptor antagonist CNQX (50 µM). The rise time (6.2 ± 1.5 ms, n = 6, Fig. 5a) and decay time constant (τ = 30.8 ± 7.7 ms, n = 6, Fig. 5a) of the kainate receptor-mediated EPSCs were slower than those of the AMPA receptor-mediated EPSCs (2.6 ± 0.3 ms, $p < 0.001$ and = 13.2 ± 5.6 ms, $p < 0.001$ respectively, n = 6, Fig. 5a) but faster than the metabotropic receptor-mediated EPSCs reported in CA1 pyramidal neurons following trains of electrical stimuli[23]. The kainate receptor-mediated EPSCs reversed at -1.3 ± 2.4 mV (n = 4, Fig. 5a4). In current clamp mode and under conditions of AMPA receptor blockade, a single stimulus to the s. radiatum evoked EPSPs in interneurons that were associated with bursts of action potentials (n = 8 out of 16, Fig. 5b). The GluR5 antagonist LY 293558 (30 µM) blocked the evoked kainate receptor-mediated synaptic responses in four out of five interneurons (Fig. 5c). A small LY 293558-resistant depolarization persisted in one out of five interneurons (not shown). Finally, EPSCs and EPSPs showed large paired-pulse facilitation (interval 50 ms, Fig. 5b and c). Therefore, interneurons in CA1 show synaptic responses that are mediated by the activation of GluR5-containing kainate receptors.

Morphological substrate of excitatory responses

We then investigated the relationship between the morphological properties of interneurons and the presence of kainate receptor-mediated currents (Table 1). Identification of interneurons according to established classification[23,24] requires the complete labeling of the axon and dendritic arbors. This was successfully done for 51 out of 98 interneurons. The remaining 47 were unequivocally identified as interneurons, but their axons were not labeled far enough for the cells to be classified. Fully identified interneurons included seven putative basket cells (Fig. 6 a and b), two putative axo-axonic cells with a soma in s. oriens, (Fig. 6c), six bistratified cells (Fig. 6a), three interneurons with a soma in s. oriens, a spiny dendritic tree and an axon restricted to this layer (O-O), seven oriens-lacunosum moleculare (O-LM) cells (Fig. 6a), fifteen Schaffer-associated s. radiatum-lacunosum moleculare interneurons (Fig. 6d and e), and five perforant path-associated s. radiatum-lacunosum moleculare interneurons (Fig. 6a). In addition, we found six extensively labeled interneurons with somata in s. oriens (n = 2) or s. radiatum (n = 4), which have not been characterized previously; these will be further described in another study.

Several points emerge from this analysis (Table 1). First, almost all the interneurons recorded (38/44) were depolarized by kainate. Second, all those whose somata were located in s. oriens (n = 9/9), including perisomatic and dendritic projecting interneurons, were also depolarized by the GluR5 agonist APTA, whereas only 20% of the s. radiatum interneurons were affected by APTA. This functional distinction between different layers is consistent with the preferential expression of the GluR5 subunit by s. oriens interneurons[4]. Third, the perisomatic projecting interneurons (n = 3/4) showed kainate receptor-mediated synaptic responses that were both more consistent and larger than in other interneurons (n = 11/22). Therefore, activation of kainate receptors on interneurons, particularly those in s. oriens that contain the GluR5 subunit, increases dendritic and somatic inhibition in principal cells.

Discussion

Kainate receptor-mediated increase of tonic inhibition

Our data demonstrate that exogenous application of kainate generates repetitive firing in interneurons, resulting in a massive increase of the frequency of spontaneous IPSCs in CA1 pyramidal neurons. These results are mimicked by the endogenous ligand glutamate, suggesting that this mechanism could be physiologically relevant. The depolarization of interneurons occurs via the activation of kainate receptors

