NMDA Receptor-Dependent Long-Term Synaptic Depression in the Entorhinal Cortex In Vitro

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Kourrich, Saïd and C. Andrew Chapman. NMDA receptor-dependent long-term synaptic depression in the entorhinal cortex in vitro. J Neurophysiol 89: 2112–2119, 2003. First published December 18, 2002; 10.1152/jn.00714.2002. The entorhinal cortex receives a large projection from the piriform (primary olfactory) cortex and, in turn, provides the hippocampal formation with most of its cortical sensory input. Synaptic plasticity in this pathway may therefore affect the processing of olfactory information and memory encoding. We have recently found that long-term synaptic depression (LTD) can be induced in this pathway in vivo by repetitive paired-pulse stimulation but not by low-frequency (1 Hz) stimulation with single pulses. Here, we have used field potential recordings to investigate the stimulation parameters and transmitter receptors required for the induction of LTD in the rat entorhinal cortex in vitro. The effectiveness of low-frequency stimulation (900 pulses at 1 or 5 Hz) and repeated delivery of pairs of stimulation pulses (30-ms interpulse interval) was assessed. Only repeated paired-pulse stimulation resulted in lasting LTD, and a low-intensity paired-pulse stimulation protocol that induces LTD in vivo was only effective in the presence of the GABA<sub>A</sub> receptor antagonist bicuculline (50 μM). LTD could also be induced in normal ACSF, however, by increasing the number of pulse-pairs delivered and by increasing the stimulation intensity during LTD induction. The induction of LTD was blocked by constant bath application of the N-methyl-d-aspartate (NMDA) glutamate receptor antagonist 2-amino-5-phosphononovaleric acid (50 μM), indicating that LTD is dependent on NMDA receptor activation. However, LTD was not blocked by the group I/II mGluR antagonist (RS)-α-ethyl-4-carboxyphenylglycine (500 μM) or by bicuculline (50 μM). The induction of LTD in the entorhinal cortex in vitro is therefore dependent on intense stimulation that recruits activation of NMDA receptors, but does not require concurrent activation of mGluRs or inhibitory synaptic inputs.

INTRODUCTION

The superficial layers of the entorhinal cortex are a major site of convergence for sensory information from a number of cortical sensory and association areas and also provide the hippocampal formation with most of its cortical sensory input (Burwell and Amaral 1998). Although a great deal of work has focused on the mechanisms of synaptic plasticity in the hippocampus, bidirectional synaptic modification in the entorhinal cortex may also play an essential role in the integration of multimodal sensory information and in the mnemonic functions of the medial temporal lobe. Long-term potentiation (LTP), an enduring increase in synaptic strength resulting from intense activation of afferents (Malenka and Nicoll 1999), has been characterized in the superficial layers of the entorhinal cortex both in vitro (Alonso et al. 1990; de Curtis and Llinás 1993; Yun et al. 2002) and in vivo (Chapman and Racine 1997a,b; Racine et al. 1983). Synaptic depression effects are thought to complement synaptic potentiation during the formation of memory (Abbott 2000; Bear 1996; Brauneck and Manahan-Vaughn 2001; Christie et al. 1994), and we have also recently demonstrated the induction of long-term synaptic depression (LTD) (Bear and Abraham 1996; Kemp and Bashir 2001) in piriform cortex efferents to the entorhinal cortex in the awake rat (R. Bouras and C. A. Chapman, unpublished observations).

