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Chronic buprenorphine reduces the response to sucrose-associated cues in non food-deprived rats

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Abstract

The mechanisms through which buprenorphine (BUP), a mixed opioid agonist–antagonist, reduces both heroin and cocaine taking remain unclear. Evidence suggests that chronic exposure to BUP blunts drug seeking by attenuating the salience of drug-associated cues. Here, we examined the effect of chronic BUP treatment (osmotic minipumps, 3.0 mg/kg/day) in rats on responding for sucrose pellets and associated cues on FR1, FR5, and PR schedules and on extinction and reinstatement of sucrose seeking by sucrose priming. The effect of chronic BUP treatment on the dopamine (DA) response in the nucleus accumbens (NAc) to sucrose pellets and to lab chow was also measured using in vivo microdialysis. Whereas chronic BUP treatment had only a modest effect on pellet intake on the FR1 schedule, it significantly reduced responding at the outset of sessions and reduced lever pressing during sucrose-associated cue presentations. No effect was observed in the FR5 or PR schedules. BUP slightly reduced responding during extinction and significantly reduced reinstatement. Chronic BUP did not alter the NAc DA response to either sucrose pellets or lab chow, although it did significantly increase basal DA. Consistent with previous studies with heroin and cocaine, chronic BUP reduced responding in the presence of reward-related cues.

Keywords: Buprenorphine; Sucrose; Reward; Reinstatement; Conditioned cues; Dopamine; *In vivo* microdialysis; Nucleus accumbens

1. Introduction

Buprenorphine (BUP) is a mixed opioid agonist–antagonist currently used as a treatment for opioid addiction (Tzschentke, 2002). Daily injections of BUP significantly reduce heroin intake in human opioid addicts, as shown in outpatient treatment studies and in experimental settings in which participants are allowed to self-administer heroin under medical supervision (Strain et al., 1994; Mello and Mendelson, 1980). In rhesus monkeys, daily BUP injections reduce operant responding for heroin on a second order, cue-controlled schedule of reinforcement (FR4 [VR 16:S]) (Mello et al., 1983; Mello and Negus, 1998). Interestingly, a number of reports suggest that BUP also reduces the intake of cocaine, in both humans (e.g., Strain et al., 1996; Kosten, 1989; Ling et al., 1996, but see Johnson et al., 1995; Oliveto et al., 1995) and laboratory animals. For example, in rhesus monkeys Mello and colleagues found that daily BUP treatment reduced responding for cocaine on a second order schedule of reinforcement (Mello et al., 1992), as well as for a heroin-cocaine 'speedball' combination (Mello and Negus, 1998). In rats, chronic exposure to BUP via osmotic minipumps reduces self-administration of cocaine on fixed ratio (FR) 1 and FR5 schedules, and reduces breakpoints on a progressive ratio (PR) schedule (Sorge and Stewart, 2006).

Although the clinical efficacy of BUP in treating opioid addiction has been repeatedly demonstrated, the mechanism by which BUP reduces intake of abused drugs such as cocaine remains unknown. For example, Sorge and colleagues (Sorge et al., 2005; Sorge and Stewart, 2006) reported that, whereas chronic BUP treatment in rats attenuates the rise in dopamine (DA) in the nucleus accumbens (NAc) induced by an acute injection of heroin, chronic BUP augments the increase in NAc

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DA following an acute injection of cocaine. The results of studies by Sorge and colleagues, however, have suggested that BUP might reduce seeking of both heroin and cocaine by diminishing the salience of cues previously paired with drugs. For example, Sorge and Stewart (2006) reported that rats trained to self-administer heroin or cocaine were slower to respond to the presentation of drug-associated cues at the start of self-administration sessions when under the influence of chronic BUP via osmotic minipump. In other experiments, BUP treatment was found to reduce drug-seeking behavior during extinction of both heroin and cocaine self-administration and to attenuate reinstatement following a priming injection of either drug (Sorge et al., 2005). Given that the common effect in these experiments appeared to be a reduction in the response to drug-associated cues, one interpretation of these findings is that BUP treatment impedes the ability of such cues to initiate and maintain motivated behavior.

