Prenatal Morphine Exposure Attenuates the Maintenance of Late LTP in Lateral Perforant Path Projections to the Dentate Gyrus and the CA3 Region In Vivo

D. M. Villarreal, B. Derrick, and I. Vathy

1Cajal Neuroscience Research Center, The Department of Biology, The University of Texas at San Antonio, San Antonio, Texas; and Departments of Psychiatry and Behavioral Sciences and Neuroscience, Albert Einstein College of Medicine, Bronx, New York

Submitted 30 August 2007; accepted in final form 11 January 2008

Villarreal DM, Derrick B, Vathy I. Prenatal morphine exposure attenuates the maintenance of late LTP in lateral perforant path projections to the dentate gyrus and the CA3 region in vivo. J Neurophysiol 99: 1235–1242, 2008. First published January 16, 2008; doi:10.1152/jn.00981.2007. Previously we reported that prenatal exposure to morphine twice daily during gestation decreases proenkephalin levels in adult progeny within the brain, including the dentate gyrus and alters µ and δ opioid receptors in the hippocampal CA3 region. The lateral aspect of the perforant path contains and releases enkephalin-derived opioid peptides, and induction of long-term potentiation (LTP) in lateral perforant path projections to both the dentate gyrus and the hippocampal CA3 region is blocked by antagonists of opioid receptors. Thus LTP induction at these synapses involves opioid receptor activation mediated by the release of proenkephalin-derived opioid peptides with lateral perforant path activation. Here we show in adult behaving animals, neither LTP induction nor the early phase of LTP (E-LTP) maintenance is altered by prenatal morphine exposure in the lateral perforant path projections to the dentate gyrus and the CA3 region. However, maintenance and longevity of late LTP (L-LTP), as reflected in the magnitude of LTP over days, was attenuated in animals prenatally exposed to morphine. In contrast, in medial perforant path projections to the dentate gyrus and CA3 region, both LTP induction and the maintenance of E- and L-LTP were unaffected by prenatal morphine treatment. Thus a brief prenatal exposure to the opiate morphine produces sustained, and possibly permanent, alterations in L-LTP in the opioidergic lateral perforant path projection. This suggests that prenatal morphine exposure disrupts LTP via disruption of opioid mechanisms involved in LTP maintenance or via disruption of opioid receptor activation during LTP induction, which can subsequently alter LTP maintenance.

INTRODUCTION

The problem of drug abuse among pregnant women is of major concern; it is estimated that >250,000 infants, children, and adults, or 1 in 1,000 people, have been exposed to opiates in early life in the United States (Zagon and McLaughlin 1992). The discovery of endogenous opioid systems that regulate somatic and neural growth raises the possibility that exogenous opiates, when present at inappropriate times or in nonphysiological concentration, can alter neural development. Thus offspring exposed to an excess of exogenous opiates as a result of maternal drug abuse may exhibit long-term deleterious alterations in psychological, behavioral, and physiological processes that may persist into adulthood.

Maternal drugs of abuse including opiates typically affect those systems in the CNS that are developing in the fetus at the time of drug exposure (Kellog 1992). Prenatal morphine exposure during the period when opioid receptors first begin to appear in the rat brain (the 14th day of fetal life or perhaps even earlier; Clendeninn et al. 1976) has a number of long-term effects on adult male progeny, including decreasing proenkephalin-derived opioid peptides in the perforant path (Schildler et al. 2004), increasing mu (µ) and delta (δ) opioid receptors in the hippocampal formation, possibly in response to enkephalin depletion (Rimanóczy and Vathy 1995; Rimanóczy et al. 2001; Vathy et al. 2000, 2002), as well as alterations in normal synaptic transmission in opioid peptide-containing afferents (Zagon and McLaughlin 1992). These findings together suggest that limited gestational exposure to opiates during early fetal development may produce long-lasting alterations in normal opioidergic function in the brain.

Long-term potentiation (LTP) is a widely studied phenomenon that is thought to reflect the synaptic mechanisms of memory formation (Bliss and Collingridge 1993; Pastalkova et al. 2006; Villarreal et al. 2002; Whitlock et al. 2006). LTP is a sustained increase in synaptic strength that follows high-frequency synaptic activity and is thought to be one of the cellular processes involved in memory storage (Bliss and Collingridge 1993). The lateral perforant path (LPP) is a projection that originates in the lateral entorhinal cortex and projects to the dentate gyrus (DG) and the CA3 region of the hippocampal formation. LTP in LPP afferents to both the DG and the hippocampal CA3 region displays features that distinguish it from LTP in medial perforant path (MPP) projection from the medial entorhinal cortex. The LPP contains and releases proenkephalin-derived opioid peptides (Chavkin et al. 1983; Commons and Milner 1995; Fredens et al. 1984; Gall et al. 1981), and induction of LTP at LPP, but not MPP, synapses, in both the DG and the hippocampal CA3 region is blocked by the opioid receptor antagonist naloxone, as well as antagonists selective for µ or δ opioid receptor antagonists (Bramham and Sarvey 1996; Bramham et al. 1988, 1991b; Breindl et al. 1994; Do et al. 2002). Moreover, LTP at LPP-DG synapses is not blocked by N-methyl-D-aspartate (NMDA) receptor antagonists in vivo (Bramham et al. 1991a). Similarly, in LPP projections to area CA3, LTP induction also is insensitive to NMDA receptor antagonists in vivo (Do et al. 2002; Kosub et al. 2005).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Address for reprint requests and other correspondence: I. Vathy, Dept. of Psychiatry and Behavioral Sciences, Albert Einstein College of Medicine, 1300 Morris Park Ave., Ullmann Bldg., 111, Bronx, NY 10461 (E-mail: ivathy@optonline.net).
Given that opioid receptor activation in the LPP is necessary for LTP induction and that proenkephalin-derived opioid peptides are decreased in the LPP by prenatal exposure to morphine, we hypothesize that prenatal exposure to morphine will have a deleterious effects on LTP induction in LPP afferents. Only one previous study has addressed the long-term effects of prenatal morphine exposure on synaptic plasticity (Velisek et al. 2000). This in vitro study found that submaximal stimulation of the mixed perforant path-DG input in hippocampal slices taken from adult animals exposed prenatally to 10 mg/kg of morphine (2 times/d) on gestation days 11–18 resulted in long-term depression (LTD), whereas neither LTP nor LTD was observed following submaximal stimulation in vehicle-treated controls.