Although LTD in the hippocampus has conventionally been induced using prolonged (15 min) 1-Hz stimulation trains, this type of stimulation has been most effective in slices taken from juvenile animals (Dudek and Bear 1992; Mulkey and Malenka 1992) and is generally ineffective in slices taken from the adult (Dudek and Bear 1993; Kemp et al. 2000; Wagner and Alger 1995) and in in vivo preparations (Doyle et al. 1997; Errington et al. 1995; Staubli and Scafaldi 1997; see Heynen et al. 1996). An alternative pattern of stimulation, in which pairs of stimulation pulses separated by a short interval (25 ms) are repeatedly delivered at 0.5–1 Hz, induces LTD more effectively. Repetitive paired-pulse stimulation induces N-methyl-d-aspartate (NMDA) receptor-dependent LTD in the adult CA1 region in vivo (Doyère et al. 1996; Thiels et al. 1994), and a similar pattern of paired-burst stimulation induces LTD in the dentate gyrus in vivo (Thiels et al. 1996) and reverses LTP of hippocampal inputs to the prefrontal cortex (Burette et al. 1997). In the CA1, LTD induced by paired-pulse stimulation is blocked by GABA<sub>A</sub> receptor antagonism (Thiels et al. 1994) and by reducing the stimulation intensity to limit activation of local inhibitory interneurons (Doyère et al. 1996). This stimulation pattern is therefore thought to be effective because the first pulse activates local interneurons that cause postsynaptic inhibition during the excitatory postsynaptic potentials (EPSPs) evoked by the second pulse.

Our previous observations have shown that LTD in piriform cortex efferents to the entorhinal cortex can be induced by repetitive paired-pulse stimulation in the awake rat, but the contribution of inhibitory mechanisms is not clear. Repetitive stimulation using a 30-ms interval that evokes strong paired-pulse facilitation induced LTD, but stimulation using a 10-ms interval that evokes
little facilitation did not. However, inhibitory postsynaptic potentials and action potential afterhyperpolarizations are also observed in vitro at the same latencies that evoke paired-pulse facilitation (Alonso and Klink 1993; Finch et al. 1998), and an inhibitory current source, which peaks at a latency of 45 ms, also occurs in the superficial layers of the entorhinal cortex in vivo after picrotorf cortex stimulation (Chapman and Racine 1997a). Therefore even though LTD was induced by stimulation that evokes paired-pulse facilitation, concurrent activation of inhibitory mechanisms may also contribute.

The induction of LTD in the entorhinal cortex in vivo is blocked by MK-801, indicating that it requires NMDA receptor activation, but metabotropic glutamate receptors (mGluR) might also play a role. In the CA1, LTD induction is typically attributed to moderate increases in intracellular Ca\textsuperscript{2+} via the NMDA receptor (Artola and Singer 1993; Kemp and Bashir 2001) but has also been linked to mGluR activation (Fitzjohn et al. 2001; Nicoll et al. 1998; Otani and Connor 1998; Watabe et al. 2002). Metabotropic receptors also contribute to LTD induction in the dentate gyrus (Camodeca et al. 1999; Manahan-Vaughan 1998) and cerebellum (Daniel et al. 1998). Further, combined mGluR and AMPA/kainate receptor activation is required for a form of LTD in the CA1 that is induced by repeated stimulation that evokes paired-pulse facilitation (Kemp and Bashir 1999).

In the present study, we have used extracellular field potential recordings to determine the effective stimulus parameters for LTD induction in layer II of the entorhinal cortex and to evaluate the involvement of excitatory and inhibitory synaptic transmission in the induction of LTD. Tests were conducted using low-frequency stimulation with single pulses (1 or 5 Hz) and with pairs of stimulation pulses using a 30-ms interpulse interval that effectively induces LTD in vivo. Neurotransmitter receptor blockers were used to assess the contribution of NMDA receptors, mGluRs, and GABA\textsubscript{A} receptor-mediated inhibition in the induction of LTD.

**METHO DS**

**Slices**

Experiments were performed on slices obtained from male Long-Evans rats (3–5 wk old) as described previously (Chapman et al. 1998). All chemicals were obtained from Sigma unless otherwise indicated. Animals were anesthetized with halothane and brains were rapidly removed and cooled (4°C) in oxygenated artificial cerebrospinal fluid (ACSF). Horizontal slices (400 μm) were cut using a vibratome (WPI, Vibroslice). ACSF consisted of (in mM) 124 NaCl, 5 KCl, 1.25 NaH\textsubscript{2}PO\textsubscript{4}, 2 MgSO\textsubscript{4}, 2 CaCl\textsubscript{2}, 26 NaHCO\textsubscript{3}, and 10 dextrose. Slices were placed on a nylon net in a gas-exchanged (DC-3 kHz) and amplified with an Axoclamp 2B amplifier (Axon Instruments), and responses were monitored using an oscilloscope (Gould, Model 1602) and digitized at 20 kHz (Axon Instruments, Digidata 1322A) for storage on computer hard-disk using the software package Clampex 8.1 (Axon Instruments).

