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Long-term opiate effects on amphetamine-induced dopamine release in the nucleus accumbens core and conditioned place preference

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Abstract

Withdrawal following chronic exposure to opiates or other drugs of abuse, administered as frequent doses, or a chronic infusion can cause reductions in mesolimbic dopamine (DA) transmission. However, mesolimbic DA transmission can be enhanced by opiates or psychostimulants administered intermittently as a single daily injection. Both enhanced and attenuated responsiveness of the mesolimbic DA system may have important implications for substance abuse disorders. Previous studies have shown that procedures that use electrical stimulation or drug treatments to augment neurotransmitter release are more effective for demonstrating declines in mesolimbic DA transmission that persist for extended periods following opiate withdrawal. The present study evaluated the effects of pretreatment with noncontingent morphine on amphetamine-induced DA release in the nucleus accumbens core and conditioned place preference (CPP). Morphine pretreatment was administered as a constant infusion, which was gradually increased to a dose of 50 mg/kg/day over a 1-week period in Wistar rats. At 10 days after cessation of morphine pretreatment, baseline dialysate DA levels in the nucleus accumbens core were unchanged, but amphetamine-induced increases in DA were attenuated by greater than 50% in morphine-pretreated animals. Morphine pretreatment did not modify locomotor activity during conditioning sessions, expressed as absolute values or change in activity counts between saline and morphine injections. Place preference, conditioned by two morphine pairings at 10 and 11 days after the onset of opiate withdrawal, was enhanced by opiate pretreatment between 12 and 33 days after the onset of withdrawal. In conclusion, morphine pretreatment delivered as a constant infusion can have pronounced and long-lasting effects on DA release and CPP, which may have important implications for drug-seeking behavior and treatment of substance abuse disorders.

Keywords: Amphetamine; Dependence; Dopamine; Conditioned place preference; Morphine; Nucleus accumbens; Opiate; Psychostimulant; Withdrawal syndrome

1. Introduction

Chronic exposure to opiates and other drugs of abuse has been shown to cause behavioral and neurochemical changes, which persist for extended periods following the onset of withdrawal (Pollock and Kornetsky, 1996; McCann et al., 1997). Depending on the dose, schedule of administration, brain region, and drug of abuse being studied, chronic exposure can cause mesolimbic dopamine (DA) transmission to be decreased, unchanged, or enhanced (see reviews by Stewart and Badiani, 1993; Robinson and Berridge, 1993). Increased responsiveness of the DA system (sensitization) usually occurs after intermittent exposure to opiates or psychostimulants (Post, 1980), while the attenuation of DA transmission has most often been associated with the administration of multiple daily injections or as a chronic infusion (Tjon et al., 1995; Schenk and Partridge, 1997). For example, the release of DA by tissue slices from the nucleus accumbens is enhanced after pretreatment with a single daily injection of cocaine over 14 days, and is attenuated when the same dose of cocaine is administered as a constant infusion (King et al., 1993).

Abbreviations: ANOVA, analysis of variance; CPP, conditioned place preference; DA, dopamine.

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Three studies have shown that unstimulated baseline DA levels in the ventral striatum, estimated through microdialysis, are diminished for up to 1 week after withdrawal from chronic treatment with twice-daily injections of morphine (Acquas et al., 1991; Acquas and Di Chiara, 1992; Crippens and Robinson, 1994). Following a similar schedule of morphine administration, another study failed to demonstrate changes in baseline unstimulated dialysate DA levels in the nucleus accumbens on Day 3 or 30 of opiate withdrawal (Spanagel et al., 1993). Additional studies have shown that the firing rate of mesolimbic dopaminergic neurons is also decreased during the initial week of withdrawal following twice-daily morphine injections and returns to control values at 14 days following the onset of withdrawal (Diana et al., 1995, 1999). These findings indicate that chronic treatment with multiple doses of morphine per day can lead to mesolimbic DA transmission that is either unchanged or diminished under unstimulated conditions.

