Long-Term Potentiation in Direct Perforant Path Projections to the Hippocampal CA3 Region In Vivo

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Long-term potentiation in direct perforant path projections to the hippocampal CA3 region in vivo. J Neurophysiol 87: 669–678, 2002; 10.1152/jn.00938.2000. The perforant path constitutes the primary projection system relaying information from the neocortex to the hippocampal formation. Long-term synaptic potentiation (LTP) in the perforant path projections to the dentate gyrus is well characterized. However, surprisingly few studies have addressed the mechanisms underlying LTP induction in direct perforant path projections to the hippocampus. Here we investigate the role of N-methyl-D-aspartate (NMDA) and opioid receptors in the induction of LTP in monosynaptic medial and lateral perforant path projections to the CA3 region in adult pentobarbital sodium–anesthetized rats. Similar to LTP observed at the medial perforant path–dentate gyrus synapse, medial perforant path–CA3 synapses display LTP that is blocked by both local and systemic administration of the competitive NMDA receptor antagonist (±)-3-(2-carboxypiperazin-4-yl) propyl-1-phosphonic acid ([±]-CPP). By contrast, LTP induced at the lateral perforant path–CA3 synapses is not blocked by either local or systemic administration of this NMDA receptor antagonist. The induction of LTP at lateral perforant path–CA3 synapses, which is blocked by the opioid receptor antagonist naloxone, is also blocked by the selective μ (µ) opioid receptor antagonist Cys²-Tyr³-Orn⁵-Pen⁷-amide (CTOP), but not the selective δ (δ) opioid receptor antagonist naltrexonole (NTI). CTOP was without effect on the induction of medial perforant path–CA3 LTP. The selective sensitivity of lateral perforant path–CA3 LTP to μ-opioid receptor antagonists corresponds with the distribution of μ-opioid receptors within the stratum lacunosum-moleculare of area CA3 where perforant path projections to CA3 terminate. These data indicate that both lateral and medial perforant path projections to the CA3 region display LTP, and that LTP induction in medial and lateral perforant path–CA3 synapses are differentially sensitive to NMDA receptor and μ-opioid receptor antagonists. This suggests a role for opioid, but not NMDA receptors in the induction of LTP at lateral perforant path projections to the hippocampal formation.

INTRODUCTION

Information flow through the hippocampal formation classically is described as a sequential activation of the dentate gyrus, CA3 and CA1 regions of the hippocampal formation, with the perforant path projection to the dentate gyrus serving as the primary target of neocortical input to the hippocampus (Andersen et al. 1971). However, both anatomical and physiological data indicate that direct perforant path projections to pyramidal cells of the CA3 and CA1 regions of the hippocampus are substantial (Amaral et al. 1990) and capable of driving pyramidal cells (Andersen et al. 1966; Yeckel and Berger 1990). Additionally, studies by others (Yeckel and Berger 1990) and ourselves (Breindl et al. 1994) demonstrate that perforant path activation of CA3 pyramidal cells elicits firing of pyramidal cells in the CA3 region that precedes the firing of dentate granule cells. Thus CA3 pyramidal cells are the first cells within the hippocampal formation to respond to extrinsic cortical input. In addition, both medial and lateral perforant path-CA3 synapses display activity-induced increases in synaptic efficacy (long-term potentiation or LTP) (Breindl et al. 1994). These data, taken together with models of information processing that ascribe important roles to these direct perforant path-CA3 projections (Marr 1971; Morris and McNaughton 1987; Treves and Rolls 1994), indicate strongly that the monosynaptic perforant path projections to the hippocampus are likely to play important roles in hippocampal information processing.

Long-term synaptic potentiation (LTP) remains the most plausible and intensively studied model of the cellular mechanisms of synaptic plasticity that may underlie memory (Bliss and Lomo 1973). LTP is a persistent increase (days to weeks in freely moving animal) (Barnes 1979) in the amplitude of synaptic responses following frequency-specific activation of afferent fibers. The existence of LTP in variety brain structures implicated in learning and memory, such as the hippocampal formation, together with findings that pharmacological (Morris et al. 1986), physiological (Moser et al. 1998), and genetic (Giese et al. 1998) manipulations that alter LTP also alter learning and memory, provide substantial convergent, although indirect, evidence in support of the hypothesis that LTP is one mechanism underlying learning and memory within the vertebrate nervous system (Martinez and Derrick 1996).