Responses to test pulses were monitored every 20 s using an intensity adjusted to evoke fEPSPs with an amplitude of ~60–70% of the maximum. This intensity was determined by delivering pulses from 25 to 400 μA in 25-μA increments. Tests for LTD were conducted only on slices with stable fEPSPs (less than ±5%) during the 20-min baseline period.

**RESULTS**

Field potentials evoked in layer II of the entorhinal cortex by stimulation of layer I afferents were similar to those observed in previous in vitro studies (Alonso et al. 1990; Yun et al. 2002) and contained an initial fiber volley followed by a negative fEPSP with a mean amplitude of 0.57 ± 0.02 mV (e.g., Fig. 1A, inset). Neither transient nor lasting depression effects observed in the fEPSPs were associated with changes fiber volley amplitude.

**Low-frequency stimulation**

Fifteen minutes of 1-Hz stimulation caused a small, transient depression that peaked 5 min after the stimulation [93.8 ± 5.0% of baseline; n = 11; F(3,72) = 4.94, P < 0.01; Neuman-Keuls, P < 0.05] but did not cause a lasting depression of fEPSPs (P = 0.47; Fig. 1A). Response amplitudes were 101.8 ± 4.3% of baseline after 60 min.
To determine if a higher frequency of stimulation could induce LTD more effectively, 5-Hz stimulation trains were delivered, and the train duration was set to 3 min to hold the number of pulses (900) constant. Five-hertz stimulation resulted in a transient depression of fEPSPs that peaked after 5 min (n = 6; P < 0.05; Fig. 1B). Although the size of the transient depression (85.9 ± 3.3% of baseline) was larger than that induced by 1-Hz stimulation, there was no significant LTD either 30 (95.3 ± 2.4%) or 60 min (98.8 ± 3.2%; P = 0.85) after 5-Hz stimulation. Responses in control slices remained stable throughout the recording period (99.9 ± 1.5% of baseline after 60 min; Fig. 1C).

Repetitive paired-pulse stimulation

An interpulse interval of 30 ms was chosen for tests of LTD induction because this interval effectively induces LTD in piriform cortex inputs to the entorhinal cortex in vivo. In awake animals, a 10-ms interval causes no net paired-pulse facilitation, and intervals of 30–50 ms cause the greatest paired-pulse facilitation (Chapman and Racine 1997a). In slices, there was a paired-pulse depression at the 10-ms interval (58.3 ± 7.3%), and no net facilitation at the 30-ms interval (96.2 ± 6.8%, not shown). Thus although responses evoked by the second pulses were smaller in slices, the responses showed a pattern that was similar to that observed in vivo, with the 10-ms interval reflecting the greatest inhibition and the 30-ms interval evoking the largest paired-pulse responses. In all groups, a stable paired-pulse depression developed during the first 2–3 min of repetitive stimulation for LTD induction, and the size of this depression was not significantly affected by the intensity of paired-pulse stimulation or drug treatment (84.6 ± 9.8% in control medium vs. 82.2 ± 7.8% in APV, 72.6 ± 11.0% in E4-CPG, and 90.8 ± 8.5% in bicuculline).