Evaluation of tissue slices obtained from brain regions that receive terminal projections from dopaminergic neurons has allowed a more detailed analysis of the mechanisms through which drugs of abuse modify neurotransmitter release (Tjon Tien Ril et al., 1993). In tissue slices obtained from the striatum for up to 21 days after withdrawal of chronic treatment with three morphine injections per day, baseline efflux of DA is unchanged, but electrically stimulated release is attenuated (Tjon et al., 1994b). Withdrawal following chronic treatment with two or more injections of morphine per day does not modify baseline DA efflux, but can attenuate release that is actively stimulated in tissue slices obtained from the nucleus accumbens at 3 to 28 days after the onset of withdrawal (Grasing and Ghosh, 1998; Ghosh et al., 1998). In tissue slices obtained from the nucleus accumbens during naloxone-precipitated withdrawal, baseline DA efflux is also unchanged, but release stimulated by several different mechanisms is attenuated (Ghosh and Grasing, 1999). These results show that procedures which use electrical stimulation or drug treatments to augment neurotransmitter release are more effective for demonstrating declines in mesolimbic DA transmission that persist for extended periods following opiate withdrawal. Even so, we are not aware of any published studies that have examined the effects of opiate pretreatment on changes in amphetamine-induced DA release measured through microdialysis.

The purpose of the present study was to test the hypothesis that chronic morphine treatment attenuates amphetamine-induced DA release in the nucleus accumbens core at 10 days after the onset of opiate withdrawal, without modifying the unstimulated dialysate levels of DA. To maximize the potential for opiate-induced attenuation of DA release, morphine was delivered as a continuous infusion, which was gradually increased to a final dose of 50 mg/kg/day over a 1-week period. We also examined the effects of opiate pretreatment on morphine-induced locomotor activity and conditioned place preference (CPP), initiated at 10 days after the onset of opiate withdrawal. Because the attenuation of mesolimbic DA transmission is a neuroadaptation that may promote drug-seeking behavior in substance abuse disorders (Dackis and Gold, 1985; Schulteis and Koob, 1996; Di Chiara, 2002), our hypothesis was that opiate pretreatment would enhance morphine-induced CPP.

2. Materials and methods

2.1. Overview

Fig. 1 shows a schematic representation of the timing for microdialysis and CPP procedures. For Experiment 1, the amphetamine-induced release of DA in the nucleus accumbens core was evaluated in two groups of animals, at 10 days after completion of pretreatment with saline ($n = 10$) or morphine ($n = 10$). Experiment 2 evaluated morphine-induced locomotor activity and CPP in two groups of animals. As outlined below, locomotor activity was measured after injections of saline or morphine were administered during conditioning sessions conducted twice daily at 10 and 11

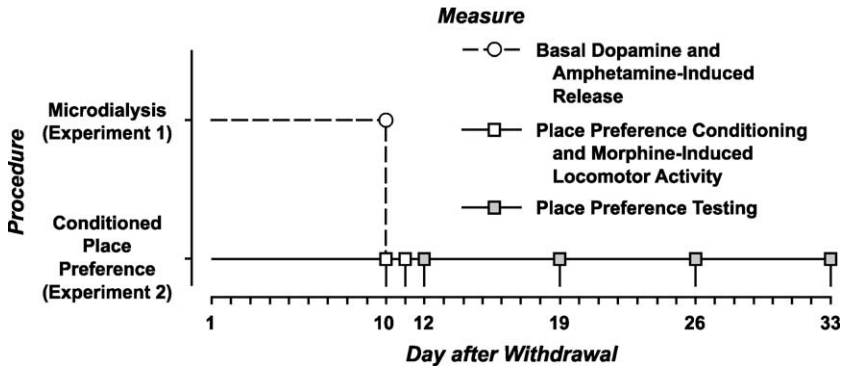

Fig. 1. Schematic representation of the timing of Experiments 1 and 2, which utilized microdialysis and CPP procedures, respectively. All animals received pretreatment with a chronic infusion of saline or morphine. The number of days following termination of saline or morphine pretreatment for different measures is graphed on the horizontal axis.

days after completion of saline ($n = 8$) or morphine ($n = 12$) pretreatment. Preferences for morphine-paired compartments were determined at test sessions performed at 12 to 33 days after completion of pretreatment.

2.2. Subjects

Experimental procedures were approved by the local Animal Care and Use Committee. Laboratory facilities were accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, and the Principles of Laboratory Animal Care (NIH publication no. 86–23, 1996) were followed.