We sought to investigate this idea by examining how chronic BUP treatment would affect responding for a non-drug reward (sucrose pellets) and for cues paired with this non-drug reward. Others have examined the interaction of BUP with motivation for non-drug rewards, such as food or sucrose, although the results of these studies are mixed. In some cases, BUP treatment reduced responding for these rewards in both non-food-deprived and deprived monkeys (e.g., Comer et al., 2002; Dykstra, 1983), but this effect has been found to gradually diminish with prolonged BUP administration (e.g., Lukas et al., 1988; Mello et al., 1992). Notably, however, these studies did not specifically examine the influence of BUP on responding to food-or sucrose-related cues by using measures of reward seeking under extinction conditions or in tests for reinstatement of responding. It is also not clear from these studies the extent to which the complex schedules of reinforcement used contributed to the effects of BUP on responding.

We conducted three experiments using methods similar to those used by Sorge and colleagues to explore the generality of the effect of BUP on responding to non-drug rewards and associated cues. First, we tested the effects of chronic BUP treatment in non-deprived rats trained to obtain sucrose pellets on different schedules of reinforcement (FR1, FR5, and PR). Second, we examined the influence of BUP on sucrose-seeking behavior under extinction conditions and during reinstatement. Third, we used in vivo microdialysis to investigate how chronic BUP would affect the rise in DA in the NAc in response to consumption of sucrose or standard lab-chow pellets.

2. Methods

2.1. Experiments 1 and 2: lever pressing for sucrose pellets

2.1.1. Subjects

A total of 35 male Long Evans rats (Charles River, St. Constant, QC) weighing about 350 g at the beginning of the experiments was used. In a preliminary study, seven rats were trained on an FR1 schedule and continued on this schedule for one week after BUP-filled osmotic minipumps were implanted. Data from these rats were combined with those from Experiment 1 ($n=7+14$), which were tested on both the FR1 schedule and the FR5 and PR schedules. An additional 14 rats were used for Experiment 2. All rats were housed individually in plastic shoebox cages in the university colony under a reverse 12:12 h light–dark cycle (lights off at 0800 h) and had access to lab chow (Rat Chow, Purina Foods) and water *ad libitum* throughout the experiments. Experimental procedures were in accordance with the guidelines of the Canadian Council on Animal Care and were approved by the Animal Care Committee of Concordia University.

2.1.2. Surgery

Rats were exposed to a continuous level of BUP by the use of osmotic minipumps (Alzet, model 2ML2, Durect Corp., Cupertino, CA), which were implanted subcutaneously. Rats were anaesthetized using isofluorane gas (Vetoquinol NA Inc., Lavaltrie, QC) and a small incision was made between the scapulae. A small pocket was formed under the skin using a hemostat and a BUP-filled minipump was inserted with the flow moderator pointed away from the opening of the incision to prevent leakage of the drug. The incision was closed using stainless steel wound clips. Pumps were removed in a similar fashion under isofluorane anesthesia. Those animals assigned to the sham condition (SHAM) underwent the same surgical procedure as did BUP-exposed animals but did not receive a pump. As we have noted previously (Sorge and Stewart, 2006), the decision to not implant saline-filled osmotic minipumps in SHAM rats was made on the basis of previous experiments conducted in this laboratory. In these studies concerning the effects of methadone on reinstatement of cocaine and heroin seeking, control rats received saline-filled osmotic minipumps. These rats readily tolerated the minipumps and no effects on their ongoing behavior were observed. Similar findings of a lack of effect of saline-filled minipumps on behavior have been reported elsewhere (e.g., Kunko et al., 1998; Rada et al., 2001; Vann et al., 2006).

