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High-Dose Methadone Maintenance in Rats: Effects on Cocaine Self-Administration and Behavioral Side Effects

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It has been demonstrated that high-dose methadone maintenance is efficacious in reducing cocaine abuse in opioid-dependent individuals, but it is not clear whether this is caused by an action of methadone on the direct reinforcing properties of cocaine or on cocaine seeking. Also, it is not clear whether high-dose methadone maintenance may induce behavioral side effects, which could limit its clinical use. Here, we report that high-dose methadone maintenance (20–40 mg/kg/day) does not reduce, and even enhances cocaine (10–30 mg/kg, i.p.)-induced elevation in dopamine concentration in the ventral striatum measured by *in vivo* microdialysis. In parallel, however, rats maintained on high-dose methadone (30 mg/kg/day) seek and consume significantly less cocaine than controls when tested for intravenous cocaine (0.5 mg/kg/infusion) self-administration on a progressive ratio schedule of reinforcement. This reduction in cocaine self-administration does not result from impaired sensory-motor functioning as rats maintained on high-dose methadone show normal locomotor activity. Furthermore, the reduction in responding for cocaine does not seem to result from general behavioral deficits as male rats maintained on high methadone doses respond normally to palatable food and thermal pain, although their sexual responses to receptive females are greatly suppressed. Taken together, these results from studies in rats support the usefulness of larger doses of methadone to reduce severe cocaine abuse in opioid-dependent individuals and possibly in the management of pure-cocaine addiction. *Neuropsychopharmacology* (2007) **32,** 2290–2300; doi:10.1038/sj.npp.1301357; published online 21 February 2007

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INTRODUCTION

Two interesting issues arise when considering the evidence that high-dose methadone maintenance can effectively reduce cocaine use in opioid-dependent individuals (Stine *et al*, 1991; Strain *et al*, 1993; Borg *et al*, 1999; Schottenfeld *et al*, 2005; Peles *et al*, 2006a). First, clinical studies have not determined whether the observed reductions in cocaine intake are due to the effects of high methadone doses on the direct reinforcing effects of cocaine or on cocaine seeking. Second, although methadone maintenance at high doses may effectively reduce cocaine intake, it may also produce a range of undesired behavioral side effects which would limit its clinical use.

With respect to the first issue, experimental studies in humans and animals suggest that high-dose methadone maintenance can reduce cocaine abuse not by altering its acute reinforcing and stimulatory actions, but rather by reducing cocaine seeking. In fact, patients maintained on doses of methadone ranging between 50 and 100 mg/day report either no changes, or even increases, in subjective reports of liking and stimulation induced by acute cocaine administration (Foltin *et al*, 1995; Foltin and Fischman, 1996; Preston *et al*, 1996). In primates, methadone maintenance has been shown to suppress cocaine self-administration on a variety of reinforcement schedules, but these effects have been attributed to impaired sensory-motor functions (Negus and Mello, 2004) such as impaired ability to initiate motor behavior toward the self-administration lever. In rats, high-dose methadone maintenance neither alters the stimulatory action of cocaine (Leri *et al*, 2004, 2006) nor reduces the intravenous self-administration of cocaine on a continuous schedule of reinforcement (Leri *et al*, 2006). However, rats maintained on high-dose methadone show significant reductions in cocaine seeking as indexed by reduced reinstatement of operant responding after priming injections of cocaine (Leri *et al*, 2004), as well as reduced formation and expression of cocaine-induced place preference (Leri *et al*, 2006).

To explore further the basis of changes in cocaine-induced behaviors brought about by methadone, we first used *in vivo* microdialysis (Experiment 1) to assess the effects of maintenance on high-dose methadone on elevations of extracellular dopamine in the ventral striatum induced by acute injections of cocaine (Di Chiara and Imperato, 1988; Koob and Nestler, 1997; Wise *et al*, 1995).

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Having found that methadone actually enhances the dopaminergic response to cocaine, we next studied its effects on self-administration of cocaine on a progressive ratio (PR) schedule of reinforcement (Experiment 2) to determine whether the reinforcing effects of cocaine might also be enhanced by high-dose methadone maintenance, leading to the reduced responding seen previously on a fixed ratio 1 schedule, but to greater responding when cocaine is more difficult to obtain (Barr and Phillips, 1999; Richardson and Roberts, 1996).

The other experiments reported in this paper were designed to study the possible side effects of high-dose methadone maintenance on responses of male rats to receptive females (Experiment 3), palatable food (Experiment 4), and painful stimulation and its modulation by cocaine (Experiment 5). Loss of sex drive is a side effect commonly reported by patients on methadone maintenance (Daniell, 2002b; Fischer *et al*, 2002), but it is not clear whether sexual dysfunctions result from a dose-dependent pharmacological effect of methadone (Daniell, 2002a; de la Rosa and Hennessey, 1996) or from preexisting hypogonadism induced by heroin addiction (Mirin *et al*, 1980). Another side effect reported by some methadone-maintained patients is general anhedonia (Fischer *et al*, 2002), and in primates chronic exposure to methadone reduces operant responding for palatable food (Negus, 2006; Negus and Mello, 2004). However, because acute methadone can have rate-suppressing effects on operant behavior (Macenski *et al*, 1994; McMillan *et al*, 1980; Nader and Thompson, 1987, 1989), its effect on consumption of palatable food needs to be examined using a test that does not involve operant responding. Finally, there is evidence that individuals maintained on methadone for the management of opiate addiction show hyperalgesia (Compton *et al*, 2000; Doverty *et al*, 2001a, b). Most of these studies, however, examined individuals with a history of dependence on illicit opioids, and there are reasons to believe that some may have been hyperresponsive to pain before methadone treatment (Pud *et al*, 2006). Furthermore, although it is well established that methadone-maintained individuals require higher doses of acute opioid agonists for pain control (Alford *et al*, 2006b; Scimeca *et al*, 2000), it has never been established whether high methadone maintenance produces cross-tolerance or enhances the analgesic effect of cocaine (Lin *et al*, 1989; Waddell and Holtzman, 1999).