Nine-week old, male Wistar rats (Charles River Laboratories, Raleigh, NC) were housed individually and maintained on a reversed light–dark cycle (14 h of darkness beginning at 9:00 a.m., and 10 lighted hours), with ad lib drinking water. Microdialysis and behavioral sessions were conducted during the dark phase of the light–dark cycle. Because changes in food intake can modify the CPP (Bell et al., 1997) and DA levels in the nucleus accumbens (Pothos et al., 1995), intake was limited to 13 g of standard rat chow daily, administered following CPP or microdialysis procedures. Rats maintain a stable body weight on this level of food restriction, with less than a 3.0% change in body weight over a 4-week period. All rats received an intravenous catheter and pretreatment with saline or morphine as outlined in the following two sections.

2.3. Catheter and probe placements

For microdialysis studies, an intravenous catheter and microdialysis probe guide was implanted during the same procedure, with connections to catheters made through a connector attached to the skull with dental acrylic. For CPP studies, intravenous catheters were implanted and passed subcutaneously to an exit wound on the back, with connections made by a shoulder harness (model CIH105AV, Instech Laboratories, Plymouth Meeting, PA).

All surgeries were performed while the rats were anesthetized with 50 mg/kg of pentobarbital, administered intraperitoneally. For catheter placement, the internal jugular vein was then exposed and dissected free of surrounding connective tissue. A small incision was made in the vein, and a commercially available silastic catheter (model S25, IITC, Woodland Hills, CA) was inserted and fastened in place by silk suture and cyanoacryllic cement.

Microdialysis probe guides were implanted stereotactically, with the tip of the probe guide aimed at the dorsal surface of the nucleus accumbens core, at coordinates of 1.4 mm rostral to bregma, 1.5 mm lateral from midline, and 5.8 mm below dura. Probes extended 2.0 mm past this depth when inserted during a subsequent procedure. Probe guides were secured with three stainless steel screws, cemented in place with dental acrylic, and closed with a stylet.

2.4. Morphine pretreatment

Morphine sulfate was donated by the National Institute of Drug Abuse (Bethesda, MD) and was dissolved in 0.9% saline. All morphine doses are expressed as the sulfate salt. Three days after surgery for placement of intravenous catheters and probe guides for animals undergoing microdialysis studies, all animals received 1 week of a continuous intravenous infusion of noncontingent saline or morphine. This infusion was administered while the animals were maintained in 'shoe box' style Plexiglas cages. As outlined in Table 1, the infusion was initiated at a starting dose of 16.8 mg/kg/day, which was increased by 20% every 24 h, arriving at a final dose of 50.0 mg/kg/day on the last day of morphine pretreatment. This schedule for dose escalation has been shown to produce a high level of opiate dependence (Grasing et al., 2003). Each home cage was equipped with an infusion pump (model A, Razel, Stanford, CT), liquid swivel, counterbalanced arm, and steel-spring tether, as described above. After the completion of the infusion, the rats were evaluated through microdialysis or morphine-induced CPP.

2.5. Microdialysis

Microdialysis studies were conducted 10 days following chronic saline or morphine pretreatment as outlined above. Animals were placed in Plexiglas chambers (24 cm wide, 25 cm deep, 26 cm tall) located within sound attenuating boxes, under dim lighting (approximately 1.0 lx). White noise was provided for each chamber by a ventilating fan. Each chamber was equipped with a quartz-lined, two-channel liquid swivel (model 375/D/22QM, Instech Laboratories) to allow free movement of the animal in the chamber.

On the morning of microdialysis experiments, commercially available concentric microdialysis probes with a 2.0-mm active length (part number 0128309562, CMA Microdialysis, Acton, MA) were inserted through guide cannulae and were perfused at 1.0 μl/min with 145 mM NaCl, 2.8 mM KCl, 1.22 mM $CaCl_2$, 1.20 mM $MgSO_4$, 5.4 mM glucose, and 0.2 mM phosphate buffered saline (final pH of 7.40) with a Harvard 22 syringe pump (Harvard

Table 1
Dose, concentration, and infusion rate for morphine pretreatment

Day of infusion	Dose (mg/kg/day)	Concentration (mg/ml)	Volume (ml/kg/day)
1	16.8	3.0	5.59
2	20.1	3.0	6.70
3	24.1	3.0	8.04
4	28.9	3.0	9.65
5	34.7	4.0	8.68
6	41.7	4.0	10.42
7	50.0	4.0	12.51

Infusions were administered continuously, 24 h a day. Control animals received saline, delivered at an identical rate and volume.