The effectiveness of repetitive paired-pulse stimulation for the induction of LTD was found to be dependent on the level of synaptic inhibition. In normal ACSF, 450 pairs of pulses delivered once every 2 s, which induces LTD in piriform cortex inputs to the entorhinal cortex in vivo, did not induce LTD in vitro (n = 5; Fig. 2A). Field EPSP amplitude was
the intensity of stimulation pulses during LTD induction (to 90% of asymptotic levels) increased the amount of LTD induced by 900 pairs at 1 Hz (n = 9; Fig. 3B). Field EPSP amplitude was reduced to 62.9 ± 5.2% of baseline levels 5 min after conditioning stimulation and remained at 81.0 ± 3.3% of baseline after 60 min (0.38 ± 0.03 vs. 0.48 ± 0.04 mV; P < 0.001). To determine the influence of the number of pulse-pairs on LTD induction, another group of slices received 450, rather than 900, high-intensity pulse-pairs delivered over a 7.5-min period (n = 7; Fig. 3C). The smaller number of pulse-pairs induced LTD, but the size of the depression after 60 min (89.1 ± 3.8%) was significantly less than that induced by 900 pulse-pairs (P < 0.05). Because the greatest LTD was induced by intense paired-pulse stimulation at 1 Hz for 15 min (Fig. 4), this stimulation pattern was used in further tests to assess the neurotransmitter receptors that are required for LTD induction.

**NMBA and mGluR antagonism**

Because NMDA receptor antagonism blocks LTD induction in the entorhinal cortex in vivo, the role of NMDA receptors in the induction of LTD in slices was tested using constant bath application of the NMDA receptor antagonist APV (50 μM). The induction of LTD by intense paired-pulse stimulation at 1 Hz was blocked by APV (n = 8; Fig. 5A). There was no significant depression 5 min after paired-pulse stimulation, and responses remained at 99.3 ± 5.4% of baseline levels after 30 min (0.46 ± 0.05 vs. 0.47 ± 0.04 mV).

The role of mGluRs in LTD induction was tested by delivering intense paired-pulse stimulation during constant bath application of the group I/II mGluR antagonist E4-CPG (500 µM). Increasing the intensity of the stimulation pattern in normal ACSF, by delivering 900 (vs. 450) pairs of pulses at a frequency of 1 Hz, also induced LTD (n = 8; F(9,91) = 10.00, P < 0.001; Fig. 3A). Field EPSP amplitude was reduced to 77.3 ± 3.0% of baseline levels 5 min after conditioning stimulation and remained at 90.1 ± 2.7% of baseline levels at the end of the 60-min follow-up period (0.52 ± 0.07 vs. 0.57 ± 0.08 mV; P < 0.01). Further increasing the intensity of the stimulation pattern by raising the intensity of stimulation pulses during LTD induction (to 90% of asymptotic levels) increased the amount of LTD induced by 900 pairs at 1 Hz (n = 9; Fig. 3B). Field EPSP amplitude was reduced to 62.9 ± 5.2% of baseline levels 5 min after conditioning stimulation and remained at 81.0 ± 3.3% of baseline after 60 min (0.38 ± 0.03 vs. 0.48 ± 0.04 mV; P < 0.001). To determine the influence of the number of pulse-pairs on LTD induction, another group of slices received 450, rather than 900, high-intensity pulse-pairs delivered over a 7.5-min period (n = 7; Fig. 3C). The smaller number of pulse-pairs induced LTD, but the size of the depression after 60 min (89.1 ± 3.8%) was significantly less than that induced by 900 pulse-pairs (P < 0.05). Because the greatest LTD was induced by intense paired-pulse stimulation at 1 Hz for 15 min (Fig. 4), this stimulation pattern was used in further tests to assess the neurotransmitter receptors that are required for LTD induction.

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**FIG. 3.** Intense patterns of repetitive paired-pulse stimulation induce LTD in the entorhinal cortex. A: delivery of 900 pairs of pulses at a frequency of 1 Hz caused a reduction of fEPSP amplitude to 90.1 ± 2.7% of baseline levels after 60 min (n = 8). B: increasing the pulse intensity (↑μA) during conditioning stimulation increased the amount of LTD that was induced (81.0 ± 3.3% of baseline after 60 min; n = 9). C: reducing the number of pairs of pulses, from 900 to 450, also reduced the amount of LTD that was induced (89.1 ± 3.8% of baseline after 60 min; n = 7).