2.1.3. Drugs

Buprenorphine HCl was purchased from Reckitt Benckiser Healthcare Limited (Hull, UK) and was prepared in nanopure water. The dose of BUP (3.0 mg/kg/day) used in all experiments was chosen on the basis of previous research conducted in this laboratory. This dose has a significant effect on drug-seeking behavior in rats trained to self-administer heroin and cocaine and has a mild stimulatory effect on locomotion (Sorge et al., 2005).

2.1.4. Apparatus

Seven custom-made operant chambers (Concordia University, Montreal, QC) enclosed within sound-attenuating plywood chambers were used for the self-administration experiments. Each operant chamber contained one retractable or 'active' lever and one stationary or 'inactive' lever (Med Associates, Lafayette, IN). Levers were located on one wall of the chamber, approximately 12 cm apart and each was positioned 7 cm above the floor. The active lever was connected to a food pellet hopper (Med Associates, Lafayette, IN). Completion of a specified

number of responses on the active lever resulted in activation of the hopper and in the release of a single 45-mg sucrose pellet (Research Diets, New Brunswick, NJ) into a magazine within the chamber. The magazine was approximately 5 cm wide by 5 cm high by 4 cm deep and was located 3 cm above the floor of the chamber. Operant chambers were also equipped with a white cue light positioned 5 cm above the active lever. The sound-attenuating plywood chambers were equipped with a red houselight that was illuminated at the beginning of every session and remained on throughout each session.

3. Procedures

3.1. Self Administration on FR1, FR5 and PR schedules (Experiment 1)

Before self-administration training began, rats were given about 100 sucrose pellets per day in heavy ceramic containers in their home cages for four days to reduce any neophobia in response to this food and thereby facilitate training. For each self-administration session, rats were transported from the colony to the operant chambers in the laboratory. One min after the rats were placed in the chamber, the red house light was illuminated to signal the start of the session. Ten seconds after the illumination of the house light, the active lever extended into the operant chamber and the white cue light was turned on for 30 s. This cue light remained on for the full 30-s period unless a response was made on the active lever, after which the cue light would remain on for 5 s only.

An FR1 schedule of reinforcement was used during self-administration training. On this schedule, a single response on the active lever resulted in one sucrose pellet being dispensed into the magazine and the illumination of the cue light for 5 s. During this 5-s period following the dispensing of each pellet, additional responses on the active lever were recorded but had no consequences. Responses on the inactive lever had no programmed consequences, but provided a measure of discrimination between the two levers and a rough measure of general activity in the chamber. At the end of the 180-min session, the active lever retracted and the houselight was extinguished.

Following eight days of self-administration training when sucrose pellet intake had remained constant for three days, rats were switched to an FR5 schedule for one session (day 9) and then to a PR schedule on the following day for one session (day 10). Fixed ratio 5 sessions were conducted in the same way as were the FR1 sessions with the exception that rats had to make five responses on the active lever instead of one to activate the white cue light and receive one sucrose pellet. Similarly, PR sessions were the same as FR1 sessions except that the response requirement for the cue light to be activated and a pellet to be dispensed increased with each pellet earned. This increase was determined by the equation $5e^{(0.2 \times \text{pellet } \#)-5}$, such that one active lever response produced the first pellet, one response produced the second, two responses produced the third, three responses produced the fourth, and so on (Fletcher et al., 1999).

On days 11 and 12, rats were switched back to an FR1 schedule to verify that the experience of the FR5 and PR schedules had not altered the level of responding they previously demonstrated on the FR1 schedule. The number of active lever responses made during sessions 11 and 12 was used to assign rats to either the BUP or SHAM conditions so that the two groups were matched in this respect. Immediately after completing session 12, rats underwent either sham surgery or surgery to implant BUP pumps.