METHODS

Subjects

These experiments were performed in different institutions, using different strains of rats (see below). All rats were purchased from Charles River (St Constant, QC, Canada), housed singly, and maintained on a reverse light–dark cycle (0800 lights off; 2000 lights on) with free access to food and water except during behavioral testing, which always occurred during the dark cycle. Experimental procedures were approved by the Animal Care Committees of the universities where the work was conducted: the University of Guelph and Concordia University. All experiments were carried out in accordance with the recommendations of the Canadian Council on Animal Care.

Methadone Doses

The primary objective of these experiments was to provide a profile of neurochemical and behavioral effects of high-dose methadone maintenance in rats (methadone HCL, Pharmascience, Montreal, QC, Canada). In this species, we have recently found that 30 mg/kg/day methadone delivered via subcutaneously implanted osmotic minipumps yields a mean ($\pm$SEM) plasma level of 489.3 ($\pm$27) ng/ml (unpublished data collected in collaboration with Dr Y Zhou and Dr MJ Kreek). In humans, a dose of 100 mg/day results in an average daily concentration of about 240 ng/ml (Kreek, 2000), but steady-state plasma concentrations above 420 ng/ml appear to be necessary to reduce effectively cocaine abuse in opioid-dependent individuals (Peles *et al*, 2006a). Thus, in rats, 30 mg/kg/day closely corresponds to the high end of therapeutic dosages used for the management of opiate addiction (Dole, 1988; Maxwell and Shinderman, 1999) with concurrent cocaine dependence.

In Experiment 1, a wide range of methadone doses (20, 30, and 40 mg/kg/day) was employed to detect possible synergistic interactions between methadone maintenance and acute cocaine administration on extracellular dopamine concentration in the ventral striatum. In the other experiments (except Experiment 3, see below), a single dose of 30 mg/kg/day was used because this methadone dose has been previously shown to (1) increase locomotor activity, (2) block cocaine-induced reinstatement of cocaine seeking, (3) block formation and expression of cocaine place conditioning, (4) not alter cocaine self-administration on a continuous schedule of reinforcement, and (5) not alter stress-induced reinstatement of cocaine seeking (Leri *et al*, 2004, 2006).

Surgery

Osmotic minipumps. Methadone maintenance was achieved by implanting osmotic minipumps subcutaneously (Alzet model 2ML2, 0.5 l/h for 14 days, Durect Corporation, Cupertino, CA, USA). Isoflurane (Pharmaceutical Partners of Canada Inc., Richmond Hill, ON, Canada) was used to anaesthetize the rats and a small incision between the scapulae was made in the skin. Subcutaneous connective tissues were spread apart using a hemostat to make a small pocket for the pump. Osmotic pumps were placed into the pocket with flow moderator directed away from the incision. Wound clips kept the incision closed. An identical procedure was carried out for the animals receiving sham surgery. Using the same protocol for anesthesia, the pumps were removed upon completion of the delivery duration.

Intravenous self-administration. Rats were surgically implanted with intravenous silastic catheters (Dow Corning, Midland, MI, USA) in the right jugular vein, under general anesthesia induced by a combination of sodium pentobarbital (18.5 mg/kg i.p., MTC Pharmaceutical, Cambridge, ON, Canada), morphine (5 mg/kg s.c., Ontario Veterinary College, Guelph, ON, Canada) and diazepam (1 mg/kg s.c., Sabex Inc., Boucherville, QC, Canada). Rats were given

atropine sulfate (4.5 mg/kg s.c., Ontario Veterinary College, Guelph, ON, Canada) just before surgery and Depocillin (300 000 IU, 0.1 ml/rat IM, Intervet Canada, Whitby, ON, Canada) immediately after surgery. The catheter was secured to the vein with silk sutures and was passed subcutaneously to the top of the skull where it exited into a connector (a modified 22-gauge cannula; Plastics One, Roanoke, VA, USA) mounted to the skull with jeweler's screws and dental cement. A plastic blocker was placed over the opening of the connector when not in use. Catheters were flushed daily with saline and every second day with 0.1 ml of a saline–heparin solution (0.2 mg/ml Hepalean 1000 IU, Organon, Toronto, ON, Canada). Animals were allowed 6 days of recovery after surgery before testing.

Intracranial cannulation. For the microdialysis experiment, bilateral guide cannulas (22 gauge; Plastics One, Roanoke, VA, USA)—aimed at the nucleus accumbens—were implanted under sodium pentobarbital anesthesia (65 mg/kg i.p., MTC Pharmaceutical, Cambridge, ON, Canada). Just before surgery, rats were given atropine sulfate (0.6 mg/ml; 0.3 ml/rat s.c., MTC Pharmaceutical, Cambridge, ON, Canada). With the arm of the stereotaxic inclined at a 10° angle, the following coordinates were used: AP +1.6 mm, ML ±2.8 mm, and DV −5.5 mm (Paxinos and Watson, 2005). Animals were allowed at least 8 days of recovery after surgery before testing.

Ovariectomy. Females were ovariectomized bilaterally through lumbar incisions under general anesthesia induced by ketamine hydrochloride (50 mg/kg, i.p.) and xylazine hydrochloride (4 mg/kg, i.p.) mixed in a ratio of 4:3, respectively. All females were given 1 week of postsurgical recovery and maintained for the duration of the experiment on hormone replacement by subcutaneous injections of estradiol benzoate (10 μg in 0.1 ml of sesame oil) 48 h and progesterone (500 μg in 0.1 ml of sesame oil) 4 h before testing.

Histology

At the end of the microdialysis experiment, animals were perfused transcardially with saline and formaldehyde (formalin 10% (v/v); Anachemia, Montreal, QC, Canada) under sodium pentobarbital anesthesia. Brains were removed and fixed in formaldehyde for at least 24 h before sectioning. Brains were sectioned at 30 μm, and every third section through the ventral striatum was mounted and stained with Cresyl Violet. Data from individual subjects were discarded if the microdialysis probe was positioned beyond the boundaries of the intended site.