In this study, we investigated the effect of prenatal morphine exposure on the induction and maintenance of LTP in both the MPP and the enkephalinergic LPP to both the DG and the CA3 region of the hippocampus in awake, freely moving, adult rats with permanently implanted electrodes. We report that in utero exposure to opiates during 11–18 days of gestation dramatically attenuates the maintenance and longevity, but not the initial induction of LPP LTP in adult morphine-exposed progeny. These results indicate that exposure to opiates during mid to late gestation may produce sustained, and perhaps permanent, dysfunction in brain opioidergic systems. Because opioid peptides are suggested to be involved in both the induction (Bramham et al. 1988, 1991b) and maintenance of LPP LTP (Bramham 1992), this effect may underlie the sustained impairments in learning and memory seen in rats exposed prenatally to morphine (Šlamberová et al. 2001).

METHODS

Drug treatment of pregnant rats

Sprague-Dawley female rats were purchased from Taconic Farms (Germantown, NY) on the eighth day after conception. On arrival, pregnant rats were housed individually in maternity cages with food and water available ad libitum and maintained in a temperature-controlled colony room with free access to food and water on a reversed 14-h (light)-10-h (dark) cycle with lights off at 1100 h. Each pregnant rat was randomly assigned to receive morphine or saline (control) injections. Beginning on day 11 and continuing through day 18 postconception, pregnant females were injected twice daily, once at 0800 h and once at 2000 h, with morphine sulfate or saline (0.025 mm) were placed in both the DG (AP −3.5, ML −2.0; DV −3.2 mm) and the hippocampal CA3 region (AP −3.5, ML −3.5; DV −3.5 mm; Fig. 1) (Paxinos and Watson 1994). Stimulation of the LPP and MPP evoked monosynaptic responses in the DG and CA3 as described previously (Do et al. 2002) and was verified by decreased field excitatory postsynaptic potential (EPSP) slopes as the stimulating electrode passed the more dorsal MPP fibers, as well as paired pulse facilitation with 30- and 50-ms intervals, which is observed only in the LPP (Breindl et al. 1994; Do et al. 2002; McNaughton 1980). Electrodes were fixed into position and mounted to the skull with dental acrylic cement. Following surgery, animals were treated with a bolus injection intramuscularly of an antibiotic (penicillin G/gentamycin, 1,500 IU) and free access to Rimadyl (carprofen, 4 mg/kg/d for 3 days, Bio-Serve, Frenchtown, NJ), and allowed to recover for 2 wk with daily handling.

Responses were evoked in awake, behaving animals using constant current biphasic pulses (30–400 μA, 0.1 ms/pulse) delivered at 0.0667 Hz. Input/output (I/O) curves generating response size as a function of current intensity were collected before each experiment to determine the current intensities that evoked field EPSPs that were 50% of the maximal (asymptotic) DG field EPSP response. All responses were evoked using a current intensity that elicited responses 50% of maximal amplitude. Responses were preamplified with junction gate field-effect transistor preamplifiers (DMV, PCB Express), amplified 500–1,000 times, filtered (0.1 Hz to 10 kHz), digitized (10 kHz), and recorded for off-line analysis. Response magnitudes were calculated by determining the slope of the field EPSP (dV/dt) of responses, as calculated by measurement of the