Apparatus, Holliston, MA). Dialysate was collected at 20-min intervals for on-line analysis using a Bio-Analytic Laboratories Pollen-8 programmable injection system (West Lafayette, IN). At 3 h following insertion of microdialysis probes, three samples of dialysate were obtained under baseline, unstimulated conditions. Through the use of a liquid switch (#MD-1508, Bioanalytical Laboratories), perfusion with artificial cerebrospinal fluid containing 10 μM amphetamine was initiated and continued over 140 min, after which, 32 μM amphetamine was perfused over an additional 80 min.

DA concentration was determined by high-performance liquid chromatography (HPLC) with electrochemical detection using a Bio-Analytic Laboratories LC-4C system, with a potential of 0.6 V, maintained between the glassy carbon-working electrode and an Ag–AgCl reference electrode. Mobile phase consisted of 0.126 M phosphate buffer (pH 5.0), 0.525 mg/ml sodium octyl sulfate, 0.35 mg/ml EDTA, 9.0% methanol, and 0.4% tetrahydrofuran. After the experiments, the animals were deeply anesthetized with 100 mg/kg of pentobarbital and were perfused with 4.0% paraformaldehyde. Coronal sections were then cut on a cryostat and stained with cresyl violet to verify probe locations.

2.6. Conditioned place preference

Sessions were conducted in Plexiglas shuttle boxes (60 cm long, 30 cm wide, and 30 cm tall), under conditions of dim illumination (1.0 lx) in the presence of white noise. Holes along the sides of both compartments allowed infrared sensors to quantify activity and location. For conditioning sessions, the boxes were divided into two equal-sized compartments by means of a removable partition. One compartment was white with a white-textured plexiglass floor. The other was black, with a smooth black plexiglass floor. For test sessions, a 1.0-cm tall partition was inserted along the border between white and black compartments, which prevented the animals from spending time at this location, but was allowed access to either side of the shuttle box.

Rats first received chronic saline or morphine pretreatment as outlined above. At 10 and 11 days after the end of pretreatment, rats were injected with saline or 5.0 mg/kg of morphine and were confined to either the white or black compartment for 30 min. At least 6 h later, the animals received an additional injection of the opposing treatment and were confined to the opposite side of the shuttle box. Treatment compartment and presentation order were counterbalanced for the control and morphine-pretreated groups. Two test sessions were conducted at 12 days after pretreatment, in the morning and afternoon. Additional morning test sessions were conducted at 19, 26, and 33 days after pretreatment. During test sessions, time spent in morphine- and saline-paired compartments were quantified by infrared beam interruptions.

2.7. Data analysis

Statistical tests were performed by a Systat software (version 5, Evanston, IL). For Experiment 1, recovery for individual probes was calibrated prior to microdialysis, and data were then corrected for probe efficiency. A complete set of freshly made standards was run prior to each experiment. After log transformation, peak heights were fitted to standard values by simple linear regression. The effects of morphine pretreatment on microdialysis were evaluated by one-way analysis of variance (ANOVA), with post hoc comparisons made by Bonferroni *t* test. Based on the comparisons of DA concentration for saline- and morphine-pretreated animals during baseline and stimulation with two concentrations of