Rats were returned to the operant chambers after a 24-h recovery period and continued self-administration on the FR1 schedule for nine days. On day 10, rats were switched to the FR5 schedule for one session. On day 11, rats were switched to the PR schedule for one session. Immediately after completing the PR session, rats underwent another surgery to remove the BUP pumps (or underwent sham surgery again). After a 24 h recovery period, the rats resumed self-administration on the FR1 schedule for an additional 10 days. A final FR5 session was run on day 11 and a final PR session was run on day 12.

3.2. Study of extinction and reinstatement (Experiment 2)

3.2.1. Training

Fourteen rats were trained to self-administer sucrose for eight sessions on an FR1 schedule. These sessions were conducted in the same manner as described above except that the session length was reduced to 60 min from 180 min. The decision to reduce the session length was made because it was observed during Experiment 1 that rats obtained the majority (about 60–70%) of the total sucrose pellets normally taken in 180-min sessions during the first 60 min. Immediately following the final training session, rats were assigned to either the BUP or SHAM condition and underwent surgery to implant osmotic minipumps on the basis of active lever responding during the final training session.

3.2.2. Extinction

After a 24-h recovery period, rats were returned to the operant chambers and began extinction training. Extinction sessions were 60 min in length and were conducted in the same manner as were the FR1 sessions with the exception that sucrose pellets were not available; each session began with the illumination of the red house light, extension of the active lever into the operant chamber and the illumination of the white cue light for 30 s. Responses on the active lever continued to result in the illumination of the cue light for 5 s and the sound of activation of the dispenser. Extinction training continued for six days. By this time all rats met the criterion of less than 15 responses in 60 min.

3.2.3. Reinstatement

On the seventh day, rats were first given one 60-min extinction session under the same conditions as described above. Their responding during this session could then be compared to that on the reinstatement session during which sucrose pellets were used to prime responding. A 15-min time-out period elapsed between the end of the extinction session and the beginning of the reinstatement session.

The reinstatement procedure used in this experiment was based on that used by Sun et al. (2005) and began as did all other

training and extinction sessions with the illumination of the houselight, extension of the active lever, and illumination of the cue light for 30 s. Three min after the extension of the active lever into the chamber, 3 sucrose pellets were dispensed into the magazine, each spaced by 10-s intervals. The cue light was illuminated for 10 s coincident with the delivery of each pellet. This train of 3 pellets and 3 cue light illuminations was repeated every 3 min throughout the course of the 60 min session. Responses on the active lever during this session had no programmed consequences, but were recorded by the computer along with inactive lever responses.

3.3. Experiment 3: in vivo microdialysis

3.3.1. Subjects

Thirteen male Long–Evans rats (about 350 g; Charles River, St. Constant, QC.) were used in this experiment to study extracellular levels of DA in the NAc in response to sucrose pellets. Rats were housed in the university colony according to the conditions described in the methods for Experiments 1 and 2. Microdialysis testing was conducted between 0900 h and 1700 h.

3.4. Surgery

3.4.1. Intracranial cannulation

Rats were anaesthetized with sodium pentobarbital (Somnotol, 65 mg/kg i.p.; MTC Pharmaceuticals Cambridge, ON) and treated with atropine sulphate (0.11 mg/kg; Sabex, Boucherville, QC.) to reduce respiratory stress during surgery. Unilateral stainless steel cannulae (20 g; Plastics One, Roanoke, VA) were aimed for the nucleus accumbens (NAc) in either the left or right hemisphere using the coordinates AP+1.6 mm, ML+2.8 mm, DV-5.5 mm, from bregma (Paxinos and Watson, 1986). Cannulae were angled at 10° laterally to avoid puncturing the ventricle directly above the NAc. The choice of left or right hemisphere was counterbalanced across all rats. Dental acrylic was used to secure the cannulae to the surface of the skull and all animals were given an intramuscular injection of penicillin (Pen G, Vetoquinol, Lavaltrie, QC) at the end of surgery.