Apparatus

Intravenous self-administration. Each of the 20 Plexiglas operant chambers (model ENV-008CT, Med Associates, Lafayette, IN, USA) was enclosed in larger sound-attenuating plywood chambers (model ENV-018M, Med Associates). Each operant box had a house light (28 V) and two levers: one retractable and one stationary, located 10 cm apart and 8 cm above the floor of the box. The retractable lever (active lever) was connected to an infusion pump for the delivery of drugs (Razel Scientific Instruments, Stamford, CT, USA), positioned outside the sound-insulating chamber. The stationary lever served to control for baseline, non-reinforced operant behavior; pressing this lever had no consequence (inactive lever), but all presses were recorded. A white-light (28 V) stimulus located 3 cm above the active lever was illuminated for 30 s at the beginning of the session, and for the duration of each drug infusion (5 s), serving as a discrete stimulus for drug delivery.

Locomotion. Locomotor activity was monitored using 12 chambers (30 × 40 × 26 cm, custom made, University of Guelph, Gulph, ON, Canada) made of dark gray PVC. The entire set of chambers was located in the center of the floor of a laboratory room and covered with black wire mesh to allow video tracking of the rats during testing. The tracking software used was EthoVision (version 3, Noldus Information Technology, The Netherlands).

Microdialysis. Microdialysis was conducted in four hexagonal chambers (42 × 39 × 33.5 cm, custom made, Concordia University, Montreal, QC, Canada) built from Plexiglas with wooden ceilings and stainless steel grid floors. The cages were individually housed in wooden cubicles and lighting was provided on a reverse cycle by overhead lights.

The dialysis probes consisted of a 2.5 mm length of semi-permeable dialysis membrane (Fisher Scientific, 240 μm OD, 13 000 MW cutoff), closed at one end and attached to a 21 mm long, 26-gauge stainless steel tubing. A 40–50-cm-long piece of PE tubing connected the other end of the stainless steel shaft to a single channel liquid swivel stationed above the testing chamber that was, in turn, connected to a variable speed infusion pump. Small-diameter fused silica tubing extended internally through the probe with one end resting 0.5 mm from the tip of the probe and the other end exiting the PE tubing 5–8 cm above the stainless steel shaft. The probe was secured in place by stainless-steel collars that were screwed onto the guide cannula. The external length of the PE tubing was protected from chewing by steel spring casing. The probes were inserted the day before the beginning of microdialysis testing. To prevent occlusion, artificial cerebral spinal fluid (CSF: 145 mM Na^+, 2.7 mM K^+, 1.2 mM Ca^{2+}, 1.0 mM Mg^{2+}, 150 mM Cl, 0.2 mM ascorbate, 2 mM Na_2HPO_4, pH 7.4 + 0.1) was perfused overnight at a rate of 0.03 μl/min.

A 10 μl volume of dialysate was extracted from each sample and analyzed immediately on the high-performance liquid chromatography systems with electrochemical detection (HPLC-EC). The dialysate analysis has been described previously (Sorge *et al*, 2005). Briefly, dopamine and metabolites (dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) and 5-hydroxyindole acetic acid (HIAA)) were measured simultaneously on one of the two systems and were adjusted to allow for the separation and quantification of dopamine, DOPAC, HVA, and HIAA in a single run. The peaks obtained for dopamine, DOPAC, HVA, and HIAA were integrated and quantified by EZChrom Chromatography Data System (Scientific Software Inc., San Ramon, CA, USA).

Bi-level chambers. Six custom-made (Concordia University) bi-level chambers were constructed of Plexiglas (outside dimensions of $18 \times 25 \times 65$ cm) with a platform (40 cm in length) elevated by a set of ramps at each end dividing the chamber into two levels (for further details see Pfaus *et al*, 1990).

Food consumption chambers. Six custom-made (University of Guelph) dark gray PVC boxes were used to test for consumption of highly palatable food. In our laboratory, this apparatus is employed normally for place-conditioning experiments. The boxes are located in the center of the laboratory room and are comprised of three compartments: two large ($30 \times 40 \times 26$ cm) and one smaller, middle ($23 \times 30 \times 26$ cm) compartment. Removable inserts, with or without small archway openings (10×10 cm), form the central compartment. Food cups (ceramic dishes) were secured in one corner of each large compartment, opposite to the archway openings. The entire set of chambers was covered with black wire mesh to allow video tracking of the rats during testing. The tracking software used was EthoVision (version 3, Noldus Information Technology, The Netherlands).

Hot plate. Analgesia was assessed using a hot plate apparatus (Model 58725, Stoelting Co., Wood Dale, IL, USA). The heated surface (22×22 cm) was maintained at $50 \pm 0.2^{\circ}$C.

Procedures

Experiment 1: effect of high-dose methadone maintenance on basal and cocaine-stimulated dopamine concentrations in the ventral striatum. The procedures used were similar to those reported previously by Sorge *et al* (2005). Different groups ($n = 4$–12) of male Long–Evans rats (350–375 g) were used for each dose of methadone (0, 20 and 40 mg/kg/day) and each dose of cocaine (10, 20 or 30 mg/kg, i.p.). The same doses of cocaine were used in previous studies examining interactions with buprenorphine (Sorge *et al*, 2005). An additional group of rats ($n = 9$) was maintained on 30 mg/kg/day and challenged with 20 mg/kg cocaine because this dose combination was employed in our previous methadone–cocaine experiments. Osmotic minipumps were implanted 3 days before insertion of microdialysis probes (usually 1500–1600). Dialysate sampling began the next morning at 900. The dialysate flow rate was increased to 0.7 μl/min, and baseline dialysate samples (approximately 14 μl) were collected every 20 min and analyzed immediately. Once stability of dopamine was achieved (less than 10% variation in three consecutive samples), rats were injected with cocaine and samples were collected at 20-min intervals for 140 min. Food was removed from the chambers before sampling, but a water drinking tube was available throughout.

Experiment 2: effect of high-dose methadone maintenance on cocaine self-administration on a PR schedule of reinforcement. Fourteen Sprague–Dawley male rats (300–350 g) were initially trained to self-administer 0.5 mg/kg/infusion cocaine (Cocaine HCL, Dumex, Toronto, ON, Canada) for five consecutive daily sessions, each lasting 3 h. The same dose of cocaine has been used in previous experiments employing Sprague–Dawley male rats to investigate the effect of methadone maintenance on cocaine self-administration on a continuous schedule of reinforcement and on cocaine-seeking behavior (Leri *et al*, 2006).