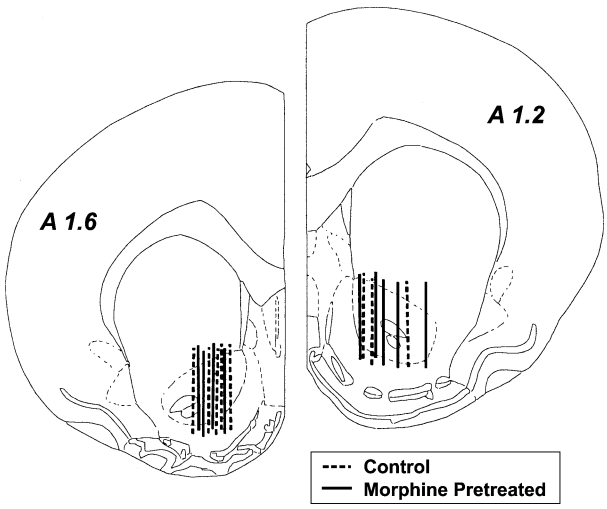

Fig. 2. Composite representation of microdialysis probe placements in the nucleus accumbens core. Vertical lines indicate locations of the 2.0-mm active area of individual probes for the animals pretreated with saline ($n = 8$, dashed lines) or morphine ($n = 9$, solid lines). Panels A 1.2 and A 1.6 correspond to sections 1.2- and 1.6-mm anterior to bregma according to the atlas of Paxinos and Watson (1986). The area of the nucleus accumbens core is shown by a dashed line.

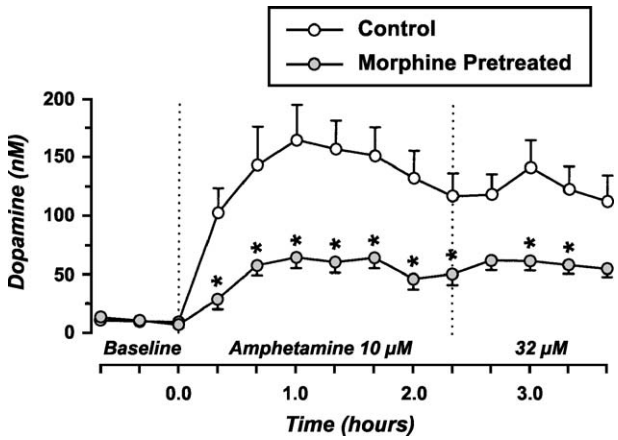

Fig. 3. Results from microdialysis performed at 10 days following morphine pretreatment. Mean values(± S.E.M.) for extracellular DA are plotted against time. Beginning at time 0, 10 μM amphetamine was infused through microdialysis probes placed in the nucleus accumbens core. At the 2-h and 20-min time point, the amphetamine concentration was increased to 32 μM. $*P<.004$ based on the comparison between saline- and morphine-pretreated animals.

amphetamine, $P<.02$ was used as a criteria for statistical significance of post hoc comparisons.

For Experiment 2, locomotor activity was quantified during conditioning sessions by recording the number of infrared beam interruptions. Time spent in morphine- and saline-paired compartments during test sessions was calculated by a software that measured intervals between infrared beam interruptions. To determine whether conditioned preferences occurred at different time points, time spent on the saline- and morphine-paired sides of the shuttle boxes for animals that received the same pretreatment was compared by *t* test. The effects of morphine pretreatment