3.4.1.1. Osmotic minipumps. Rats were exposed to a continuous level of BUP (3.0 mg/kg/day) with the use of osmotic minipumps as described above.

3.4.2. Apparatus

3.4.2.1. Testing chambers. The dialysis experiment was conducted using four custom-made hexagonal chambers (42×39×33.5 cm^3; Concordia University). Chambers were comprised of Plexiglas walls, wooden ceilings, and stainless steel rod flooring. Each chamber was housed within a sound-attenuating plywood chamber and a single fluorescent tube was mounted at the top of the plywood chamber to provide lighting on a reverse cycle.

3.4.2.2. Microdialysis probes. Probes were custom made from a 2.5 mm length of semipermeable dialysis membrane (Fisher Scientific, 240 um OD, 13 000 MW cutoff) and a 21 mm long section of 25 g stainless steel tubing. A 40–50 cm section of polyethylene tubing (PE), flared at one end, was connected to the stainless steel tubing. The complete experimental setup has been previously described (Sorge et al., 2005). Probes were inserted into the guide cannulae the day before testing and artificial cerebrospinal fluid (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 0.5 μl/min to prevent the probes from blocking.

3.4.2.3. High-performance liquid chromatography. Ten μl of dialysate were withdrawn from each sample and analyzed immediately using an HPLC system with electrochemical detection (HPLC-EC). The samples were loaded onto C-18 reverse-phase columns (5 um, 15 cm) through manual injection ports (Reodyn 7125; 20 μl loop). Dual-channel ESA coulometric detectors (Coulochem III, with model 5011 analytical cell) were used to measure reduction currents for DA. The mobile phases (20% acetonitrile, 0.076 M SDS, 0.1 M EDTA, 0.058 M NaPO4, 0.27 M citric acid, pH 3.35) were circulated through each closed system at a flow rate of 1.1 mL/min by Waters 515 HPLC pumps. The peak obtained for DA was quantified by the EZChrom Chromatography Data System (Scientific Software Inc., San Ramon, CA).

3.5. Procedures

Rats underwent surgery to implant guide cannula in the NAc and then remained in their home cages for a one-week recovery period. For six days before the start of microdialysis testing, rats were given approximately 100 sucrose pellets per day in heavy ceramic containers in their home cages to reduce the novelty associated with this food. Rats were randomly assigned to either the BUP or SHAM condition four days before beginning microdialysis testing and underwent surgery for osmotic minipumps or sham surgery.

During the afternoon of the fourth day after the pumps were implanted, four rats were transported to the microdialysis testing chambers and probes were inserted into the guide cannula. Rats were provided with food and water *ad libitum* overnight and dialysate was infused through the probes at a rate of 0.5 μl/min. At 0900 h the next day, food was removed from the chambers and the dialysate flow rate was increased to 0.7 μl/min. Baseline samples (of about 14 μl total volume) and locomotor activity scores were taken every 20 min. A baseline of DA was said to be established once there was less than 10% variation in these readings across three consecutive samples.

Following baseline sampling, rats were presented with either two full-sized lab chow pellets or 100 sucrose pellets in a heavy ceramic container in their dialysis testing chambers. The chow or sucrose was left in the chamber for 20 min; after this period, any uneaten food or sucrose was removed and the amount eaten by each rat was recorded. Dialysate samples were taken at 20-min intervals for 120 min after the presentation of chow or sucrose. After completing dialysis testing for the day, rats were left in the dialysis chambers overnight with the dialysate flow

rate reduced to 0.5 μl/min. Lab chow and water were available *ad libitum*. The next day, rats underwent the same protocol but received the opposite 'food' so that all rats were given both chow and sucrose pellets on separate days. The order of chow and sucrose presentation across the two days of dialysis testing was counterbalanced within the groups.