and not AMPA receptors, because kainate-induced currents were unaffected by the presence of the AMPA receptor antagonist GYKI 53655. Our data also indicate that the kainate receptor-dependent increase of sIPSCs frequency depends primarily upon the activation of kainate receptors containing the GluR5 subunit on interneurons, because the effect of kainate was mimicked by the GluR5 agonist ATPA and blocked by the GluR5 antagonist LY293558. (One should keep in mind, however, that the specificities of these drugs have been established using cloned receptors expressed in artificial systems[20] rather than native receptors, for which the subunit composition and stoichiometry are unknown.) However, other subunits of kainate receptors could also be involved, because some interneurons depolarized in response to kainate but not to ATPA. Although nearly all types of interneurons are depolarized by kainate, functional GluR5-containing kainate receptors are primarily present in s. oriens interneurons, in keeping with the laminar distribution of this subunit[4] and despite the morphological and functional heterogeneity of s. oriens interneurons[23]. Finally, this kainate receptor-dependent circuitry with apparent GluR5 pharmacology is reminiscent of other pathways that when activated also increase tonic inhibition on principal neurons. These pathways rely on the activation of glutamate metabotropic-[25], nicotinic-[26], noradrenergic-[27,28], dopaminergic-[29] or peptidergic-[30,31] receptors. Because kainate receptors, including those containing GluR5 subunits, are widely expressed in principal cells and inhibitory interneurons throughout the brain[2,4], a similar increase of inhibition on target cells of GABAergic neurons is expected to occur in other regions.

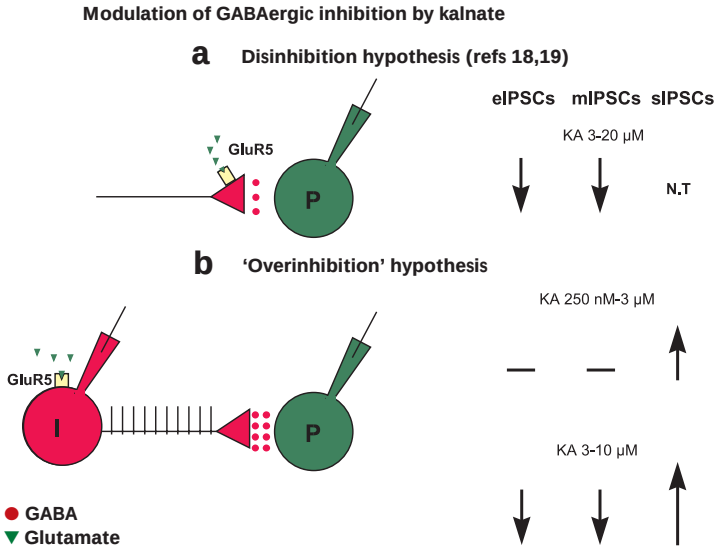


Fig. 7. Proposed schemes for the modulation of GABAergic inhibition by kainate in the CA1 region of the hippocampus. **(a)** In the disinhibition hypothesis[19,20], glutamate activates kainate receptors present on GABAergic presynaptic terminals leading to a decrease of GABA release on pyramidal cells and an enhanced excitability. **(b)** In the 'overinhibition' hypothesis (present report), glutamate activation of kainate receptors containing the GluR5 subunit enhances the firing of interneurons leading to an increase of GABA release on pyramidal cells and a reduced excitability. P, pyramidal neuron; I, interneuron; eIPSCs, evoked IPSCs; mIPSCs, miniature IPSCs; sIPSC, spontaneous IPSCs; N.T., not tested.

Physiological role of interneuronal kainate receptors

Our results provide the first demonstration of kainate receptor-mediated synaptic responses in interneurons. Several arguments suggest that these EPSPs are mediated by kainate receptors with an apparent GluR5 pharmacology. The EPSP was blocked by the GluR5 antagonist LY293558 and by the broad-spectrum AMPA/kainate receptor antagonist CNQX. The involvement of metabotropic glutamate receptors may be ruled out because the kainate receptor-mediated EPSCs were CNQX-sensitive, did not require repetitive stimulation to be generated and displayed kinetics significantly shorter than those of metabotropic receptor-mediated EPSCs[32]. The synaptic responses we observed in CA1 interneurons are also markedly different from those mediated by GluR6-containing kainate receptors in CA3 pyramidal cells[16,17], in that they display both larger amplitudes and faster kinetics. These differences could be due to a higher density of receptors, larger ion channel conductances or different distributions of the receptor channel complex[6,16]. The development of new specific antagonists of kainate receptors and of their different subunits will make it possible to determine the contribution of these receptors to synaptic transmission in interneurons.