Although synaptic potentiation in the main afferent systems of the hippocampal trisynaptic circuit are well characterized, the physiology of the direct perforant path projections to the hippocampal CA3 region have remained largely uncharacterized until recently. Barrionuevo and colleagues (Berzhanskaya et al. 1998) demonstrate both monosynaptic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl-D-aspartate (NMDA) receptor–mediated excitatory postsynaptic...
 currents (EPSCs), and disynaptic inhibitory postsynaptic currents (IPSCs) in CA3 pyramidal cells generated by activation of the lateral and medial aspects of the perforant path. Direct perforant path projections to area CA3 also display LTP (Berger and Yeckel 1991; Breindl et al. 1994), although few studies have addressed the mechanisms underlying LTP induction in these projections. Previously, we reported that the induction of LTP in lateral perforant path–CA3 synaptic responses is blocked by local application of the nonselective opioid receptor antagonist naloxone, whereas the induction of medial perforant path–CA3 LTP is insensitive to this antagonist (Breindl et al. 1994). This is significant in that the lateral, but not medial perforant path contains and releases proenkephalin-derived opioid peptides (Chavkin et al. 1983, 1985; Gall et al. 1981; McLean et al. 1987; Neumaier and Chavkin 1989; Stengaard-Pedersen 1983). At its dentate gyrus target, lateral perforant path projections display LTP that is blocked by antagonists of both mu (μ) and delta (δ) opioid receptors (Bramham and Survev 1996; Bramham et al. 1988, 1991a), the two opioid receptor types activated by proenkephalin-derived opioid peptides (Lutz and Pfister 1992). However, studies have yet to address the contribution of NMDA receptors or specific opioid receptors to LTP induction in the direct medial and lateral perforant path projections to the CA3 region.

In the present study, we characterized LTP in the direct medial and lateral perforant path projections to the CA3 region in anesthetized adult rats, and the contributions of NMDA receptors and μ- and δ-opioid receptors to LTP induction in these pathways. Some of these data were presented earlier in preliminary form (Derrick et al. 1993).

**Methods**

Adult male Sprague-Dawley rats (350–450 g, Harlan Laboratories, Indianapolis, IN) were anesthetized with pentobarbital sodium (60 mg/kg ip) and mounted in a stereotaxic frame. Rats were maintained at 39°C with a thermal pad, and a surgical level of anesthesia was maintained by supplementary doses of pentobarbital (20 mg · kg⁻¹ · h⁻¹). All experiments were performed under National Institutes of Health guidelines for the care and use of animals in research.

In the intact animal, afferents of the medial and lateral entorhinal cortex that comprise the perforant path remain segregated within the angular bundle. This allows for the selective stimulation of these aspects of the perforant path in vivo (McNaughton 1980; McNaughton and Barnes 1977). Perforant path responses were evoked by stimulation of the extreme dorsomedial aspect of the angular bundle for medial perforant path activation [AP – 8.1, ML 4.0, DV 2.3 mm from Bregma, using the coordinates of Paxinos and Watford (1989)], or the extreme ventrolateral aspect of the angular bundle for lateral perforant path activation [AP – 8.0, ML 5.0, DV 2.8 mm (see Bramham et al. 1991a)]. Stimulation (monophasic constant current pulses, 0.2 ms duration) was delivered via electrodes constructed from twisted Teflon-coated stainless steel wire (0.008 mm diam, A-M Systems).