For each self-administration session, rats were placed in the chambers and their connector was attached to the infusion line. Each session started with the activation of the house light, the entry of the retractable lever and the illumination of the light stimulus for 30 s. Subsequently, lever-presses on the active lever led to drug infusions according to a fixed ratio 1 (FR1) schedule of reinforcement. Cocaine was infused at a volume of 150 μl over a 5-s period, and during this period, the light stimulus was illuminated. Responses on the active lever made during the infusion were recorded, but did not lead to further infusions. Drug concentration was adjusted for differences in body weight.

Following the last session of acquisition of self-administration (ie, FR1 5), rats were implanted with osmotic minipumps filled either with vehicle ($n = 6$) or 30 mg/kg/day methadone ($n = 8$), and after a 5-day recovery period, they received a 2-h test of locomotor activity. Twenty-four hours after this test, self-administration for the same dose of cocaine resumed using a PR schedule of reinforcement. In this schedule, response requirements escalated through steps calculated by the following equation: response ratio $= (5e^{(0.2 \times \text{infusion number})}) - 5$, rounded to the nearest integer. Animals were tested under these conditions for 4 consecutive days, in 3-h long sessions.

Experiment 3: effect of high-dose methadone maintenance on male sexual behavior. Thirty sexually experienced Long–Evans male rats (600–700 g) were randomly assigned to three groups receiving pumps filled with vehicle, 10 or 30 mg/kg/day methadone, although one rat from the high-methadone group was subsequently removed because of poor health. After 5 days of recovery from methadone-pump implantation, each male rat was introduced in the bi-level chamber followed by the introduction of a sexually receptive and experienced female, 5 min later. Subsequent male sexual behavior was videotaped for 30 min. During this period, frequency of the following behaviors was recorded (1) appetitive level changes (number of level changes in the 5-min period before the introduction of the female), (2) pursue level changes (number of level changes during pursue of the female), (3) genital investigation, (4) mounts, (5) intromissions, and (6) ejaculations. Eight days later, the minipumps were removed, and rats received two additional tests 24 h (early withdrawal) and 14 days (late withdrawal) after pump removal.

Experiment 4: effect of high-dose methadone maintenance on consumption of palatable food. The 24 Sprague–Dawley male rats (300–350 g) used in this experiment were not food deprived. Because of this, food pre-exposure sessions were given to establish reliable food consumption within the limited time period of the test.

The experiment began with a session of habituation to the entire apparatus. On this day, the inserts with openings were used, and rats had free access to the three compart-

ments for 20 min. Then, rats received 5 consecutive days of food exposure training. Each day, the inserts with openings were replaced with solid inserts, and rats were confined for 30 min in one of the two large compartments (a.m. session: between 1130 and 1230). At least 4 h later, the same animal was confined to the alternate large compartment for another 30 min (p.m. session: between 1630 and 1830). Ten rings of froot-loop cereal were placed in the ceramic dish within one compartment only. The specific compartment chosen to contain food was counterbalanced across rats. In addition, the time of food session (AM or PM) was counterbalanced across rats and, for each rat, across days of training. Three days after the last exposure session, 12 rats received sham surgery whereas 12 rats received minipumps dispensing 30 mg/kg/day methadone. Finally, 5 days after surgery, rats were tested for food consumption. During this test, rats had access to the entire apparatus and 10 froot-loops were placed in the appropriate food compartment. The pumps were removed 8 days later to verify the effect of methadone withdrawal on body weight.

Experiment 5: effect of high-dose methadone maintenance on basal and cocaine-induced analgesia. An initial study was carried out in 30 Sprague–Dawley male rats (300–350 g) to establish baseline latency in the hot-plate test. Time to lick one hind paw was used as the end point (Carter, 1991). In the subsequent methadone experiment, the cutoff latency to prevent tissue damage was 3 times that observed in rats tested without treatment (Franklin and Abbott, 1989).

In the methadone experiment, 17 Sprague–Dawley male rats (300–350 g) were assigned to two groups receiving either vehicle-filled minipumps ($n=8$) or 30 mg/kg/day methadone minipumps ($n=9$). Five days after implanting the minipumps, all rats received the first test of baseline analgesia 20 min after an injection of vehicle (saline, i.p.). Three days later, a second test of analgesia was administered 20 min after an injection of 5 mg/kg cocaine (i.p.) and, 3 days later, a final test was administered 20 min after an injection of 20 mg/kg cocaine (i.p.). These two doses of cocaine have been found to produce minimal and maximal analgesia in male Sprague–Dawley rats (Lin *et al*, 1989; Waddell and Holtzman, 1999). All tests were carried out between 1600 and 1700. The pumps were removed 3 days after the last test to verify the effect of methadone withdrawal on body weight.

Statistical analyses. One- and two-factor ANOVAs with independent and repeated measures were used as appropriate for the design of each experiment. In case of significant interactions or significant main effects, multiple comparisons were performed using the Newman–Keuls method to identify individual mean differences ($\alpha=0.05$). The specific values of negative findings are not reported. All statistical analyses were performed using SigmaStat (version 3.0 for Windows, SPSS Inc.).

RESULTS

Experiment 1

Histological analysis revealed that the probes were located in the medial nucleus accumbens, encroaching on both the shell and the core region, and in some cases, in the dorsal striatum. The functional tip of the probe (ie, 2.5 mm long), however, was confined to the ventral striatum in all cases (see Figure 1 for 26 rats with near identical probe locations, $n=10$, 5, 7, and 4 for 0, 20, 30, and 40 mg/kg/day methadone, respectively).

Basal levels of dopamine within the ventral striatum were measured in four consecutive samples before injections of cocaine. Although there was no significant effect of methadone dose on basal dopamine levels, there was a trend (see Figure 2a; ($F(3,22)=2.5$, $p=0.09$)). *Post hoc* analysis revealed that the basal dopamine level for the rats with vehicle-filled minipumps was significantly different from that of rats with 30 mg/kg/day methadone minipumps ($p<0.05$). Thus, methadone had a small dose-dependent effect on basal dopamine levels.