Presynaptic modulation of inhibition by kainate

The massive increase of the frequency of spontaneous IPSCs in CA1 pyramidal neurons, or 'overinhibition' (Fig. 7), is observed over a wide range of kainate concentrations, including low concentrations that do not produce other pre- or postsynaptic effects on $GABA_A$ receptor-mediated currents. These results do not support the hypothesis that activation of kainate receptors has disinhibitory effects on pyramidal neurons[19]. This hypothesis is based on the observation that high concentrations of kainate (3–20 μM) reduce the amplitude of monosynaptic evoked IPSPs[18,20,33] and the frequency of miniature IPSCs[19,33]. The core of this hypothesis is that glutamate acting directly on kainate receptors located on GABAergic terminals reduces GABA release[33,34]. However, we find an overall disinhibitory action of kainate unlikely for three reasons. First, there is no evidence for functional presynaptic kainate receptors on GABAergic terminals. Second, when area CA1 is surgically isolated, kainate (10 μM) does not generate epileptiform activity in pyramidal cells (Bernard, unpublished results). Third, our results show that, even if disinhibition occurs, the activation of kainate receptors on interneurons by high concentrations of kainate considerably enhances the inhibitory drive on principal cells (3000%) and overcomes the small (25%) presynaptic reduction of miniature IPSCs (Fig. 5). Interestingly, frequency-dependent synaptic depressions of inhibitory unitary synaptic connections have been described recently in several brain regions; these activity-dependent processes do not involve presynaptic glutamate receptors[35] (Galarreta, M. & Hestrin, S., *Soc. Neurosci. Abstr.*, 1998). Further studies are needed to determine whether kainate receptor activation is more efficient than other ionotropic glutamate receptors present on interneurons in dampening excitation in pyramidal cells. Interestingly, synaptic activation of GluR5-containing kainate receptors evokes bursts of action potentials in CA1 interneurons. These bursts may boost the AMPA/NMDA receptor-mediated EPSPs in these interneurons and lead to a

burst of $GABA_A$ receptor-mediated IPCSs in pyramidal neurons.

In conclusion, we suggest that kainate may exert opposite effects on hippocampal excitability via different types of kainate receptors that are present on pyramidal neurons and interneurons, respectively. The epileptogenic effects of kainate are likely to be mediated by receptors containing the GluR6 subunit at the mossy fiber/CA3 pyramidal cell synapse[12–15]; consistent with this, CA3 has a lower threshold than other parts of the hippocampus for the generation of seizures by kainate[10,11], whereas the epileptogenic effects of kainate are reduced either by lesion of mossy fibers[12–14] or by the lack of GluR6 in knockout mice[15]. In area CA1, by contrast, activation of GluR5-containing receptors on interneurons causes an increase in tonic GABAergic inhibition on pyramidal cells. This may decrease the postsynaptic membrane time constant, input resistance and cell excitability, as has recently been shown in the cerebellum[36]. The GluR5-mediated EPSCs in interneurons show paired-pulse facilitation, which suggests a build-up of inhibitory responses during high frequency activity. Further studies are required to determine whether this 'overinhibition' acts as an endogenous regulatory mechanism to prevent epileptogenesis. Interestingly, in animal models of temporal-lobe epilepsy, there is a selective degeneration of GABAergic s. oriens interneurons[37] that express GluR5. This neuronal loss is associated with a 50% reduction of tonic inhibition in CA1 pyramidal cells (Bernard, unpublished results) that may lead to a permanent reduction of the GluR5-dependent increase in GABAergic inhibition.