Responses evoked in the dentate gyrus by medial and lateral perforant path are well defined (McNaughton 1980; McNaughton and Barnes 1977) and can be identified by differences in field excitatory postsynaptic potential (EPSP) slopes and responses to paired pulses. The CA3 region receives direct medial and lateral perforant path input from the same layer II stellate cells that project to the dentate gyrus. However, in the CA3 region, differences in medial and lateral perforant path field EPSP slopes are less obvious, possibly due to the proximity of the termination zones in the most distal dendritic regions of CA3 pyramidal cells. Therefore lateral or medial perforant path responses were first isolated by recording from the hilar region of the dentate gyrus. Following placement of stimulating electrodes at depths that produced field responses corresponding to stimulation of the medial or lateral perforant path, a cannula, constructed from an Expoxylite-insulated 33 g stainless steel cannula exposed by cutting the tip of the cannula, was lowered into the pyramidal layer of area CA3b region of the hippocampus. The cannula allowed for the recording of field EPSPs and local application of drugs at the same site (Fig. IA). Responses were amplified ×1,000 by a differential AC amplifier with a skull screw used as the indifferent electrode.

Measurements of the magnitude of CA3 responses were confined to the initial slope of field EPSPs measured between 2 and 4 ms following response onset. Previous studies by ourselves and others demonstrate the monosynaptic nature of early (<5 ms) components of the perforant path responses recorded in the CA3 region (Breindl et al. 1994; Yeckel and Berger 1990). CA3 responses phase reverse on penetration of the CA3 pyramidal cell layer (as determined by audio monitoring of injury-induced cell discharge), and display extracellular population spikes peak latencies that are earlier than those observed in the dentate gyrus, allowing verification of responses generated locally in the CA3 region (Breindl et al. 1994; Yeckel and Berger 1990).

However, because the perforant path projects to both the CA3 region and the dentate gyrus, a potential problem in recording perforant path–CA3 responses is that activation of dentate granule cells could result in CA3 pyramidal cell activation disynaptically via granule cell axons (the mossy fibers), leading to a contamination of monosynaptic perforant path–CA3 responses by disynaptic mossy fiber–CA3 responses. We therefore confined our measures of synaptic activity to the rising phase (slope) of the CA3 field EPSP. This component of the evoked response occurs prior to dentate gyrus spike generation, allowing for monosynaptically evoked perforant path–CA3 field EPSP slopes to be measured in vivo without the possibility of contamination from disynaptically elicited mossy fiber–CA3 responses. Furthermore, because dentate population spikes cannot follow stimulation trains >20 Hz (Breindl et al. 1994), it is unlikely that field EPSP slopes of the perforant path–CA3 response were contaminated by disynaptic activation of the mossy fibers during either low- or high-frequency stimulation.

**Experimental design, drug application, and data analysis**

Low-frequency responses were evoked at 0.066 Hz using a current intensity that elicited responses that were 50% of the asymptotic field EPSP amplitudes. Responses were amplified, filtered at 0.1 Hz to 10 kHz, digitized (10 kHz), and then stored for off-line analysis.

For each experiment, baseline perforant path responses were collected for a minimum of 20 min. Following collection of baseline responses, drugs were applied over a 5-min period (1 μl at a rate of 0.2 μl/min). For studies employing opioid receptor antagonists, we used the μ-receptor–selective antagonist Cys²-Tyr³–Omr⁻Pen⁻amide (CTOP; RBI/Sigma, St. Louis, MO) in 3-nmol quantities dissolved in lactated Ringer. This quantity was found to be effective in blocking mossy fiber LTP in the CA3 region in vivo (Derrick et al. 1992), and reversing the effects of μ-agonists applied to the CA3 region (Derrick and Martinez 1994). We also used the δ-receptor–selective antagonist naltrindole HCl [NTI; (22) RBI/Sigma, St. Louis, MO] in 3- and 10-nmol quantities dissolved in a 10% DMSO/lactated Ringer solution, the competitive NMDA receptor antagonist (±)-3-(2-carboxypropyl)propaconitrile-4-yl)propyl-1-phosphonic acid [±]-CPP, RBI 3 nmol], or the lactated Ringer vehicle alone (pH 7.4). All drugs were delivered to the CA3 pyramidal cell region through the 33 gauge stainless steel cannula via pressure ejection. In studies in which systemic administration

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of the NMDA receptor antagonist was employed. (±)-CPP was dissolved in water, and a single dose (10 mg/kg) (Abraham and Mason 1988) was administered intraperitoneally 90 min prior to LTP induction.