Methadone enhanced the dopamine response to acute injections of cocaine in the ventral striatum except at the highest dose of cocaine (see Figure 2b–d). As not all doses of cocaine were tested with every dose of methadone, separate ANOVAs were performed for each dose of cocaine. For the 10 mg/kg dose of cocaine (panel b; $n=4$, 4, and 6 for the 0, 20, and 40 mg/kg/day of methadone, respectively), the ANOVA revealed significant main effects of methadone dose ($F(2,11)=6.2$, $p<0.05$) and postinjection time ($F(6,66)=29.3$, $p<0.001$). *Post-hoc* analysis revealed that the dopamine response to cocaine was significantly greater in the 40 mg/kg methadone group than in either 20 mg/kg/day or vehicle group ($ps<0.05$). At 20 mg/kg dose of cocaine (panel c; $n=12$, 8, 9, and 8 for 0, 20, 30 and 40 mg/kg/day doses of methadone, respectively), the ANOVA revealed significant main effects of methadone dose ($F(3,33)=3.9$, $p<0.05$), postinjection time ($F(6,198)=$

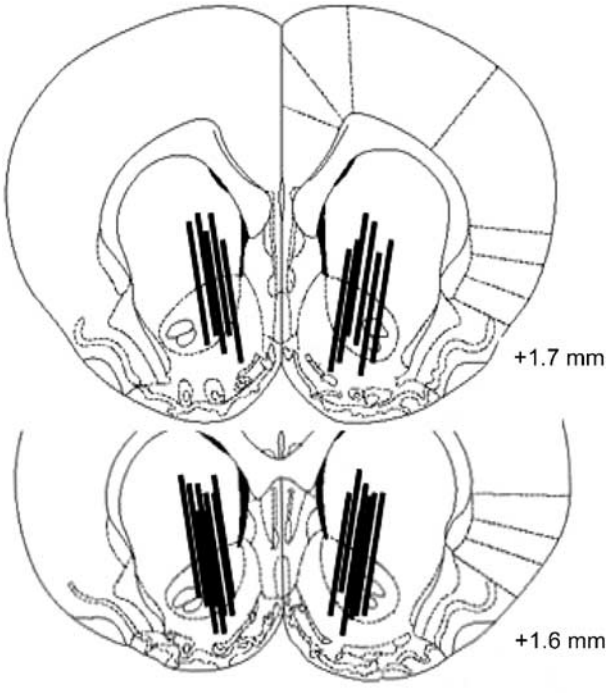

Figure 1 Verified location of microdialysis probe implanted in the ventral striatum, plotted on drawings for coronal sections from the atlas of Paxinos and Watson (2005).

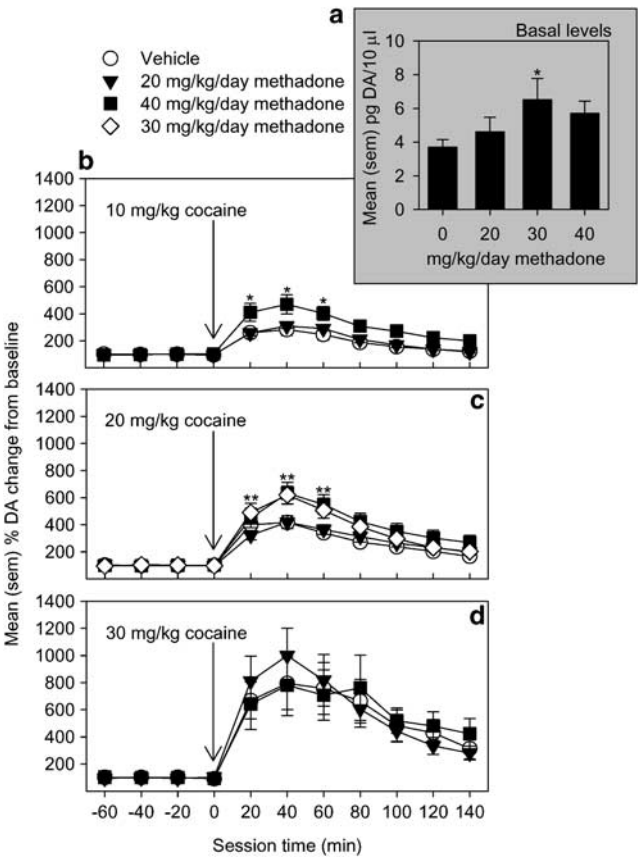

Figure 2 (a) Mean (SEM) basal dopamine concentrations sampled in the nucleus accumbens of rats chronically exposed to different doses of methadone released by osmotic minipumps implanted subcutaneously. The * indicates a significant difference from the 0 mg/kg/day methadone group. (b–d) Mean (SEM) percent increase in dopamine concentration in the ventral striatum caused by cocaine injections (i.p.) given to rats maintained on vehicle and different methadone doses. The * indicates a significant difference between rats maintained on 40 mg/kg/day methadone and vehicle. The ** indicates a significant difference between rats maintained on 30 and 40 mg/kg/day methadone and rats maintained on vehicle.

60.9, $p<0.001$), and a significant methadone dose by postinjection time interaction ($F(18,198)=2.1$, $p<0.01$). *Post-hoc* tests revealed that the dopamine response was greater in the 40 and 30 mg/kg/day methadone groups than in the vehicle group ($ps<0.05$). Only at the highest dose of cocaine given (30 mg/kg, panel d; $n=6$, 5, and 5 for 0, 20, and 40 mg/kg/day, respectively) was there no effect of methadone. The ANOVA revealed a significant main effect of postinjection time only ($F(6,78)=22.9$, $p<0.001$).