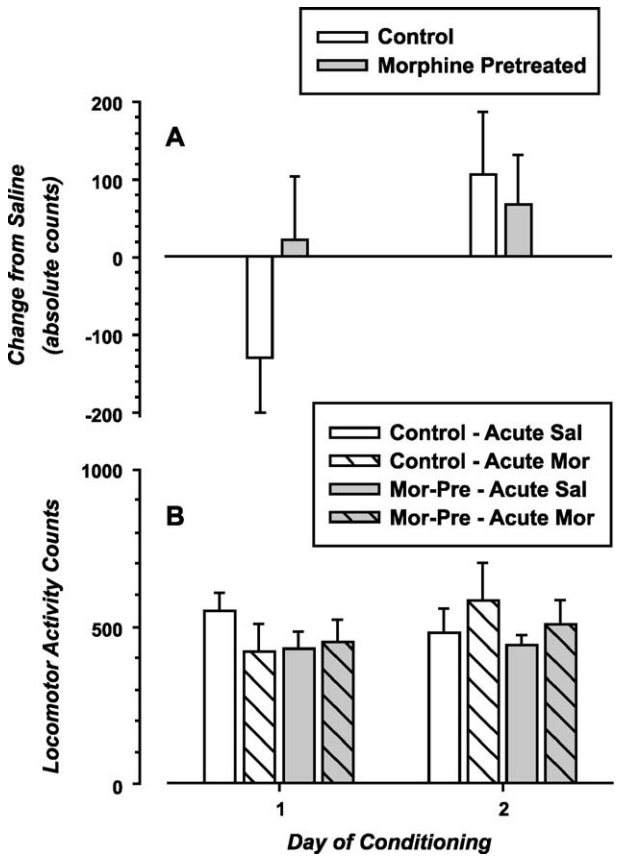

Fig. 4. (A) Mean values(± S.E.M.) for the change in locomotor activity between conditioning sessions in which animals received morphine and saline. (B) Effect of place preference conditioning on locomotor activity. Mean locomotor activity counts (± S.E.M.) for saline (control)- and morphine (Mor-Pre)-pretreated rats during conditioning with saline (Acute Sal) or morphine (Acute Mor).

on locmotor activity and preferences were evaluated by one-way ANOVA, with post hoc comparisons made by Bonferroni *t* test. Based on evaluations made at five different time points, $P < .01$ was used as a criteria for statistical significance of post hoc comparisons.

3. Results

3.1. Experiment 1: Amphetamine-induced DA efflux

A schematic representation of the microdialysis probe locations for the subjects included in the present study is shown in Fig. 2. Probes for eight and nine animals that received pretreatment with saline and morphine, respectively, were implanted in the core region of the nucleus accumbens. Of the 20 animals implanted, data from 2 animals were excluded because of locations outside of the nucleus accumbens core, and data from an additional subject were excluded because a stable baseline was not obtained prior to amphetamine infusion. Baseline dialysate DA levels at time 0 (immediately prior to infusion of amphetamine) were 9.24 ± 2.59 (mean $\pm$ S.E.) and 6.90 ± 2.44 nM for saline- and morphine-pretreated rats, respectively [$t(15) = 0.74$, n.s.]. ANOVA showed a significant main effect [$F(27,210) = 10.3$, $P < .001$] for dialysate DA levels (Fig. 3). Post hoc comparisons indicated that morphine pretreatment decreased dialysate DA levels for most time points evaluated during stimulation with 10 or 32 μM amphetamine. Relative to control subjects, amphetamine-induced increases in dialysate DA levels were attenuated by 50% to 60% following morphine pretreatment.

3.2. Experiment 2: Morphine-induced locomotor activity and conditioned place preference

Values for locomotor activity during conditioning sessions are shown in Fig. 4. Morphine pretreatment did not modify locomotor activity during CPP conditioning, expressed as absolute values or change in activity counts between saline and morphine injections. Results of place preference conditioning are shown in Fig. 5. Significant preferences for the morphine-paired sides of the shuttle boxes were observed for morphine-pretreated animals [$t(22) = 3.23$, 4.26, 4.13, 7.87, and 4.35 corresponding to $P < .01$, $P < .001$, $P < .001$, $P < .0001$, and $P < .001$ for testing at Day 12 (a.m.), Day 12 (p.m.), Day 19, Day 26, and Day 33, respectively], but not after saline pretreatment. ANOVA demonstrated a significant effect on the differences for time spent in morphine- and saline-paired sides of the shuttle boxes [$F(9,90) = 2.06$, $P < .05$], with post hoc comparisons showing that morphine pretreatment increased values on Day 26.

4. Discussion

Our finding that morphine pretreatment did not modify baseline dialysate DA levels in the nucleus accumbens core at day 10 of abstinence is in agreement with earlier studies, which have reported that morphine-induced reductions in mesolimbic DA transmission under unstimulated conditions are observed most often during the initial week of withdrawal, described in the Introduction section. As outlined in the Introduction, decrements in DA release stimulated by