After cessation of local drug delivery, medial or lateral perforant path responses were collected for an additional 10 min at 0.066 Hz to assess effect of each drug on synaptic responses. At the end of this 10-min period, medial or lateral perforant path fibers were tetanized using five trains composed of 10 50-ms, 400-Hz bursts (20 pulses/burst) delivered every 200 ms (train duration of 2.3 s), with a 15-s interval between each train. Electroencephalograph (EEG) was monitored following delivery of each train, and none of the animals displayed afterdischarges following tetanization. Following high-frequency tetanization, evoked responses were again collected at the rate of 0.066 Hz for 1 h.

For statistical analysis, the magnitude of LTP or long-term depression (LTD) is expressed as the percent change in the slope of the field EPSP measured at 25–30 min period posttetanus as compared with the predrug baseline. Treatment effects on LTP magnitude were compared with animals receiving only the vehicle and evaluated using a single-df ANOVA (Keppel and Zedeck 1991). Electrode placements were verified by both characteristic responses evoked in the dentate gyrus and CA3 region, and randomly in 10% of the subject population using standard histological techniques employing Nissl stains.

RESULTS

Characteristics of medial and lateral perforant path–CA3 responses in vivo: comparison with dentate gyrus responses

Consistent with previous reports (Breindl et al. 1994; Yeckel and Berger 1990), stimulation of either the lateral or medial perforant path elicited field EPSPs in both the dentate and CA3 regions. Responses observed in both the CA3 and dentate regions were similar in several respects. First, medial perforant path responses recorded in vivo displayed paired-pulse depression, with responses of the second of two stimuli at pulse intervals of 30–50 ms showing a depression of field EPSP slopes. By contrast, paired lateral perforant path responses showed a pronounced facilitation of field EPSPs, similar to perforant path–dentate responses (Fig. 1C). These characteristics were reported previously for both lateral and medial responses in vitro (Berzhan-skaya et al. 1998; Wu and Leung 1999) and in vivo (Breindl et al. 1994).

Medial and lateral perforant path–CA3 responses displayed important differences when compared with responses
observed simultaneously in the dentate gyrus. Consistent with previous in vivo studies (Breindl et al. 1994; Yeckel and Berger 1990; but see Wu and Leung 1999), the latency of field EPSP onset in the dentate and CA3 regions were identical (2–3 ms); however, population spikes observed in responses recorded in the CA3 region displayed onsets and peak latencies that preceded those observed in the dentate gyrus. The disparity in lateral perforant path–CA3 versus dentate gyrus population spikes is particularly striking (Fig. 1B). This finding, taken along with previous demonstrations that both lateral and medial perforant path-CA3 responses phase reverse as the electrode penetrates the CA3 pyramidal cell layer, and follow stimulation at frequencies of 50 Hz (Breindl et al. 1994), indicating that perforant path responses observed in the CA3 region are monosynaptic and locally generated.

**Induction of medial, but not lateral, perforant path LTP is blocked by both local application and systemic administration of the NMDA receptor antagonist (±)-CPP**

We first assessed the effect of local application of a 3-nmol quantity of the competitive NMDA receptor antagonist (±)-CPP. Application of a 1-μl quantity had no consistent effect on the magnitude of field EPSP slopes of either medial or lateral perforant path responses (Fig. 2, A and B). However, local application of (±)-CPP was effective in blocking the induction of LTP in medial perforant path–CA3 responses (Fig. 2, A and C), with a mean increase in medial perforant path responses of 109 ± 5% following application of (±)-CPP, and 132 ± 8% (mean ± SE) following application of the lactated Ringer vehicle (F [1,10] = 6.02, P < 0.05). By contrast, application of the same quantity of (±)-CPP that is effective in blocking LTP