Electrophysiology

Animals were allowed to acclimate to their environment for ≥1 wk before surgery. Between postnatal days 109 and 120, male Sprague-Dawley rats prenatally exposed to morphine or saline were anesthetized with sodium pentobarbital (65 mg/kg, ip) and mounted in a stereotaxic frame. Teflon-coated stainless steel wire was used to construct bipolar (0.035 mm) stimulating electrodes. Under sterile conditions, stimulating electrodes were placed in either the ventrolateral aspect of the angular bundle (AP 8.1, ML −2.5, DV 2.7 mm) or the dorsomedial aspect of the angular bundle or medial (AP 8.0, ML −2.3, DV 2.5 mm) to stimulate lateral and medial perforant path fibers, respectively. In each animal, monopolar recording electrodes (0.025 mm) were placed in both the DG (AP −3.5, ML −2.0; DV −3.2 mm) and the hippocampal CA3 region (AP −3.5, ML −3.5; DV −3.5 mm; Fig. 1) (Paxinos and Watson 1994). Stimulation of the LPP and MPP evoked monosynaptic responses in the DG and CA3 as described previously (Do et al. 2002) and was verified by decreased field excitatory postsynaptic potential (EPSP) slopes as the stimulating electrode passed the more dorsal MPP fibers, as well as paired pulse facilitation with 30- and 50-ms intervals, which is observed only in the LPP (Breindl et al. 1994; Do et al. 2002; McNaughton 1980). Electrodes were fixed into position and mounted to the skull with dental acrylic cement. Following surgery, animals were treated with a bolus injection intramuscularly of an antibiotic (penicillin G/gentamycin, 1,500 IU) and free access to Rimadyl (carprofen, 4 mg/kg/d for 3 days, Bio-Serve, Frenchtown, NJ), and allowed to recover for 2 wk with daily handling.

Responses were evoked in awake, behaving animals using constant current biphasic pulses (30–400 μA, 0.1 ms/pulse) delivered at 0.0667 Hz. Input/output (I/O) curves generating response size as a function of current intensity were collected before each experiment to determine the current intensities that evoked field EPSPs that were 50% of the maximal (asymptotic) DG field EPSP response. All responses were evoked using a current intensity that elicited responses 50% of maximal amplitude. Responses were preamplified with junction gate field-effect transistor preamplifiers (DMV, PCB Express), amplified 500–1,000 times, filtered (0.1 Hz to 10 kHz), digitized (10 kHz), and recorded for off-line analysis. Response magnitudes were calculated by determining the slope of the field EPSP (dV/dt) of responses, as calculated by measurement of the

FIG. 1. Diagram of permanent electrode placement for perforant path-dentate gyrus (DG) and CA3 responses. Recording electrodes were placed in the hilus below the DG and in the stratum pyramidale of the hippocampal CA3 region and affixed to the skull of anesthetized rats. Two weeks after recovery, responses in both the DG and the CA3 region were evoked by stimulation of either the dorsolateral [lateral perforant path (LPP)] or ventromedial [medial perforant pathway (MPP)] aspect of the angular bundle.
rational phase of the field EPSP slope over a 2- to 3-ms period beginning 2 ms after field EPSP onset.

Daily responses were collected in the animal’s home cage while in a soundproof isolation box. Daily responses were measured by collecting 10 responses at a rate of 0.33 Hz using the current intensity that elicited a response 50% of maximum. After ≥3 days of daily baseline responses, LTP was induced. Daily recordings were collected first, followed by 15 min of baseline responses evoked at 0.05 Hz. Following baseline response collection, LTP was induced by delivering five “theta burst” stimulation trains (five 400-Hz, 50-ms bursts delivered over an 850-ms period, interburst interval of 200 ms). Five theta burst trains (25 bursts total) were delivered three times at 5-min intervals (75 bursts total). All trains delivered while the animal was awake and alert. Responses were collected at the 0.05-Hz rate for an additional 1 h after tetanus. Data were analyzed and, where indicated, normalized and presented as the percent change with respect to amplitude of baseline responses collected 15 min before tetanus. Daily responses were collected for ≥7 days following LTP induction. LTP magnitude is presented as the percent change in field EPSP slopes relative to the average magnitude of baseline responses collected 3 days before tetanus.

Data analysis

LTP processes are typically defined as induction (the occurrence of LTP) and maintenance (the magnitude and longevity of induced LTP), with the latter divided into early LTP (E-LTP, <3 h) and late LTP (L-LTP, >24 h) phases. To assess the effects of prenatal morphine exposure on LTP induction, successful LTP induction was defined as the proportion of animals receiving theta bursts stimulation that showed increases in field EPSP slopes >25% above baseline after 1 h.

To assess the effects of prenatal morphine exposure on LTP maintenance, LTP magnitude was compared at 1 h (E-LTP) and daily (L-LTP) 1–7 days following LTP induction. Only animals in which LTP was observed at 1 h were used for comparisons of E- and L-LTP magnitude. E-LTP magnitude was calculated as the average percent increase from baseline measured 50–60 min following delivery of theta bursts and compared in drug-exposed and control animals using a one-way ANOVA. For measures of L-LTP maintenance, both LTP magnitude and longevity were measured. For L-LTP magnitude, response magnitudes were collected daily over the 1-wk period following LTP induction and compared among groups using a two-way mixed design repeated-measures ANOVA, with daily LTP magnitude as a single factor repeated measure. For measures of L-LTP longevity, post hoc Tukey tests for all pairwise comparisons from each group were used to compare average daily LTP magnitudes with baseline responses at day –1. LTP longevity was defined as the time to LTP decay, defined as the first day when daily averaged response did not differ significantly from baseline response magnitudes collected 1 day before LTP induction (Tukey tests, P > 0.05).