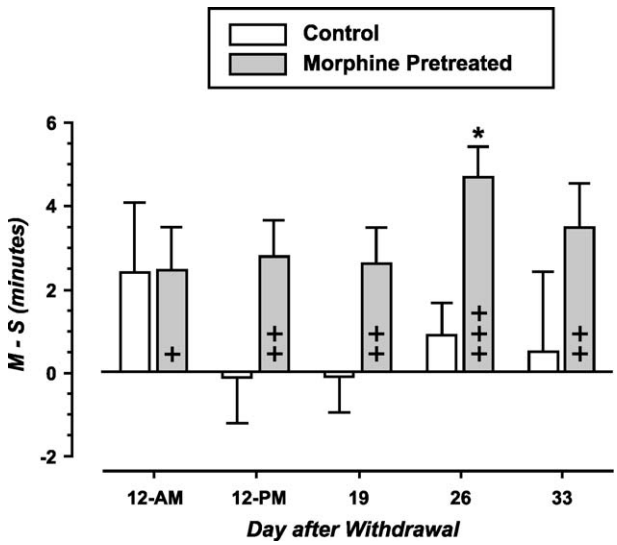

Fig. 5. Results from CPP testing. Test sessions for CPP were conducted at times shown on the horizontal axis. The first two test sessions after conditioning, conducted on the morning and afternoon of day 12 following pretreatment, are denoted as 12-AM and 12-PM, respectively. Mean values($\pm$ S.E.M.) for the difference between time spent in morphine- and saline-paired compartments, in minutes, are graphed on the vertical axis. $^{*}P < .01$ based on comparison between saline- and morphine-pretreated animals. $^{+}P < .004$, $^{++}P < .001$, and $^{+++}P < .0001$ based on comparison between time spent on saline- and morphine-paired sides, for animals that received the same pretreatment (no significant differences were observed for saline-pretreated animals).

different mechanisms occur for tissue slices obtained after the initial week of opiate withdrawal from the nucleus accumbens or striatum. Results of the present study show that the latter approach can be extended to the measures of DA release obtained through microdialysis.

For striatal or nucleus accumbens tissue slices obtained on Days 3 to 5 of spontaneous withdrawal, morphine pretreatment does not modify DA release produced by an initial in vitro exposure to 4-aminopyridine, but attenuates values obtained during a second exposure to 4-aminopyridine by approximately 30% (Ghosh et al., 1998). 4-Aminopyridine nonselectively prolongs action potential duration through the blockade of voltage-sensitive potassium channels and the facilitation of calcium ion entry (Rogawski and Barker, 1983). For tissue slices obtained from the nucleus accumbens during naloxone-precipitated withdrawal, morphine-induced declines in DA efflux also occur only after two or more in vitro exposures to 4-aminopyridine, amphetamine, or cocaine (Ghosh and Grasing, 1999). Amphetamine blocks DA re-uptake (Wayment et al., 1998) and causes DA to be released from intracellular vesicles into the cytosol (Sulzer et al., 1995) and be reverse transported through the plasmalemmal DA transporter (Pifl et al., 1995). Cocaine also blocks DA re-uptake and has local anesthetic properties (Povlock and Schenk, 1997). For tissue slices obtained from the nucleus accumbens during the second or third week of spontaneous withdrawal, opiate pretreatment does not modify DA release stimulated by initial exposure to amphetamine or cocaine but attenuates release stimulated with a second exposure to either compound by values of 19.3% and 30.0%, respectively (Grasing and Ghosh, 1998). In the latter study, no effects of morphine pretreatment were observed for DA release by nucleus accumbens slices stimulated with 4-aminopyridine or for DA release by striatal slices stimulated with 4-aminopyridine, amphetamine, or cocaine. These results, along with findings from the present study, support the use of stimulation with compounds that produce a net increase in DA efflux for demonstrating the ability of chronic morphine pretreatment to attenuate DA release in the nucleus accumbens. Although microdialysis was conducted over a much longer time period than tissue slice studies, significant declines in DA release were observed in the present study for most of the values measured during amphetamine treatment. An additional important difference is that DA release was attenuated by greater than 50% in the present study. These findings indicate that microdialysis procedures may be more reliable than brain slice techniques for demonstrating long-term declines in DA release following opiate pretreatment.