3.6. Histology

After completing dialysis testing, rats were given an overdose of sodium pentobarbital and perfused intracardially with saline and formaldehyde (4% formalin V/V, Anachemia, Montreal, QC). Brains were removed, frozen, and sectioned at 40 μm using a cryostat to determine the placement of guide cannulae and dialysis probes. Tissue was stained with cresyl violet to aid visualization of the dialysis probes.

3.7. Statistical analyses

Data from Experiments 1 and 2 were analyzed using analyses of variance (ANOVAs) for groups by session. The comparison between the two groups on the test for reinstatement was made using two-tailed *t*-tests for independent samples. For Experiment 3, group by sample ANOVAs were carried out on the raw quantities of DA (in picograms/10 μl of dialysate) in the final baseline sample and the two samples that followed presentation of either lab chow or sucrose pellets. An additional ANOVA was carried out on the raw quantities of DA in each baseline sample for BUP and SHAM groups. This analysis was conducted because previous findings from this laboratory indicate that BUP increases basal DA levels in the NAc (Sorge et al., 2005). An alpha level of .05 was used in all the analyses.

4. Results

4.1. Experiment 1

4.1.1. Self-administration on an FR1 schedule

The results from the preliminary FR1 study and from the FR1 schedule portion of Experiment 1 were not found to be significantly different, so these data were pooled to yield sample sizes of 11 rats in the BUP group and 10 rats in the SHAM group. The intervening FR5 and PR sessions during the FR1 sessions in Experiment 1 had no effect on the performance of either the BUP or SHAM groups on subsequent FR1 sessions. Fig. 1a shows the mean number of pellets obtained by the BUP and SHAM groups over FR1 sessions. The left panel of the figure shows that BUP and SHAM groups obtained comparable numbers of sucrose pellets prior to BUP treatment ($F(1, 19) = 0.41$, ns), whereas during BUP treatment (middle panel), the BUP group took fewer pellets than the SHAM group ($F(1, 19) = 4.36$, $p = 0.05$). This reduction in intake by BUP rats persisted for several sessions following the removal of the osmotic minipumps (right panel) as reflected in the significant group by session interaction ($F(9, 171) = 2.89$, $p < 0.01$).