R E S U L T S

We first compared basal synaptic transmission in perforant path synapses in both the DG and CA3 region in rats exposed prenatally to either saline or morphine. As an estimate of basal synaptic strength, we used absolute values of the maximal (asymptotic) field EPSP slope (dV/dt in mV/ms) taken from the initial I/O curves collected from each animal. Variability in electrode placement results in maximal field EPSPs of varying magnitudes. However, this variability is likely homogeneous among animals. Thus any robust differences in measures of the average asymptotic (maximal) response as reflected in the 50% maximal field EPSP slope within groups can serve as an approximation of basal synaptic efficacy. Animals exposed prenatally to morphine showed similar basal synaptic responses in both LPP and MPP responses compared with saline-exposed animals. In the LPP, DG responses did not differ among saline-exposed animals [2.30 ± 0.57 (SE) mV/ms, n = 10] and morphine-exposed animals [2.10 ± 0.55 mV/ms; n = 11; F(1,19) = 0.06, P > 0.05]. In LPP-CA3 responses, there also was no significant difference among saline-exposed (1.40 ± 0.58 mV/ms; n = 10) and morphine-exposed animals [1.60 ± 0.057 mV/ms; n = 10; F(1,17) = 0.07, P > 0.05]. Differences in synaptic strength also were not observed in MPP-DG projections, with basal responses for saline-exposed animals (slope = 1.21 ± 0.34 mV/ms; n = 6) not differing from morphine-exposed animals [1.13 ± 0.20 mV/ms; n = 7; F(1,11) = 0.04, P > 0.05]. In MPP-CA3 responses, no differences in basal synaptic strength were observed among saline-exposed (slope = 1.17 ± 0.27 mV/ms; n = 6) and for morphine-exposed animals [slope = 0.08 ± 0.21 mV/ms; n = 7; F(1,11) = 0.84, P > 0.05].

We determined whether LTP induction is altered in morphine-exposed animals. Following a 15-min period of baseline responses, LTP was induced using a modified theta burst stimulation protocol (Villarreal et al. 2002). We assessed LTP induction by determining the number of animals displaying LTP (defined as an increase in responses >25% of baseline as measured 1 h following theta bursts). In LPP projections to the DG, LTP was observed in 7/10 of the saline-exposed control animals, whereas in the LPP-DG, LTP induction was observed in 7/10 of morphine-exposed animals. In LPP projections to the CA3 region, LTP was observed in 6/9 saline-exposed animals and 7/10 morphine-exposed animals. For MPP-DG responses, LTP was observed in 4/6 saline-exposed animals and 5/6 animals exposed prenatally to morphine, whereas MMP-CA3 LTP was observed in 3/6 saline-exposed and 5/7 morphine-exposed animals. Thus the occurrence of LTP following tetanus showed no apparent or significant differences in LTP induction in rats exposed prenatally with morphine.

Next, we assessed the maintenance of E-LTP by comparing LTP magnitude over the 1-h period following LTP induction. To assess E-LTP magnitude, potentiated responses were compared only among animals that displayed LTP (as defined by an increase >25% 1 h following LTP induction). For LPP-DG responses, rats exposed to morphine in utero showed no attenuation of E-LTP magnitude relative to saline-exposed controls as measured over 1 h (Fig. 2A), with field EPSP slope magnitudes for LPP-DG responses in morphine-exposed animals (177 ± 20%; n = 7) similar to saline-exposed animals [172 ± 15%; n = 7; F(1,12) = 0.03, P > 0.05]. Likewise, E-LTP at MPP-DG synapses was not altered by prenatal morphine exposure (Fig. 2B), and field EPSP slope magnitudes for LPP-DG responses in morphine-exposed animals (165 ± 8%; n = 5) did not differ from saline-exposed animals [199 ± 46%; n = 4; F(1,7) = 0.66, P > 0.05]. Perforant path-CA3 E-LTP also was not altered by morphine exposure. For LPP-CA3 responses (Fig. 2C), the magnitude of E-LTP in morphine-exposed animals (156 ± 19%; n = 7) did not differ from saline-exposed animals [222 ± 64%; n = 6; F(1,11) = 1.10, P > 0.05]. In MPP projections to the CA3 region, E-LTP magnitude among animals exposed prenatally to morphine (159 ± 10%; n = 5) did not differ from saline-exposed animals [170 ± 42%; n = 3; F(1,7) = 0.10, P > 0.05; Fig. 2D]. Thus prenatal morphine exposure did not alter either LTP induction or E-LTP magnitude as measured over a 1-h period.
We also assessed L-LTP maintenance by determining the magnitude and longevity of the later, protein synthesis-dependent phase (>24 h) of LTP over the course of 1–7 days (Fig. 3). In the DG, animals normally maintain an increase in synaptic strength following theta burst stimulation for 3–7 days (Barnes 1979; Villarreal et al. 2002). Morphine-exposed animals displayed a significant reduction in LPP-DG LTP magnitude over the week of collection compared with saline controls \(F(1,13) = 7.92; P < 0.05\); Fig. 3A]. In contrast, in MPP projections to the DG, no effect of prenatal morphine exposure was observed on LTP magnitude \(F(1,9) = 0.099; P > 0.05\); Fig. 3B). A similar pattern was observed at LPP-CA3 synapses. However, whereas L-LTP of LPP-CA3 responses in morphine-exposed animals did not show a significant overall effect relative to saline-exposed controls \(F(1,13) = 2.38, P = 0.15\); Fig. 3C], a significant interaction was observed among these groups, with a significant reduction in LTP magnitude observed in morphine-exposed animals on days 1–4 \(F(1,7) = 2.37, P < 0.05\), Tukey test; Fig. 3C]. In contrast, prenatal morphine exposure had no significant effects on MPP-CA3 L-LTP compared with saline-exposed animals \(F(1,9) = 0.23; P > 0.05\); Fig. 3D).