Experiment 2

During the acquisition of cocaine self-administration, rats displayed significant increases in responding on the active lever compared with significant decreases in responding on the inactive lever (data not shown; Session by Lever interaction ($F(4,52)=7.1$, $p<0.001$) and main effect of Lever ($F(1,13)=5.2$, $p<0.05$). Along with increases in responding on the active lever, number of cocaine infusions also increased significantly (data not shown; ($F(4,52)=6.8$, $p<0.001$)).

Following implantation of osmotic minipumps, it was found that methadone-maintained rats showed a higher level of locomotion (mean $\pm$ SEM $=20\,464.1\pm2144.4$) than the vehicle-maintained rats (mean $\pm$ SEM $=15\,421.9\pm1442.5$), but this difference was not significant ($p=0.09$). In self-administration, methadone maintenance had a substantial impact on PR responding for cocaine (Figure 3a; Group by Session interaction ($F(4,48)=6.3$, $p<0.001$), main effect of Group ($F(1,12)=7.1$, $p<0.05$)], and main effect of Session ($F(4,48)=6.6$, $p<0.001$)). In fact, as a result of implementation of this schedule, methadone-maintained rats showed no increase in responding from the last day of self-administration on FR1 before pump implantation (FR1 (5)) to the first PR session after pump implantation (PR (1)). Moreover, in comparison to vehicle rats, methadone-maintained rats showed significantly lower levels of responding on each PR session. Similarly, although the implementation of the PR led to significant decreases in cocaine intake in both groups, methadone-maintained rats obtained significantly fewer infusions of cocaine than the vehicle-treated rats on each PR session (Figure 3b; main effect of Group ($F(1,12)=5.1$, $p<0.05$) and main effect of Session ($F(4,48)=8.5$, $p<0.001$)).

Experiment 3

In this experiment, it was observed that most aspects of male sexual behavior were suppressed during methadone maintenance in a dose-dependent manner. Some behaviors returned to normal ranges within 24 h of pump removal, and all aspects of sexual behavior recovered by 10 days of withdrawal from methadone. Thus, as it can be seen in Figure 4, the frequency of appetitive level changes was significantly suppressed in animals maintained on the highest methadone dose, and returned to control levels on the subsequent tests during early and late withdrawal (Group by Drug exposure interaction ($F(4,52)=3.1$, $p<0.05$), and main effect of Drug exposure ($F(2,52)=8.6$, $p<0.001$)). Very similar findings were observed for frequencies of pursuit (significant main effect of Group ($F(2,26)=4.8$, $p<0.05$) and Drug exposure ($F(2,52)=8.2$, $p<0.001$)), mounts (significant main effect of Group ($F(2,26)=4.6$, $p<0.05$) and Drug exposure ($F(2,52)=23.5$, $p<0.001$)), intromissions (data not shown; significant Group by drug exposure interaction ($F(4,52)=11.2$, $p<0.001$), main effect of Group ($F(2,26)=22.6$, $p<0.001$), and main effect of Drug exposure ($F(2,52)=3.6$, $p<0.05$)), and ejaculations (data not shown; significant Group by Drug exposure interaction ($F(4,52)=17.5$, $p<0.001$), main effect of Group ($F(2,26)=9.5$, $p<0.001$), and main effect of Drug exposure ($F(2,52)=17.6$, $p<0.001$)). The only aspect of male sexual behavior that was not significantly reduced by methadone maintenance was frequency of genital investigation (only a significant effect of Drug exposure ($F(2,52)=15.2$, $p<0.001$)).

Experiment 4

There was no difference in the number of froot-loops consumed by non-food-deprived control rats (mean $\pm$

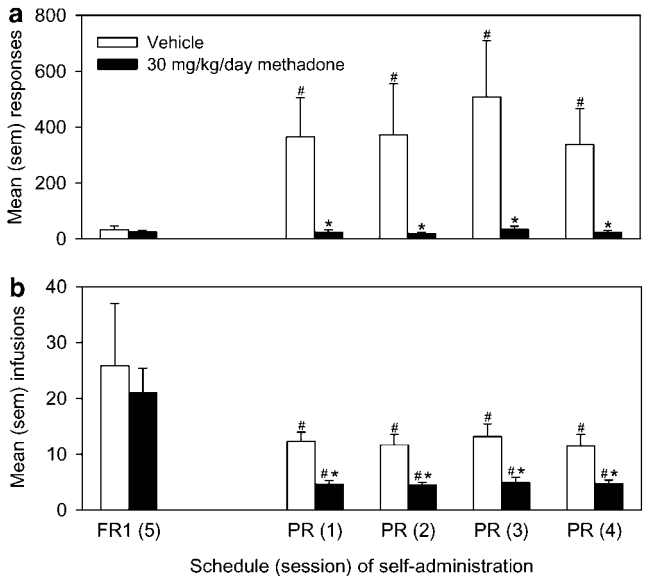

Figure 3 (a) Mean (SEM) responses emitted by rats implanted with vehicle- and methadone-filled minipumps on the last day of cocaine (0.5 mg/kg/infusion) self-administration on a continuous schedule of reinforcement (FR1 (5)), and on the four subsequent sessions of cocaine (same dose) self-administration on a PR schedule. (b) Mean (SEM) infusions in the same rats, under the same conditions. The # indicates significant within-group differences from the last day of self-administration on a continuous schedule of reinforcement (FR1 (5)). The * indicates a significant difference between rats maintained on 30 mg/kg/day methadone and rats maintained on vehicle.

SEM = 6.6 ± 1.2) and methadone-maintained rats (6.8 ± 0.7). This lack of effect of methadone maintenance on consumption of palatable food cannot be attributed to faulty operation of the methadone-filled minipumps as a significant loss of body weight was observed 24 h after their removal (data not shown; Group by Time interaction ($F(1, 22) = 190.5$, $p < 0.001$), main effect of Group ($F(1, 22) = 37.7$, $p < 0.001$), and main effect of Time ($F(1, 22) = 132.1$, $p < 0.001$)).