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\text{CPP blocked medial, but not lateral, perforant path LTP induction as compared with vehicle control (for medial perforant path, n = 6 for the Ringer vehicle, n = 6 for CPP; for the lateral perforant path, n = 9 for the Ringer vehicle; n = 4 for CPP, \ast \ P < 0.05).}
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![Graph A](image1.png)  
**A** Comparison of the effects of local application of a 3-nmol quantity of the N-methyl-D-aspartate (NMDA) receptor antagonist (±)-3-(2-carboxypiperazin-4-yl) propyl-1-phosphonic acid [(±)-CPP] vs. lactated Ringer vehicle on field responses and long-term potentiation (LTP) induction at the perforant path–CA3 synapse. Point plot of field EPSP slopes of medial perforant path responses (A) and lateral perforant path-CA3 responses (B) showing baseline responses (a) and responses after application of a 3-nmol quantity (1-μl volume) of (±)-CPP (■) or vehicle (○), and following theta burst stimulation (b). Insets are representative responses for a and b. Calibration: 0.5 mV, 5 ms. C: summary of the magnitude of potentiation as measured from 25–30 min following LTP induced in the presence of CPP or vehicle. CPP blocked medial, but not lateral, perforant path-CA3 LTP induction as compared with vehicle control (for medial perforant path, n = 6 for the Ringer vehicle, n = 6 for CPP; for the lateral perforant path, n = 9 for the Ringer vehicle; n = 4 for CPP, *P < 0.05).
at medial perforant path–CA3 synapse was ineffective in blocking the induction of LTP in lateral perforant path–CA3 responses (Fig. 2, B and C). In fact, the magnitude of LTP in the presence of (±)-CPP appeared greater than the vehicle alone, although this difference was not significant (Fig. 2C; mean increase in lateral perforant path responses = 146 ± 8% following application of (±)-CPP, 131 ± 6% following application of the lactated Ringer control; F[1,7] = 2.3, P > 0.05).

We also assessed the effect of systemic administration of CPP on lateral perforant path LTP using a systemic dose (10 mg/kg) found effective in blocking NMDA receptor–dependent LTP at other hippocampal synapses (Abraham and Mason 1988). Ninety minutes following intraperitoneal administration of 10 mg/kg CPP, LTP was induced by stimulation of either the lateral or medial perforant path, and LTP was assessed 25–30 min posttetanus. We found that while CPP was effective in blocking medial perforant path LTP, this dose was ineffective in blocking LTP in lateral perforant path afferents (Fig. 3, A and B; mean change in medial perforant path responses = 106 ± 8% following (±)-CPP administration, mean change in lateral perforant path responses = 140 ± 5%; F[1,7] = 2.3, P > 0.05).

**Induction of lateral, but not medial, perforant path LTP is blocked by the µ-opioid receptor–selective antagonists CTOP**

Local application of a 3-nmol quantity of the competitive µ-opioid receptor antagonist CTOP did not alter either medial or lateral perforant path–CA3 responses; however, CTOP effectively blocked LTP induction in lateral perforant path–CA3 responses (Fig. 4, B and C; mean increase in lateral perforant path responses = 103 ± 9% following application of CTOP, 129 ± 6% following application of vehicle, F[1,8] = 8.0, P < 0.05). Application of this quantity of CTOP did not block LTP induction in medial perforant path–CA3 responses (Fig. 4, A and C), although a nonsignificant reduction in the overall magnitude of medial perforant path–CA3 LTP was observed (mean increase in medial perforant path responses = 117 ± 6% following application of CTOP, 132 ± 8% following application of vehicle only, F[1,13] = 2.20, P > 0.05).