We also estimated L-LTP longevity by determining the first day after LTP induction when averaged daily responses did not differ significantly from baseline responses collected on day 1 \(P > 0.05\). In the LPP projections to the DG, potentiated responses in animals exposed to morphine returned to baseline on average by day 1, whereas in saline-exposed animals, potentiation persisted until day 4 \(P > 0.05\) compared with day 1, Tukey test; Fig. 3A]. In the MPP-DG projection, potentiated responses in saline-treated animals returned to baseline by day 5 \(P > 0.05\), whereas potentiated responses in saline-treated animals returned to baseline by day 4 (Fig. 3B). In the LPP-CA3 projection, potentiated responses in animals exposed to morphine returned to baseline by day 1, whereas in saline-exposed animals, potentiated responses returned to baseline by day 4 \(P > 0.05\), Tukey test; Fig. 3C]. In
the MPP-CA3, the potentiated LTP responses returned to baseline by day 4 in morphine-exposed animals and by day 5 in saline-exposed animals \((P > 0.05; \text{Fig. 3D})\). Thus prenatal morphine exposure attenuated both L-LTP magnitude and L-LTP longevity in LPP-DG and -CA3 responses but not in the MPP-DG and -CA3 responses.

**DISCUSSION**

These results indicate that, although prenatal exposure to the opiate morphine had no effect on LTP induction or its initial maintenance, prenatal morphine attenuated the magnitude and longevity of L-LTP in opioidergic LPP projection to both the DG and the CA3 region of the hippocampus. In contrast, the maintenance of both E- and L-LTP at MPP-DG and -CA3 synapses was unaffected by prenatal exposure to morphine. This attenuation of L-LTP magnitude and longevity was observed in both the DG and CA3 targets of the LPP but not MPP, suggesting a selective effect of prenatal morphine exposure on LTP maintenance in the opioidergic LPP projection.

The LPP contains and releases proenkephalin-derived opioid peptides (Chavkin et al. 1983; Gall et al. 1981), and LPP projections to both the DG and CA3 region display LTP that is modulated by endogenous opioid peptides (Bramham 1992; Bramham and Sarvey 1996; Bramham et al. 1988, 1991b; Breindl et al. 1994; Do et al. 2002). Because prenatal exposure to morphine reduces opioid peptides (Schindler et al. 2004) and increases both \(\mu\) and \(\delta\) opioid receptors (Rimanóczy and Vathy 1995; Rimanóczy et al. 2001; Vathy et al. 2000, 2002), the prenatally morphine-exposed animals returned to baseline by day 4 in morphine-exposed animals and by day 5 in saline-exposed animals \((P > 0.05; \text{Fig. 3D})\). Thus prenatal morphine exposure attenuated both L-LTP magnitude and L-LTP longevity in LPP-DG and -CA3 responses but not in the MPP-DG and -CA3 responses.

**DISCUSSION**

These results indicate that, although prenatal exposure to the opiate morphine had no effect on LTP induction or its initial maintenance, prenatal morphine attenuated the magnitude and longevity of L-LTP in opioidergic LPP projection to both the DG and the CA3 region of the hippocampus. In contrast, the maintenance of both E- and L-LTP at MPP-DG and -CA3 synapses was unaffected by prenatal exposure to morphine. This attenuation of L-LTP magnitude and longevity was observed in both the DG and CA3 targets of the LPP but not MPP, suggesting a selective effect of prenatal morphine exposure on LTP maintenance in the opioidergic LPP projection.

The LPP contains and releases proenkephalin-derived opioid peptides (Chavkin et al. 1983; Gall et al. 1981), and LPP projections to both the DG and CA3 region display LTP that is modulated by endogenous opioid peptides (Bramham 1992; Bramham and Sarvey 1996; Bramham et al. 1988, 1991b; Breindl et al. 1994; Do et al. 2002). Because prenatal exposure to morphine reduces opioid peptides (Schindler et al. 2004) and increases both \(\mu\) and \(\delta\) opioid receptors (Rimanóczy and Vathy 1995; Rimanóczy et al. 2001; Vathy et al. 2000, 2002), the prenatally morphine-exposed animals returned to baseline by day 4 in morphine-exposed animals and by day 5 in saline-exposed animals \((P > 0.05; \text{Fig. 3D})\). Thus prenatal morphine exposure attenuated both L-LTP magnitude and L-LTP longevity in LPP-DG and -CA3 responses but not in the MPP-DG and -CA3 responses.