Experiment 5

Methadone maintenance at 30 mg/kg/day for 5 days did not affect the latency to lick the hind paws on the hot–plate; that is, methadone maintenance did not induce analgesia or hyperalgesia in this test. In fact, the ANOVA revealed no differences in latencies between rats that did not receive pump surgery (mean ± SEM = 24.3 ± 2.1), rats implanted with pumps containing vehicle (see Figure 5), and rats on methadone (see Figure 5). Similarly, although cocaine dose dependently increased paw-lick latency, there was no effect of methadone treatment: the ANOVA revealed a significant effect of Test ($F(2, 30) = 20.6$, $p < 0.001$), but no group effect. As shown in Figure 5, 20 mg/kg cocaine significantly elevated response latencies over baseline whereas 5 mg/kg cocaine had no effect, and this effect was equivalent in the two groups. This lack of effect of methadone maintenance on basal and cocaine-induced analgesia cannot be attributed to faulty operation of the methadone-filled minipump as a significant loss of body weight was observed 24 h after the removal of methadone-filled minipumps (data not shown; Group by Time interaction ($F(1, 15) = 75.0$, $p < 0.001$), main effect of Group ($F(1, 15) = 116.2$, $p < 0.001$), and main effect of Time ($F(1, 15) = 152.7$, $p < 0.001$)).

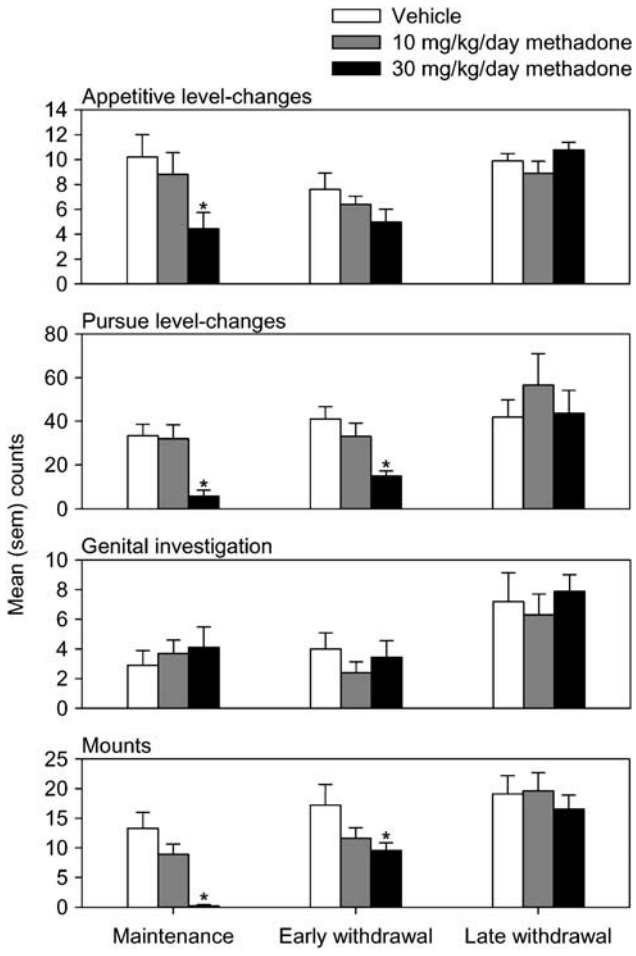

Figure 4 Mean (SEM) frequency of sexual behaviors displayed by male rats during the maintenance on 0, 10 or 30 mg/kg/day methadone, 24 h after pump removal (early withdrawal) and 10 days after pump removal (late withdrawal). The * indicates a significant difference between rats maintained on methadone and rats maintained on vehicle.

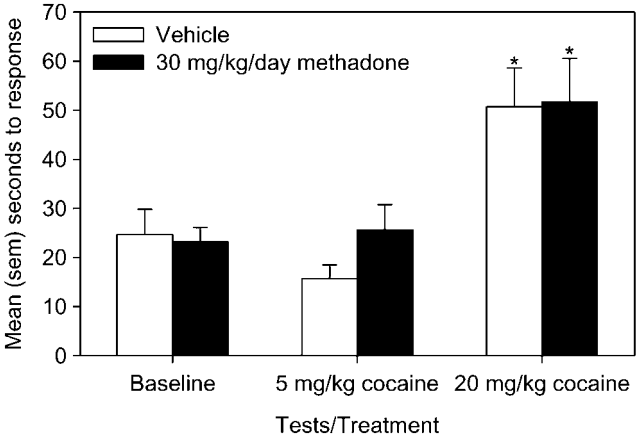

Figure 5 Mean (SEM) latency to lick the hind paws on the hot plate in rats implanted with vehicle- and methadone-filled minipumps after an injection of saline (baseline) or 5 and 20 mg/kg cocaine (i.p.). The * indicates significant within group differences from baseline latencies.

DISCUSSION

In these experiments performed in rats, it was found that maintenance on high doses of methadone reduced both self-administration of cocaine on a PR schedule, as well as the sexual responses of male rats to estrous females. Methadone did not, however, reduce the consumption of highly palatable food in non-deprived animals, alter pain responses to thermal stimulation or affect cocaine-induced analgesia. In tests of the acute effects of cocaine on the levels of extracellular dopamine in the ventral striatum, it was found that maintenance on high doses of methadone enhanced the responses to low doses of cocaine, but were without affects at the highest dose of cocaine. Finally, although there was no evidence that dopamine responses to cocaine were reduced, there was some evidence that maintenance on high doses of methadone increased basal levels of dopamine within the ventral striatum.

Heavy cocaine use at admission in methadone-maintenance programs is a significant predictor of continued cocaine use and of poor retention in treatment (Marsch *et al*, 2005). However, at doses above 100 mg/day, methadone maintenance is efficacious in reducing cocaine use (Maxwell and Shinderman, 1999; Stine *et al*, 1991; Peles *et al*, 2006a) and in promoting long-term (ie, 11 months) retention in treatment (Peles *et al*, 2006b). Our studies in laboratory rats suggest that this reduction in cocaine use can result from a pharmacological action of high–dose methadone maintenance on cocaine seeking. In fact, during cocaine self-administration on a PR (Experiment 2), it was found that rats maintained on 30 mg/kg/day methadone failed to adjust to the increased work requirement to obtain drug infusions and, as a result, obtained significantly less cocaine than control animals. These results are consistent with previous findings from our laboratories showing that rats maintained on doses of methadone above 20 mg/kg/day do not respond for cocaine-conditioned cues, do not approach a cocaine-conditioned environment, and do not show reinstatement of cocaine seeking after priming injections of cocaine (Leri *et al*, 2004, 2005, 2006). It is important to note that methadone-induced blockade of cocaine seeking observed in our experiments is not an artifact of impaired sensory-motor functioning. In fact, rats tested in Experiment 2 displayed normal locomotor activity and those tested in our previous experiments showed no reduction of cocaine self-administration on a continuous schedule of reinforcement, and showed significant reinstatement of heroin and cocaine seeking after exposure to foot-shock stress (Leri *et al*, 2004, 2006).