**Induction of lateral perforant path–CA3 LTP is not blocked by δ-opioid receptor antagonists**

Previous studies in the dentate gyrus indicate that both µ- and δ-antagonists are effective in blocking LTP induction at lateral perforant path–dentate synapses (Bramham and Survey 1996). We therefore assessed the effect of the δ-opioid receptor antagonist naltrindole HCl (NTI) in 3- and 10-nmol quantities on LTP induced at lateral perforant path–CA3 synapses (Figs. 5 and 6). Because of the limited solubility of NTI in aqueous solutions, in solutions used for application of 3- and 10-nmol quantities of NTI, NTI was first dissolved in dimethylsulfoxide (DMSO), and diluted with lactated Ringer (yielding a 10% concentration of DMSO). NTI in quantities of 3 nmol had no significant effect on the induction of LTP in lateral perforant path responses (Fig. 5, B and C; mean increase in lateral
perforant path responses following application of a 3-nmol quantity of the \( \delta \)-opioid receptor antagonist Cys\(^2\)-Tyr\(^3\)-Orn\(^5\)-Pen\(^7\)-amide (CTOP) and lactated Ringer vehicle on field responses and LTP induction at the perforant path–CA3 synapse. Point plot of field EPSP slopes of medial perforant path responses (A) and lateral perforant path–CA3 responses (B) showing baseline responses (a) and responses following application of a 3-nmol quantity (1-\( \mu \)l volume) of CTOP (b) or vehicle (c), and following delivery of theta burst stimulation (b). Insets are representative responses for a and b. Calibration: 0.5 mV, 5 ms. C: summary of the magnitude of potentiation at 25–30 min following LTP induced after CTOP or vehicle. CTOP blocked lateral, but not medial perforant path–CA3 LTP induction as compared with vehicle control (for medial perforant path, \( n = 6 \) for the vehicle, \( n = 9 \) for CTOP; for the lateral perforant path, \( n = 9 \) for the vehicle; \( n = 5 \) for CTOP; * \( P < 0.05 \)).

Previous studies demonstrate lateral perforant path projections to the dentate gyrus display LTP that is sensitive to \( \delta \)-antagonists. To assure that \( \delta \)-antagonism has no effect on lateral perforant path LTP, we assessed the effect of a larger (10 nmol) quantity of NTI (Fig. 6). Again, lateral perforant path–CA3 LTP induction was not affected by this larger quantity of NTI (Fig. 6, A and B; mean increase in lateral perforant path responses following application of 10-nmol quantities of NTI = 122 \( \pm \)14% following application of equivalent quantities of the DMSO/lactated Ringer vehicle = 124 \( \pm \)20%, \( F [1,6] = 0.002, P > 0.05 \)).

We also assessed the effect of a 10-nmol quantity of NTI on medial perforant path–CA3 responses. Application of 10 nmol of NTI depressed medial perforant path–CA3 field EPSPs, an effect not observed in lateral perforant path–CA3 responses. The induction of medial perforant path–CA3 LTP was attenuated by 10 nmol of NTI (mean increase in medial perforant path responses following application of 10-nmol quantities of NTI = 117 \( \pm \)8% following application of equivalent quantities of the DMSO/lactated Ringer vehicle = 89 \( \pm \)6%, \( F [1,8] = 7.57, P < 0.05 \), data not shown). However, this effect was not consistent. In addition, 10-nmol quantities of naltrexone (NTX), a relatively nonselective opioid receptor antagonist from which NTI is derived, produced a similar reduction in medial perforant path baseline, but was not effective in blocking medial perforant path LTP (data not shown). Thus both the selective \( \delta \)-antagonist NTI and the nonselective opioid receptor
antagonist NTX impaired medial, but not lateral perforant path–CA3 responses, suggesting a possible effect of δ-antagonists on synaptic responses. The attenuation of medial perforant path LTP by NTI, but not NTX suggests that the action of NTI on LTP induction likely reflects nonselective effects of NTI not mediated by opioid receptors.

**DISCUSSION**

The present results confirm previous studies demonstrating that perforant path projections to area CA3 display LTP (Berger and Yeckel 1991; Breindl et al. 1994), and extend these findings to demonstrate that both lateral and medial perforant path projections to area CA3 are differentially sensitive to NMDA receptor and δ- and μ-opioid receptor antagonists. This suggests distinct mechanisms of LTP induction in cortical projections to the CA3 region of the hippocampus.