**DISCUSSION**

These results indicate that, although prenatal exposure to the opiate morphine had no effect on LTP induction or its initial maintenance, prenatal morphine attenuated the magnitude and longevity of L-LTP in opioidergic LPP projection to both the DG and the CA3 region of the hippocampus. In contrast, the maintenance of both E- and L-LTP at MPP-DG and -CA3 synapses was unaffected by prenatal exposure to morphine. This attenuation of L-LTP magnitude and longevity was observed in both the DG and CA3 targets of the LPP but not MPP, suggesting a selective effect of prenatal morphine exposure on LTP maintenance in the opioidergic LPP projection.

The LPP contains and releases proenkephalin-derived opioid peptides (Chavkin et al. 1983; Gall et al. 1981), and LPP projections to both the DG and CA3 region display LTP that is modulated by endogenous opioid peptides (Bramham 1992; Bramham and Sarvey 1996; Bramham et al. 1988, 1991b; Breindl et al. 1994; Do et al. 2002). Because prenatal exposure to morphine reduces opioid peptides (Schindler et al. 2004) and increases both \(\mu\) and \(\delta\) opioid receptors (Rimanóczy and Vathy 1995; Rimanóczy et al. 2001; Vathy et al. 2000, 2002), the prenatally morphine-exposed animals returned to baseline by day 4 in morphine-exposed animals and by day 5 in saline-exposed animals \((P > 0.05; \text{Fig. 3D})\). Thus prenatal morphine exposure attenuated both L-LTP magnitude and L-LTP longevity in LPP-DG and -CA3 responses but not in the MPP-DG and -CA3 responses.

**DISCUSSION**

These results indicate that, although prenatal exposure to the opiate morphine had no effect on LTP induction or its initial maintenance, prenatal morphine attenuated the magnitude and longevity of L-LTP in opioidergic LPP projection to both the DG and the CA3 region of the hippocampus. In contrast, the maintenance of both E- and L-LTP at MPP-DG and -CA3 synapses was unaffected by prenatal exposure to morphine. This attenuation of L-LTP magnitude and longevity was observed in both the DG and CA3 targets of the LPP but not MPP, suggesting a selective effect of prenatal morphine exposure on LTP maintenance in the opioidergic LPP projection.

The LPP contains and releases proenkephalin-derived opioid peptides (Chavkin et al. 1983; Gall et al. 1981), and LPP projections to both the DG and CA3 region display LTP that is modulated by endogenous opioid peptides (Bramham 1992; Bramham and Sarvey 1996; Bramham et al. 1988, 1991b; Breindl et al. 1994; Do et al. 2002). Because prenatal exposure to morphine reduces opioid peptides (Schindler et al. 2004) and increases both \(\mu\) and \(\delta\) opioid receptors (Rimanóczy and Vathy 1995; Rimanóczy et al. 2001; Vathy et al. 2000, 2002), the prenatally morphine-exposed animals returned to baseline by day 4 in morphine-exposed animals and by day 5 in saline-exposed animals \((P > 0.05; \text{Fig. 3D})\). Thus prenatal morphine exposure attenuated both L-LTP magnitude and L-LTP longevity in LPP-DG and -CA3 responses but not in the MPP-DG and -CA3 responses.

**DISCUSSION**

These results indicate that, although prenatal exposure to the opiate morphine had no effect on LTP induction or its initial maintenance, prenatal morphine attenuated the magnitude and longevity of L-LTP in opioidergic LPP projection to both the DG and the CA3 region of the hippocampus. In contrast, the maintenance of both E- and L-LTP at MPP-DG and -CA3 synapses was unaffected by prenatal exposure to morphine. This attenuation of L-LTP magnitude and longevity was observed in both the DG and CA3 targets of the LPP but not MPP, suggesting a selective effect of prenatal morphine exposure on LTP maintenance in the opioidergic LPP projection.

The LPP contains and releases proenkephalin-derived opioid peptides (Chavkin et al. 1983; Gall et al. 1981), and LPP projections to both the DG and CA3 region display LTP that is modulated by endogenous opioid peptides (Bramham 1992; Bramham and Sarvey 1996; Bramham et al. 1988, 1991b; Breindl et al. 1994; Do et al. 2002). Because prenatal exposure to morphine reduces opioid peptides (Schindler et al. 2004) and increases both \(\mu\) and \(\delta\) opioid receptors (Rimanóczy and Vathy 1995; Rimanóczy et al. 2001; Vathy et al. 2000, 2002), the prenatally morphine-exposed animals returned to baseline by day 4 in morphine-exposed animals and by day 5 in saline-exposed animals \((P > 0.05; \text{Fig. 3D})\). Thus prenatal morphine exposure attenuated both L-LTP magnitude and L-LTP longevity in LPP-DG and -CA3 responses but not in the MPP-DG and -CA3 responses.

**DISCUSSION**

These results indicate that, although prenatal exposure to the opiate morphine had no effect on LTP induction or its initial maintenance, prenatal morphine attenuated the magnitude and longevity of L-LTP in opioidergic LPP projection to both the DG and the CA3 region of the hippocampus. In contrast, the maintenance of both E- and L-LTP at MPP-DG and -CA3 synapses was unaffected by prenatal exposure to morphine. This attenuation of L-LTP magnitude and longevity was observed in both the DG and CA3 targets of the LPP but not MPP, suggesting a selective effect of prenatal morphine exposure on LTP maintenance in the opioidergic LPP projection.