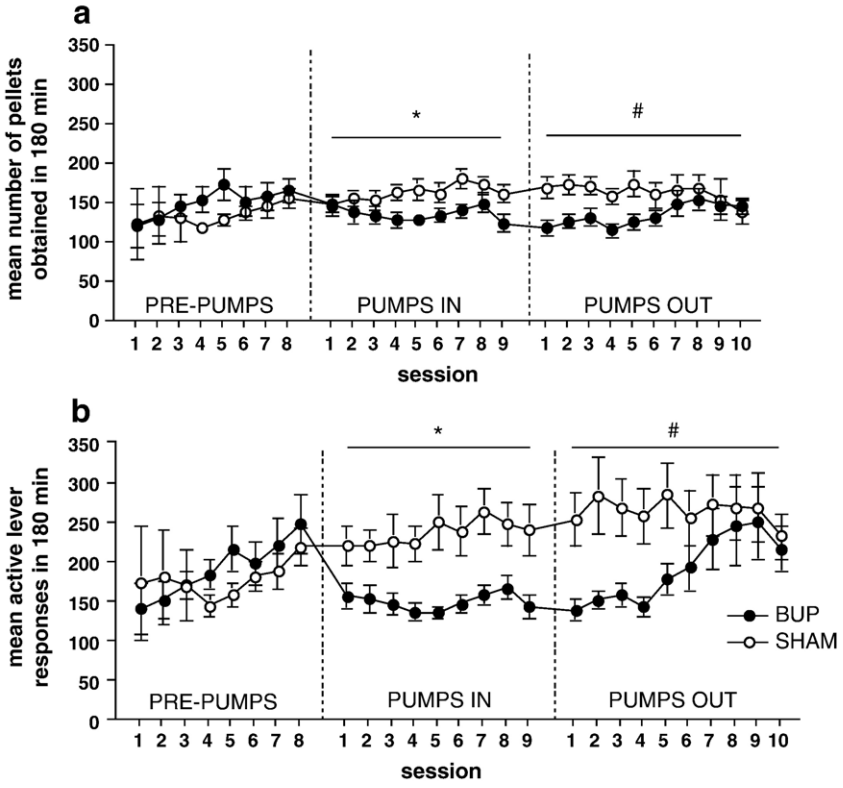

Fig. 1. (a)Mean (±SEM) daily intake of sucrose pellets on an FR1 schedule before, during, and after chronic BUP treatment in Experiment 1. (b) Mean number of active lever responses made across sessions. * group effect, $p < 0.05$; # group by session interaction, $p < 0.05$.

Both BUP and SHAM groups learned to respond reliably on the active lever and made very few inactive lever responses across the FR1 sessions (<5 per session). Fig. 1b shows the data for the number of responses on the active lever during each of the three stages of testing (i.e., before BUP treatment, during BUP treatment, and following BUP treatment). Note that in both the BUP and SHAM groups the number of lever presses increased over days such that by the end of training both the BUP and SHAM groups were making 40–50% more active lever responses than pellets obtained. These 'extra' presses would have occurred during the 5-s period immediately after pellet delivery while the cue light was illuminated. During BUP treatment, however, only the SHAM group continued to make extra responses at these high rates. This difference is apparent throughout BUP treatment (group: $F(1, 19)=9.73$, $p<0.01$) and for the first several days after removal of the osmotic minipumps (group × session interaction: $F(9, 171)=3.18$, $p< 0.01$). This effect of BUP on extra active lever responses is also shown in Fig. 2 as a ratio of lever presses to pellets received. It can be seen in the middle panel of Fig. 2 that BUP-treated rats made virtually one response per pellet received, whereas SHAM rats continued to make a high number of responses following pellet delivery when the cue light was illuminated ($F(1, 19)=9.46$, $p<0.01$). Interestingly, this extra responding in the BUP group gradually increased to SHAM group levels after the osmotic minipumps were removed (group × session interaction: $F(9, 153)=2.67$, $p<0.01$). The slow recovery of intake and responding seen in this experiment following the removal of the osmotic minipump can be explained by the slow dissociation of BUP from opioid receptors and high residual plasma levels of the drug that persist for several days as shown in our previous experiments (Sorge and Stewart, 2006).

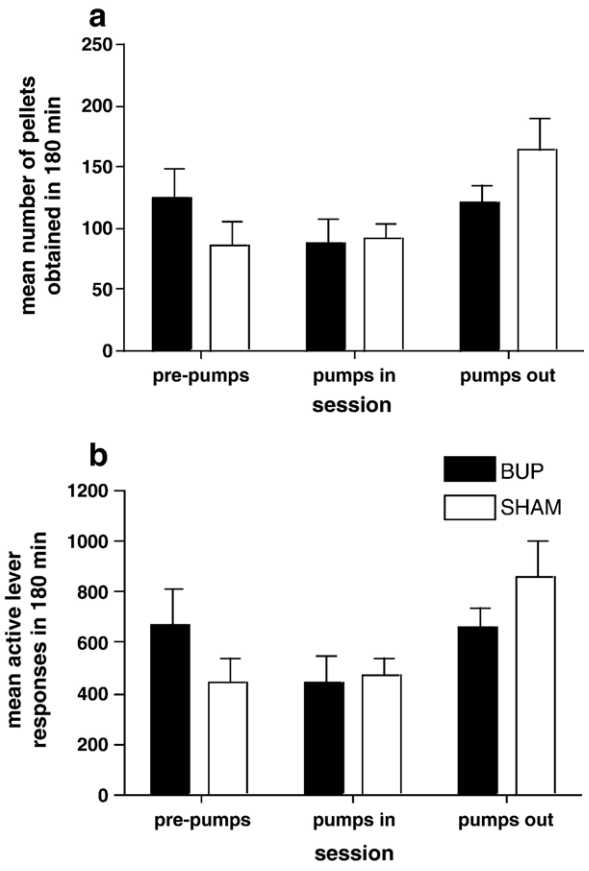

Fig. 3. (a) Mean (±SEM) intake of sucrose pellets before, during, and after chronic BUP treatment on an FR5 schedule in Experiment 1. (b) Mean number of active lever responses made across sessions.