Reduced to 90.0 ± 3.7% of baseline levels 5 min after conditioning stimulation [0.55 ± 0.07 vs. 0.50 ± 0.08 mV; F(3,39) = 4.03, P < 0.01], but responses returned to baseline levels after ~20 min and were at 97.0 ± 3.2% of baseline after 40 min. LTD was induced, however, when the same stimulation pattern was delivered during constant bath application of bicuculline (50 μM) to reduce synaptic inhibition (n = 9; Fig. 2B). Responses were reduced to 92.4 ± 3.1% of baseline levels 60 min after conditioning stimulation (0.66 ± 0.06 vs. 0.61 ± 0.06 mV; P < 0.01).

Increasing the intensity of the stimulation pattern in normal ACSF, by delivering 900 (vs. 450) pairs of pulses at a frequency of 1 Hz, also induced LTD (n = 8; F(9,91) = 10.00, P < 0.001; Fig. 3A). Field EPSP amplitude was reduced to 77.3 ± 3.0% of baseline levels 5 min after conditioning stimulation and remained at 90.1 ± 2.7% of baseline levels at the end of the 60-min follow-up period (0.52 ± 0.07 vs. 0.57 ± 0.08 mV; P < 0.01). Further increasing the intensity of the stimulation pattern by raising

**FIG. 4.** Cumulative probability distributions indicate the range of LTD effects observed after paired-pulse stimulation in individual slices. Data are taken from the same groups as shown in Fig. 2 (A) and Fig. 3 (B) and are compared with results obtained for control slices (see Fig. 1C).
inhibition as well as NMDA receptor activation (Doyère et al. 1996; Thiels et al. 1996). The dependence of entorhinal cortex LTD on GABA<sub>A</sub> receptors was therefore tested using constant bath application of the GABA<sub>A</sub> receptor antagonist bicuculline (50 μM). Bicuculline did not block the induction of LTD (n = 6; Fig. 5C). Responses were reduced to 59.6 ± 6.5% of baseline levels after 5 min and remained depressed at 74.3 ± 6.3% of baseline at the end of the 60-min recording period [0.42 ± 0.06 vs. 0.56 ± 0.06 mV; F(3,45) = 34.40, P = 0.001]. The amount of LTD induced was similar to that induced in the absence of the drug (Fig. 5C vs. 3B), indicating that LTD induction does not require activation of GABA<sub>A</sub> receptors.

**DISCUSSION**

These experiments have investigated the stimulus parameters that are effective for LTD induction in layer II of the entorhinal cortex in vitro and have assessed the roles of several neurotransmitter receptors in the induction of LTD. The successful induction of LTD was dependent on the intensity of conditioning stimulation. LTD was not induced by 1- or 5-Hz low-frequency stimulation, and low-intensity paired-pulse stimulation induced LTD effectively only in the presence of bicuculline to enhance postsynaptic excitability. LTD was induced in normal ACSF only when the number or intensity of the pulse-pairs was increased. This indicates that LTD in the entorhinal cortex slice requires strong, repetitive synaptic activation and suggests that a high degree of cooperativity is required to cause sufficient activation of postsynaptic targets (Kerr and Abraham 1995). The present results, and previous findings that the induction of LTP in the entorhinal cortex requires more intense stimulation trains than in the hippocampus (Chapman and Racine 1997b; Racine et al. 1983), suggests that the entorhinal cortex is particularly resistant to bidirectional modifications of synaptic strength.

The requirement of strong stimulation patterns is consistent with the dependence of LTD induction on NMDA receptors (Fig. 4). In the entorhinal cortex in vivo, LTD induced by paired-pulse stimulation is blocked by the NMDA receptor antagonist MK-801 (R. Bouras and C. A. Chapman, unpublished observations), and the present results show that the NMDA receptor antagonist APV blocks LTD induction in the entorhinal cortex slice. LTD was not blocked by the group I/II mGluR antagonist E4-CPG and is also not dependent on synaptic inhibition mediated by GABA<sub>A</sub> receptors. Therefore intense, repetitive synaptic stimulation leading to NMDA receptor activation, rather than concurrent activation of mGluRs or inhibitory synaptic inputs (Doyère et al. 1996; Thiels et al. 1994), is the main requirement for LTD induction in the entorhinal cortex.