Both medial and lateral perforant path–CA3 synaptic responses display similarities to perforant path dentate gyrus responses. As reported previously by ourselves and others (Berzhanskaya et al. 1998; Breindl et al. 1994), medial perforant path–CA3 responses showed a depression in field EPSP slopes using a paired pulse paradigm with pulse intervals of 30–50 ms, whereas lateral responses showed a marked facilitation at this interval, similar to medial and lateral perforant path responses that are observed in dentate gyrus responses (McNaughton 1980). Our studies also confirm differences between dentate and CA3 responses evoked by perforant path stimulation, as reported previously (Breindl et al. 1994; Yeckel and Berger 1990). Activation of CA3 pyramidal cell action potentials (as reflected in synchronous cell discharge, or population spikes) consistently preceded dentate granule cell activation by 0.5–5 ms (Fig. 1B). These data confirm that CA3 pyramidal cell activation precedes activation of dentate granule cells following medial and lateral perforant path stimulation (Breindl et al. 1994; Yeckel and Berger 1990). As noted by
Yeckel and Berger (1990), this finding suggests that pyramidal cells in the CA3 region are the first cells in the hippocampal formation to discharge in response to perforant path stimulation.

Consistent with previous studies at the medial perforant path–dentate gyrus synapse, the induction of LTP at medial perforant path–CA3 synapses is blocked by both local application and systemic administration of the NMDA receptor antagonists (±)-CPP (Bramham et al. 1991b). However, local application or systemic administration of this NMDA antagonist failed to block LTP induction in lateral perforant path responses. This suggests that NMDA receptors are not necessary for LTP induction in this pathway, as reported previously in the lateral perforant path projections to the dentate gyrus (Bramham et al. 1991b; but see Zhang and Levy 1992). However, these data are convincing in that (±)-CPP failed to block lateral perforant path LTP when applied either locally or systemically in quantities that blocked effectively LTP at adjacent medial perforant path–CA3 synapses. It therefore is unlikely that a lack of effect of locally applied (±)-CPP on lateral perforant path–CA3 LTP was the result of insufficient quantities of the drug. In addition, because lateral perforant path responses are generally less effective in depolarizing postsynaptic cells, it is unlikely that lateral, but not medial, perforant path stimulation is able to overcome a block of NMDA receptors. Thus as observed at the mossy fiber–CA3 synapse (Harris and Cotman 1986) and the lateral perforant path–dentate synapse (Bramham et al. 1991b), the lateral perforant path–CA3 synapse also appears to utilize mechanisms of LTP induction that do not require NMDA receptor activation. This does not necessarily imply that NMDA receptors may not normally contribute to LTP induction (e.g., see Milner and Drake 2001); rather, it appears that alternative, NMDA receptor–independent mechanisms can allow for the induction of lateral perforant path–CA3 LTP. For example, a µ-opioid receptor–mediated decrease in GABAergic inhibition (Bramham and Sarvey 1996) and a subsequent increase in postsynaptic depolarization normally may contribute to LTP induction by facilitating calcium influx via both NMDA receptors (Milner and Drake 2001) and other mechanisms (such as voltage-dependent calcium channels). Presumably these other NMDA receptor–independent mechanisms are sufficient for lateral perforant path–CA3 LTP induction in the presence of NMDA antagonists.

The present studies indicate differences between lateral perforant path LTP induced at CA3 and dentate targets. Similar to previous studies in the dentate gyrus (Bramham and Sarvey 1996), antagonists selective for the µ-type opioid receptor effectively blocked lateral perforant path LTP induction in area CA3. However, application of the δ-opioid receptor antagonists naltrindole in 3- and 10-nmol quantities was ineffective in blocking lateral perforant path–CA3 LTP induction. This is in contrast to previous studies demonstrating that δ-opioid receptor antagonists block lateral perforant path LTP induction in the dentate gyrus (Bramham and Sarvey 1996). The difference in sensitivities to selective opioid receptor antagonists in the dentate and CA3 corresponds with differences in opioid receptor distribution between these regions: In the dentate gyrus, both δ- and µ-receptors show similar distributions within the regions of the dentate molecular layer where both the medial and lateral perforant pathways terminate (Crain et al. 1986; McLean et al. 1987). By contrast, both labeling studies and ligand displacement studies employing activation of opioidergic projections (Crain et al. 1986; Wagner et al. 1990) demonstrate that µ-receptors are the predominant opioid receptor within the stratum lacunosum-moleculare of area CA3 where lateral perforant path afferents to CA3 pyramidal cells terminate. In fact, this region displays the highest concentration of µ-receptors within the hippocampal formation (Crain et al. 1986).
1986). Thus the blockade of lateral perforant path–CA3 LTP by \( \mu \)-opioid receptor antagonists corresponds to the localization of \( \mu \)-opioid receptors at the target of lateral perforant path projections to the stratum lacunosum moleculare of the CA3 region.