Methods

ELECTROPHYSIOLOGY. Wistar rats (15–16 days old) were prepared as described[38]. Slices, 350 µm thick, were prepared with a Leica VT 1000E tissue slicer and immediately transferred to an incubation chamber. The CA1 area was then surgically isolated from CA3. ACSF contained (in mM) 124 NaCl, 3 KCl, 1.25 KH_2PO_4, 26 $NaHCO_3$, 1.3 $MgSO_4$-$7H_2O$, 2 $CaCl_2$, 10 D-glucose and was continuously aerated with 95% O_2 and 5% CO_2. After rest (>1 h), slices were transferred to a chamber mounted on the headstage of an upright microscope (Axioscop, Zeiss) for interneuron recordings or a conventional submerged chamber for blind patch recordings of pyramidal cells. CA1 interneurons were identified using IR-DIC microscopy through a 40× water immersion objective. Whole cell measurements in current clamp or in voltage clamp mode were recorded at 32°C and filtered at 3 kHz using an EPC-9 amplifier (HEKA-Electronics, Liambrecht, Germany). Blind patch-clamp recordings of pyramidal cells were done at 32°C using an axopatch 2B amplifier (Axon Instrument, Foster City, CA). Microelectrodes had a resistance of 4–12 MΩ, and internal solutions (osmolarity 265–275 mOsm) of the following compositions were used (in mM): 1) for voltage clamp recordings, 135 Cs-gluconate, 10 $MgCl_2$, 0.1 $CaCl_2$, 1 EGTA, 2 Na_2 adenosine triphosphate, 10 HEPES, 0.5% biocytin pH 7.25; 2) for current clamp recordings, Cs-gluconate was replaced by K-gluconate. The series resistance ranged between 15 and 50MΩ and was not allowed to vary by more than 20% during the course of the experiment. Spontaneous, miniature and evoked IPSCs in pyramidal cells were measured at the reversal potentials for glutamatergic events (+10 mV) as described[38]. Electrical stimulation was applied at 0.033 Hz through electrodes placed in CA1 stratum radiatum. To measure the current-voltage (I-V) relations of responses to kainate and ATPA (in the presence of AMPA and NMDA antagonists and TTX), voltage ramps (+60 mV to -100 mV, duration 5 s) were applied to interneurons before, during and after agonist application. The net I-V relations of agonist-activated currents were obtained by digital subtraction of the ramp current recorded with or without the agonist. The current curves obtained during kainate application were averaged (5 curves averaged) and substracted from those obtained before kainate application. A picospritzer (General Valve Corporation, Fairfield, NJ) was used to puff-apply glutamate. The pressure varied from 100 to 200 kPa and the duration of the puff varied from 10 to 40 ms. During recordings, all neurons were filled passively with biocytin for *post hoc* morphological identification. *Post hoc* morphological identification is necessary, because even when IR-DIC microscopy was used to select interneurons, 5% of the recordings proved to be from pyramidal cells dendrites. Pyramidal cell apical dendrites were recorded with the blind patch technique. The recording site was indicated by a dent or a hole in the membrane after *post hoc* morphological identification. Recordings were stored on a digital tape recorder DTR 1203 (BioLogic, Claix, France) for later analysis. Data were digitized (10 kHz) with a Labmaster interface card to a personal computer and analyzed with Acquis1 program (G. Sadoc, 1996, BioLogic, Claix, France). All the experimental values are given as means ± SEM. Parameters were compared with a Student's *t*-test, $p < 0.05$ was considered significant.

MORPHOLOGY. Slices were fixed overnight at 4 °C in a solution containing 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4). After fixation, slices were rinsed in PB, cryoprotected in sucrose for 1 hr, and quickly frozen on dry ice. Then, detection of biocytin-filled neurons was performed on unsectioned slices. To neutralize endogenous peroxidase, slices were pretreated for 30 min in 1% H_2O_2. After several rinses in saline phosphate buffer (0.1 M PBS, pH 7.4), slices were incubated for 24 hrs at 4°C in 1/100 Avidin-biotinylated peroxidase complex was diluted in PBS containing 0.3% Triton-X-100. After 30 min rinses in PBS, slices were processed with 0.06 % 3,3'-diaminobenzidine tetrahydrochloride (DAB, Sigma) and 0.006% H_2O_2 diluted in PBS, rinsed, mounted on gelatin-coated slides and coverslipped in an aqueous medium (Crystal/Mount, Biomeda, Foster City, CA). Biocytin-filled interneurons were reconstructed using the Neurolucida system (Microbrightfield Inc., Colchester, VT).

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