**Low-frequency stimulation**

Similar to our previous observations in vivo, we have shown that 1-Hz stimulation does not induce LTD in entorhinal cortex slices (Fig. 1A). Although 1-Hz stimulation does induce LTD in the sensorimotor cortex in vivo (Chapman et al. 1998; Froc et al. 2000) and in CA1 slices taken from juvenile rats (Dudek and Bear 1992; Mulkey and Malenka 1992), our findings are consistent with the absence of LTD after 1-Hz stimulation in

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**GABA<sub>A</sub> receptor antagonism**

In previous studies, LTD induced by paired-pulse stimulation in the CA1 region was found to be dependent on synaptic activity as well as NMDA receptor activation (Doyère et al. 1996; Thiels et al. 1996). The dependence of entorhinal cortex LTD on GABA<sub>A</sub> receptors was therefore tested using constant bath application of the GABA<sub>A</sub> receptor antagonist bicuculline (50 μM). Bicuculline did not block the induction of LTD (n = 6; Fig. 5C). Responses were reduced to 59.6 ± 6.5% of baseline levels after 5 min and remained depressed at 74.3 ± 6.3% of baseline at the end of the 60-min recording period [0.42 ± 0.06 vs. 0.56 ± 0.06 mV; F(3,45) = 34.40, P = 0.001]. The amount of LTD induced was similar to that induced in the absence of the drug (Fig. 5C vs. 3B), indicating that LTD induction does not require activation of GABA<sub>A</sub> receptors.

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CA1 slices from adult rats (Dudek and Bear 1993; Kemp et al. 2000; Wagner and Alger 1995) and in the CA1 (Errington et al. 1995; Staubli and Scafaldi 1997) and dentate gyrus (Abraham et al. 1996; Doyère et al. 1996) in vivo.

Although 5-Hz stimulation induced a larger short-term depression than did 1-Hz stimulation, LTD was not induced (Fig. 1B). Similar to other stimulation protocols used here, 5-Hz stimulation caused a depression that peaked ~5 min after stimulation, suggesting that depression effects are mediated by gradual activation of signaling and/or expression mechanisms. Slowly developing depression effects are also observed in the hippocampal CA1 region in vivo and in vitro (Ngezahayo et al. 2000; Thielts et al. 1996). The depression induced by 5-Hz stimulation decayed slowly over a period of ~20 min and may have resulted from transient activation of mechanisms that mediate the expression of LTD. Five hertz stimulation induces a transient depression in the CA1 in hippocampal cultures that has a time course similar to that observed here (Kauderer and Kandel 2000). Although 5-Hz stimulation did induce a larger short-lasting depression than 1-Hz stimulation in these acute entorhinal cortex slices, our results show that stimulation with single pulses at these frequencies is insufficient for LTD induction (Doyle et al. 1997; Errington et al. 1995; O’Dell and Kandel 1994).

Repetitive paired-pulse stimulation

Similar to our previous findings in the awake rat, LTD was induced by repetitive stimulation with pairs of pulses. Surprisingly, however, the parameters that are effective in vivo (450 pairs over 15 min, 30-ms interval) did not induce LTD in slices maintained in normal ACSF, and LTD was induced only after pharmacological reduction of synaptic inhibition (Fig. 2) or increasing the intensity of the stimulation pattern (Fig. 3). This requirement for more intense stimulation in normal ACSF could be due to greater levels of inhibition in the slice. Although paired-pulse stimulation with a 10-ms interval evokes no net inhibition in vivo (Chapman and Racine 1997a) there was marked paired-pulse inhibition at this interval in the slice. Further, stimulation using a 30-ms interval induces a marked paired-pulse facilitation in vivo, but there was little facilitation in vitro. More intense stimulation may therefore be required for LTD induction in slices to compensate for smaller short-term synaptic facilitation effects. The requirement for more intense stimulation may also be due to the activation of a much smaller number of fibers by the concentric bipolar electrode placed on the surface of the slice. Stimulating electrodes used in vivo have a much larger tip separation (0.5 mm) and were positioned to activate a large number of fibers by straddling layer II of the piriform cortex. The number of activated fibers is likely to be much smaller in vitro than in the intact animal, and more intense stimulation is likely required to enhance activation of a smaller number of afferents. Both in vivo and in vitro, however, the dependence of LTD on intense stimulation patterns indicates that a high degree of cooperativity among afferents is required for the induction of LTD (Kerr and Abraham 1995).