The LPP contains and releases proenkephalin-derived opioid peptides (Chavkin et al. 1983; Gall et al. 1981), and LPP projections to both the DG and CA3 region display LTP that is modulated by endogenous opioid peptides (Bramham 1992; Bramham and Sarvey 1996; Bramham et al. 1988, 1991b; Breindl et al. 1994; Do et al. 2002). Because prenatal exposure to morphine reduces opioid peptides (Schindler et al. 2004) and increases both \(\mu\) and \(\delta\) opioid receptors (Rimanóczy and Vathy 1995; Rimanóczy et al. 2001; Vathy et al. 2000, 2002), the prenatally morphine-exposed animals returned to baseline by day 4 in morphine-exposed animals and by day 5 in saline-exposed animals \((P > 0.05; \text{Fig. 3D})\). Thus prenatal morphine exposure attenuated both L-LTP magnitude and L-LTP longevity in LPP-DG and -CA3 responses but not in the MPP-DG and -CA3 responses.
attenuation of LPP L-LTP may result from alterations of this opioidergic pathway as a result of prenatal exposure to opiates. How could these effects of prenatal morphine exposure on opioid peptides or their receptors explain the effects on LPP LTP observed here? Prenatal exposure to morphine alters levels of both proenkephalin-derived opioid peptides and opioid receptors in the hippocampus. A decrease in proenkephalin mRNA in the DG and μ opioid receptors in hippocampal subregions is observed in prenatally morphine-exposed rats (Schindler et al. 2004; Slamberová et al. 2004). This suggests that prenatal morphine exposure reduces the level of opioid peptides within the LPP (Schindler et al. 2004). Supporting this interpretation, autoradiograms in morphine-exposed animals showed a significant increase in μ opioid receptors in the inner molecular layer and hilus of the DG and the stratum lucidum and oriens of CA3 (Schindler et al. 2004), and such an increase in receptor number often accompanies a decrease in available neuropeptides. Thus the deficits in LTP observed here may be the result of reduced proenkephalin-derived opioid peptides and reduced μ or δ receptor activation in the LPP of adult progeny as a result of prenatal morphine exposure. Such an alteration in opioid peptides and receptors within the LPP system would explain compromised opioid receptor-dependent LTP observed here in both its DG and CA3 targets (Bramham 1992; Do et al. 2002; Velišek et al. 2000, 2003). It also would be expected that mnemonic processes mediated by LPP inputs and LTP would be compromised in adult progeny of opiate-exposed animals. This possibility is supported by our own findings that showed deficits in acquiring memory tasks that require the hippocampus (Slamberová et al. 2001).

One mechanism by which opioid peptides seem to modulate LTP involves their contribution to the induction of LTP (Bramham and Sarvey 1996). The LPP contains and releases proenkephalin-derived opioid peptides, and the induction of LTP at LPP-DG and -CA3 synapses is blocked by opioid receptor antagonists (Bramham 1992; Bramham et al. 1988, 1991b, Breindl et al. 1994; Do et al. 2002). Current evidence suggests that both the excitatory effects of opioids and their contribution to LTP induction are mediated via their actions on GABAergic inhibition. Opioids acting at both δ and μ receptors hyperpolarize GABAergic interneurons (Madison and Nicoll 1988) and block the release of GABA (Cohen et al. 1992). Reducing GABAergic inhibition greatly facilitates postsynaptic depolarization, which is critical for all known forms of LTP (Bliss and Collingridge 1993). It is currently thought that an opioid peptide-mediated reduction in GABAergic inhibition is essential to attain a sufficient level of postsynaptic depolarization for the induction of LPP LTP in vivo. In support of this, blocking GABAergic inhibition eliminates the attenuation of LPP LTP induction seen with opioid receptor antagonism (Bramham and Sarvey 1996). Thus opioid peptides are thought to contribute to LTP induction by reducing GABA release and GABAergic inhibition, thereby facilitating postsynaptic depolarization and the induction of LTP.

However, in this study, prenatal morphine exposure did not alter LTP induction, as reflected in the occurrence of LTP following theta burst stimulation. Thus it seems that the effects of prenatal morphine exposure may not involve effects on LTP induction. However, L-LTP, as reflected by both daily LTP magnitude and LTP longevity, was reduced at 1 day and until its decay in both LPP-DG and -CA3 responses in animals exposed prenatally to morphine. This indicates that prenatal exposure to morphine reduces the magnitude and duration of L-LTP once LTP has been induced.