In contrast to results obtained in the present study using amphetamine delivered to the nucleus accumbens, DA release produced by systemic injections of morphine is potentiated in the nucleus accumbens at 3 and 30 days after withdrawal from twice-daily morphine injections (Spanagel et al., 1993). Cadoni and Di Chiara (1999) observed that systemic treatment with morphine, 16 days following withdrawal from twice-daily morphine injections, potentiates the release of DA in the nucleus accumbens core and attenuates DA the release in the shell of the nucleus accumbens. Morphine excites dopaminergic neurons through the disinhibition of inhibitory gamma-aminobutyric acid (GABA) interneurons in the ventral tegmental area (Johnson and North, 1992). Differences with results from the present study may be explained by the fact that amphetamine augments DA release through different mechanisms (outlined above) and was delivered only to the nucleus accumbens core.

Although previous studies have shown that intermittent exposure to opiates can enhance morphine-induced locomotor activity (Kalivas and Duffy, 1987; Szumlinski et al., 2000), no significant effects of morphine pretreatment, delivered as a chronic infusion on locomotor activity, were observed in the present study. This finding is consistent with a previous report that the sensitization of locomotor activity is decreased by schedules that administer more frequent daily doses of morphine (Vanderschuren et al., 1997).

In previous CPP experiments, intermittent exposure to morphine, amphetamine, or cocaine intensified drug-induced place preferences (Lett, 1989; Shippenberg and Heidbreder, 1995). Pretreatment with a single daily morphine injection does not alter CPP measured 1 day later, but enhances preferences at 3, 10, or 21 days following morphine pretreatment (Shippenberg et al., 1996). Repeated, twice-daily morphine injections attenuate CPP measured 12 h later (Shippenberg et al., 1989), but effects at later time points or after opiate pretreatment administered as a constant infusion have not been reported. Results of the present experiment show that morphine pretreatment delivered as a constant infusion can enhance CPP over an extended period, from 12 to 33 days after the onset of opiate withdrawal.

The activation of the mesolimbic DA system is believed to play an important role in instrumental behavior supported by natural and drug reinforcers (Bonci et al., 2003; Salamone et al., 2003). As described above, morphine pretreatment can augment opiate-induced increases in mesolimbic DA transmission (Spanagel et al., 1993; Cadoni and Di Chiara, 1999) and attenuate increases produced by other mechanisms (results of the present study). Both neuroadaptations have the potential to enhance morphine-induced CPP. The sensitization of drug-induced increases in mesolimbic DA transmission may augment the ability of drugs to serve as positive reinforcers (Robinson and Berridge, 1993). Attenuation of mesolimbic DA transmission after chronic exposure to opiates and other drugs of abuse may be perceived as a negative emotional state (Markou et al., 1998; Cryan et al., 2003), which allows drugs of abuse to provide negative reinforcement (Dackis and Gold, 1985; Schulteis and Koob, 1996; Di Chiara, 2002). Reductions in mesolimbic DA transmission have been observed in humans with substance abuse disorders during periods of abstinence, and may result in decreased sensitivity to non-drug-related reinforcing stimuli (Volkow et al., 2002).

Neurotransmitter systems other than DA, including endogenous opioid peptides, are also involved in opiate

reinforcement (Bardo, 1998; McBride et al., 1999). Exposure to opiate-paired compartments during CPP conditioning increases met-enkephalin levels in the nucleus accumbens, while a decrease is observed with exposure to saline-paired compartments (Nieto et al., 2002). Chronic daily morphine treatment early in life causes changes in autoradiography, consistent with the enhanced release of opioid peptides in the ventral tegmental area and cortex of adult animals (Van den Berg et al., 1999), which is an additional mechanism through which opiate pretreatment may have enhanced CPP in the present study.

In summary, the present study evaluated the effects of morphine pretreatment delivered as a constant infusion that was gradually increased to a final dose of 50 mg/kg/day over 1 week. After two pairings with morphine, CPP was not observed in animals that received pretreatment with saline, but occurred in morphine-pretreated animals between 12 and 33 days after the onset of withdrawal. At 10 days following the onset of opiate withdrawal, morphine pretreatment did not modify baseline dialysate DA levels in the nucleus accumbens core, but attenuated amphetamine-induced increases in DA efflux by greater than 50%. These findings add to results of previous studies showing that procedures which stimulate neurotransmitter release are more effective for demonstrating declines in mesolimbic DA transmission that persist for extended periods following opiate withdrawal (Tjon et al., 1994a; Grasing and Ghosh, 1998).

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