Critical receptors for LTD induction

Previous studies that have induced LTD in the CA1 region with paired-pulse stimulation have used an interpulse interval of 25 ms to evoke paired-pulse inhibition of CA1 pyramidal cell firing. LTD induction by paired-pulse stimulation in the CA1 is blocked by both APV and bicuculline (Thielts et al. 1994) and is also prevented by reducing the pulse intensity to limit the activation of inhibitory circuits (Doyère et al. 1996). The induction of LTD by this stimulation pattern has therefore been attributed to the activation of NMDA glutamate receptors during a period of postsynaptic inhibition initiated by the first of the two pulses (Doyère et al. 1996; Thielts et al. 1994, 1996; see Stanton and Sejnowski 1989). The induction of LTD by manipulations that inhibit cell firing is theoretically attractive because anti-Hebbian rules for synaptic modification predict synaptic weakening when pre- and postsynaptic activities are poorly correlated. However, paired-pulse stimulation with a 10-ms interval that activates inhibitory mechanisms does not induce LTD in the entorhinal cortex in vivo (R. Bouras and C. A. Chapman, unpublished observations). Further, we have shown here that LTD was not reduced by constant bath application of the GABAA receptor blocker bicuculline (Fig. 5C). Ionotropic inhibitory synaptic transmission is therefore not required for the induction or expression of LTD in the entorhinal cortex.

Activation of NMDA receptors, but not mGluRs, is required for LTD induction in the entorhinal cortex. The NMDA receptor antagonist APV completely blocked LTD induction (Fig. 5A), and this confirms our previous findings in vivo in which LTD was blocked by the NMDA receptor antagonist MK-801. Activation of NMDA receptors is also required for LTD in the hippocampal CA1 (Dudek and Bear 1992; Mulkey and Malenka 1992; Thielts et al. 1996). We have also shown here that LTD in the entorhinal cortex is not blocked by E-4-CPG and therefore does not require activation of group I or II mGluRs. Activation of group I mGluRs, which are linked to phospholipase C, induces protein-kinase-C-dependent LTD in CA1 pyramidal cells (Otani and Connor 1998; Watabe et al. 2002), and very low-frequency stimulation combined with exposure to GABA A or GABA B receptor agonists also induces an mGluR-dependent form of LTD in the CA1 region (Yang et al. 1994). Taken together, however, our present results suggest that Ca2+ entry via NMDA receptors is a sufficient trigger for entorhinal cortex LTD and that this form of plasticity does not require mGluR activation (Sawtell et al. 1999; Selig et al. 1995).

The experiments with APV and E-4-CPG also showed that the depression of fEPSPs during paired-pulse stimulation was prevented by blocking either NMDA receptors or group I/II mGluRs. The mechanisms that produce the depression in normal ACSF may therefore depend in part on NMDA receptor-dependent Ca2+ influx and a group I mGluR-mediated reduction in transmitter release (Fass et al. 2002).

The mechanisms that mediate bidirectional synaptic modifications in the entorhinal cortex are likely to have a significant impact on the processing of olfactory information and on the neuronal representations that are processed by the hippocampal formation. The current report shows that the major characteristics of LTD induction in piriform cortex inputs to the entorhinal cortex in vivo are preserved in the slice preparation. This preparation may therefore be used effectively to identify the intracellular signal transduction mechanisms that mediate the induction and expression of LTD in the entorhinal cortex.
REFERENCES