What might account for the effect of prenatal morphine exposure on the maintenance of LPP L-LTP? It should be noted that, although prenatal morphine exposure had no effect on measures of LTP induction used here, alterations in opioid peptides and opioid receptors nonetheless may have altered LTP maintenance as a result of the absence of their effects during LTP induction. In this view, the reduced opioid peptide levels may have limited postsynaptic depolarization and subsequent LTP maintenance, by limiting postsynaptic depolarization normally afforded by the disinhibitory effects of opioid receptor activation (Bramham and Sarvey 1996). This is a viable possibility, because LTP maintenance and longevity often depend on the initial conditions during its induction, including the intensity of postsynaptic activity. For example, low-intensity bursts can induce robust E-LTP, but of short (<3 h) duration (see Straube et al. 2003). Thus a reduction in the level of postsynaptic depolarization, possibly arising from a reduced contribution of opioid peptides activating opioid receptors, may have resulted in the induction of a less robust LTP and a subsequent reduction in L-LTP maintenance. This view is supported by the finding that prenatal morphine exposure attenuated LTP at both DG and CA3 targets of the LPP. Previous studies have indicated that, although both δ and μ opioid receptors are involved in LTP induction at LPP-DG synapses (Bramham and Sarvey 1996; Bramham et al. 1991b), only μ opioid receptor antagonists alter LTP induction at LPP-CA3 synapses (Do et al. 2002). Because these findings indicate effects at both DG and CA3 targets and because these targets display distinct opioidergic mechanisms of LTP induction, the effects of prenatal morphine exposure is likely a result of the reduction in opioid peptides in the LPP that follows prenatal morphine exposure. Thus the absence of opioid peptides and their effects during LTP induction may have resulted in the induction of a less robust LTP and subsequent attenuation of the magnitude and longevity of late LTP.

Alternatively, the effects of prenatal morphine exposure on LTP magnitude could reflect a direct contribution of opioid receptors to the cellular mechanisms that are associated with LTP maintenance. It is hypothesized that δ opioid receptors contribute to the molecular mechanisms underlying LTP expression (Bramham 1992). In this view, δ receptors play a role in LTP by the activation of second messenger systems during induction that may serve to sustain LTP (Bramham 1992). In support of this view, δ opioid receptors are associated with increases in intracellular calcium (Bramham et al. 1991b; Tang et al. 1994) and increased phosphoinositide turnover (Sánchez-Blázquez and Garzón 1998). Protein kinase C and its various isoforms are activated by phosphoinositide turnover and increased calcium, and PKC is implicated in the maintenance of LTP (Pastalkova et al. 2006). Thus one explanation for the effects of prenatal morphine exposure on LTP maintenance observed here is that a reduction in opioid peptides and subsequent δ opioid receptor activation selectively impairs L-LTP, possibly by altering opioid receptor-mediated mechanisms that are associated with L-LTP maintenance. Future studies will be necessary to determine whether the effects observed here result from the reduced activation of μ or δ opioid receptors during LTP induction or a reduced contribu-
tion of δ opioid receptor activation to mechanisms that underlie LTP maintenance.

It should be noted that a number of factors affecting animals exposed prenatally to morphine might underlie the deficits in LTP observed here. For example, stress arising from transportation or from maternal or fetal opiate withdrawal could be critical factors in the effects of prenatal morphine exposure. It is unlikely that acute stress underlies this effect, because our experiments were performed 5–7 wk after transportation of morphine- and saline-exposed progeny. However, our previous studies indicate that prenatal morphine exposure can render adult progeny particularly susceptible to stress (Šlamberová et al. 2002). Thus it is possible that prior stress arising from the transportation of animals may have produced sustained changes in morphine-exposed animals, and these sustained changes may have contributed to the attenuation of LPP LTP maintenance into adulthood. Likewise, it also is possible that stress arising from maternal opiate withdrawal during pregnancy may also have had a deleterious effect on developing animals. Several studies, including our own, showed alterations in opioid systems with withdrawal (Laborie et al. 2005; Rimánóczy et al. 2003, 2006; Šlamberová et al. 2004). Moreover, long-term changes in the morphology of basal forebrain structures of offspring are observed after maternal withdrawal from morphine (Spiga et al. 2005). However, in these studies, pregnant animals did not show any overt signs of withdrawal for ≤48 h after cessation of the 8-day morphine treatment (e.g., wet dog shakes, diarrhea, or maternal neglect), making maternal opiate withdrawal an unlikely factor mediating these effects. However, we cannot rule out the possibility that the effects observed here are the result of stress arising from transportation or withdrawal, rather than from direct effects of morphine on hippocampal or DG opioid peptides, their receptors, or their development. Thus the mechanisms by which prenatal opioid exposure alters the LPP LTP remain to be defined. Further studies will be necessary to elucidate the mechanisms by which exposure to morphine during gestation alters LTP induction in opioidergic afferents, and the possible contribution of opioid withdrawal and stress in the effects on LTP maintenance in adult progeny.

Although the problem of opiate abuse among pregnant women is of major concern because of its long-term, detrimental consequences on the brain and behaviors of exposed adult offspring, these studies showed additional significance of prenatal opioid contact. With prenatal exposure to opiates, a sustained and permanent alteration occurs in synaptic plasticity in the hippocampal formation of adult animals. This may lend credence to the hypothesis that detrimental effects of drug exposure during early gestation can persist into adulthood. Such alterations engendered by opiate use during the first months in human pregnancy are likely to have profound consequences for normal hippocampal function and memory formation.

GRANTS

This work was supported by National Institutes of Health Grants DA-05833 to I. Vathy and GM-60655 and DA-11983 to B. Derrick.

REFERENCES