Numerous studies implicate opioid receptor activation in LTP induction in opioidergic synaptic systems of the hippocampal formation, including lateral perforant path projections to area CA3 and the dentate gyrus, and the mossy fiber projection to area CA3 (Bramham et al. 1988; Breindl et al. 1994; Derrick et al. 1992). Opioid peptides are known to produce excitation of pyramidal cells as a result of inhibition of GABAergic transmitter release (Cohen et al. 1992) mediated by activation of opioid receptors. Because agents that facilitate postsynaptic depolarization facilitate LTP induction (Wigstrom and Gustafsson 1985), an opioid-mediated block of GABAergic inhibition may facilitate postsynaptic depolarization, and facilitate LTP induction. Recent studies offer direct support for this view and demonstrate that the blockade of lateral perforant path–dentate LTP by opioid receptor antagonists can be reversed by GABA antagonists (Bramham and Sarvey 1996). Thus the disinhibitory effects of opioid peptides are a likely mechanism underlying the contribution of \( \mu \)-opioid receptors in LTP induction in the lateral perforant path–dentate system. It is reasonable to suspect that similar disinhibitory actions of endogenous \( \mu \)-opioid receptor agonists act in a similar manner in lateral perforant path inputs to CA3, particularly in light of the findings that the \( \mu \)-receptor is the predominant opioid receptor in the region of the stratum lacunosum-moleculare where the lateral perforant path afferents to CA3 terminate (Crain et al. 1986), and that \( \mu \)-opioid receptor agonists produce disinhibition in the CA3 region (Caudle and Chavkin 1990), and are found on GABAergic terminals, which are the suggested site of opioid actions (Cohen et al. 1992). Together, these results suggest that the activation of \( \mu \)-opioid receptors are necessary for LTP induction in lateral perforant path afferents, most likely via the actions of \( \mu \)-opioids on GABA release, and a subsequent increase in postsynaptic depolarization (Bramham and Sarvey 1996).

In models of associative memory functions of the hippocampus, direct perforant path projections are suggested to mediate distinct and essential functions (Marr 1971; Morris and McNaughton 1987; Treves and Rolls 1994). Treves and Rolls (1994) propose that plasticity induced in the direct perforant path–CA3 projection during learning may serve subsequently as the primary input initiating recall. In this view, during learning, associative changes in perforant path–CA3 and commissural/recurrent CA3 synapses result from conjunctive activity. Recall is initiated by activation of modified perforant path–CA3 connections and the subsequent reactivation of the CA3 associative/recurrent collateral system (Treves and Rolls 1994). Support for these models is provided by the present study, which indicates that direct perforant path–CA3 connections display LTP, an essential feature of these models of hippocampal information processing.

The present study demonstrates that both lateral and medial perforant path projections to the hippocampal CA3 region are capable of displaying long-term potentiation, and that \( \mu \)-opioid receptors and NMDA receptors differentially contribute to LTP induction at lateral and medial perforant path–CA3 synapses. The contributions of \( \mu \)-opioid receptors to LTP induction in the lateral perforant path projections to the CA3 region differ from those observed at the dentate targets: while both \( \mu \)- and \( \delta \)-receptor antagonists block LTP induction at lateral perforant path–dentate synapses (Bramham and Sarvey 1996), only \( \mu \)-receptor blockade was effective in blocking LTP induction at lateral perforant path–CA3 synapses. By contrast, antagonism of NMDA receptors block the induction of LTP at medial perforant path–CA3 synapses. These results suggest that opioid peptides play different roles in LTP induction not only among different afferent systems, but at different postsynaptic targets as well, and suggest complex, multifunctional roles of opioid peptides and their receptors in plastic changes in synaptic systems of the hippocampal formation.

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