Interestingly, it was found that methadone maintenance did not attenuate the rise in extracellular dopamine levels in the ventral striatum induced by acute cocaine injections. If anything, we observed a potentiation of this response, which is not surprising in light of known synergistic interactions between opiates and cocaine on the dopamine system (Brown *et al*, 1991; Hemby *et al*, 1999; Martin *et al*, 2006; Zernig *et al*, 1997). Furthermore, this effect of methadone was very similar to the effect of buprenorphine maintenance on cocaine-induced elevations in extracellular dopamine recently reported by Sorge *et al*, 2005 and Sorge and Stewart, 2006. Taken together, these results provide some support for the hypothesis that chronic maintenance on mu-opioid receptor agonists does not reduce, and can even enhance, some behavioral (Foltin *et al*, 1995; Foltin and Fischman, 1996; Leri *et al*, 2006; Preston *et al*, 1996) and neurochemical indexes of reinforcing effects of cocaine (Di Chiara and Imperato, 1988; Koob and Bloom, 1988; Wise and Bozarth, 1981).

There are a number of possible neurochemical mechanisms that could account for the effect of high-dose methadone maintenance on cocaine seeking. It is possible that the tonic elevation in basal levels of dopamine induced by methadone maintenance impaired the ability of this system to respond phasically to cocaine-related cues (Floresco *et al*, 2003; Gratton and Wise, 1994; Phillips *et al*, 2003), and thus compromised initiation and/or maintenance of motor behaviors directed toward these cues (Mogenson *et al*, 1980).

Alternatively, chronic maintenance on mu-opioid receptor agonists may have interfered with the development, maintenance or expression of cocaine-induced neural changes that promote cocaine seeking. Although cocaine exposure induces significant alterations in several neurochemical systems (Everitt and Wolf, 2002; Kalivas *et al*, 2005; Koob and le Moal, 2001; Kreek, 2001; Nestler, 2001; Robbins and Everitt, 2002; Robinson and Berridge, 2003; Self, 2004; Shaham and Hope, 2005; Stewart, 2003), our most recent work has focused on mu-opioid receptors. In fact, we have reported that rats maintained on high-dose methadone maintenance show no cocaine-place preference and no cocaine-induced up-regulation of mu-opioid receptor mRNA in the nucleus accumbens core (Leri *et al*, 2006). This is notable because the effect of cocaine exposure on mu-opioid receptor density and function in mesocorticolimbic areas (Azaryan *et al*, 1996; Unterwald *et al*, 1992; Unterwald, 2001; Yuferov *et al*, 1999) has been associated to the intensity of cocaine cravings in humans (Gorelick *et al*, 2005; Zubieta *et al*, 1996), and to a variety of behavioral, electrophysiological and neurochemical responses to cocaine in animals (Mathon *et al*, 2005a, b, 2006; Tang *et al*, 2005; Hummel *et al*, 2006).

The results of the other experiments included in this report indicate that rats maintained on high–dose methadone did not suffer from general behavioral impairments. In fact, even if not food deprived, they displayed normal approach and normal consumption of highly palatable food. However, sexual behavior was negatively affected by high-dose methadone maintenance. In fact, although sexually well experienced in the testing apparatus, male rats showed no conditioned excitement in anticipation to the arrival of the receptive female (Mendelson and Pfaus, 1989). Likewise, after the introduction of the female, male rats maintained on methadone showed no species-specific behaviors necessary for copulation such as pursue and mounting (Pfaus *et al*, 1990). Although this experiment did not identify the mechanism(s) by which methadone maintenance produced these impairments (Agmo and Paredes, 1988; Band and Hull, 1990; Ceccarelli *et al*, 2006; Cicero *et al*, 1975, 1976; Pfaus and Gorzalka, 1987; Rodriguez-Manzo *et al*, 2002; Tokunaga *et al*, 1977), the data clearly indicate that sexual dysfunctions observed in methadone-maintained individuals (Daniell, 2002b; Fischer *et al*, 2002) can result from a direct pharmacological effect of methadone (Bliesener *et al*, 2005; Daniell, 2002a; de la Rosa and Hennessey, 1996).

Maintenance on methadone has also been associated with enhanced responses to pain. In fact, there is evidence supporting increased sensitivity to experimental pain in patients receiving opioid agonist therapy (Alford *et al*, 2006a; Compton *et al*, 2000; Doverty *et al*, 2001a). In Experiment 5, rats arbitrarily assigned to groups and tested after being on a high dose of methadone or vehicle for 5 days, showed no differences in latencies of responding to painful thermal stimulation. In these rats, therefore, we noted rapid development of tolerance (Adams and Holtzman, 1990), but not hyperalgesia. Furthermore, rats tested in Experiment 5 showed unaltered analgesic response to acute injections of 5 and 20 mg/kg cocaine leading to the conclusion that, unlike acute administration (Misra *et al*, 1987; Zavala *et al*, 2003), steady-state methadone exposure does not potentiate the acute analgesic effect of cocaine.

In summary, our studies in laboratory rats show that high-dose methadone maintenance effectively reduced cocaine seeking without reducing the effect of cocaine on dopamine overflow in the ventral striatum, and without producing generalized behavioral impairments. Overall, such results in animals support the usefulness of high-dose methadone as a pharmacological tool to reduce severe cocaine abuse in opioid-dependent individuals, and possibly in the management of pure-cocaine addiction.

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