4.1.2. Self-administration on an FR5 schedule

Fourteen rats (7 BUP and 7 SHAM) were tested on the FR5 schedule once during each of the three stages of testing. Fig. 3a and b show the number of pellets obtained and active lever responses, respectively, made by BUP and SHAM groups. Although inspection of these figures suggests that the number of pellets and active lever responses were somewhat reduced during BUP treatment, there were no significant differences between groups. There were, however, significant group by session interactions (pellets: $F(2, 22)=4.16$, $p<0.05$; responses: $F(2, 22)=3.48$, $p<0.05$), which were attributable to increases in pellet intake and active lever responses by the SHAM group across the three FR5 tests. Further examination of active lever responding during the 5-s period after pellet delivery showed that both the BUP and SHAM groups made virtually no responses during this period while working on the FR5 schedule (data not shown).

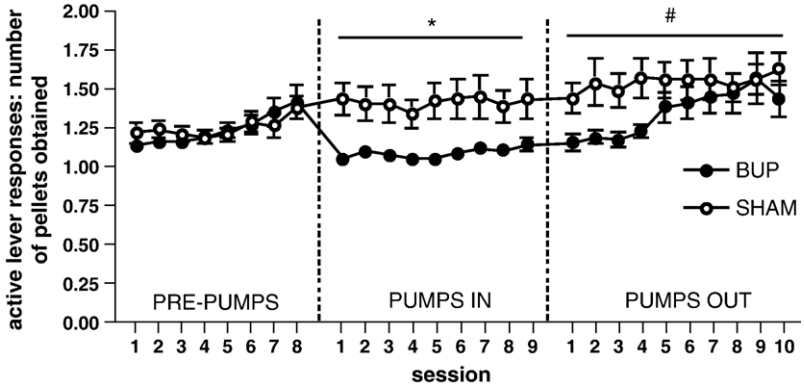

Fig. 2. Ratio of active lever responses to number of sucrose pellets obtained (±SEM) before, during, and after chronic BUP treatment on an FR1 schedule in Experiment 1. *group effect, $p<0.05$; # group by session interaction, $p<0.05$.

4.1.3. Self-administration on a PR schedule

Following each FR5 test, BUP and SHAM groups were tested for one session on a PR schedule. No differences between the groups were found (pellets: $F(1, 12)=0.55$, ns; responses: $F(1, 12)=0.04$, ns; data not shown) and, similar to responding on the FR5 schedule, both BUP and SHAM groups made essentially no extra active lever responses after pellet delivery during the PR sessions (data not shown).

4.1.4. Pattern of sucrose taking within FR1 sessions

Sorge and Stewart (2006) previously reported that rats trained to self-administer either heroin or cocaine showed a longer latency to respond to drug-associated cues at the beginning of self-administration sessions when exposed to chronic BUP. We examined our data for a similar effect. Fig. 4 shows the number of responses by BUP and SHAM groups for sucrose in 5 min bins during the first hour of four FR1 sessions: the last training session (#8), session #5 during BUP treatment, and sessions #5 and #10 after osmotic minipumps were removed. It can be seen that, in general, both BUP and SHAM groups made a substantial portion of their total responses during the first 15 min. Analyses were thus carried out on the number of active lever responses in the first 15 min of each session. Before the osmotic minipumps were implanted (Fig. 4a) the groups did not differ. During BUP treatment, however, BUP rats made significantly fewer responses during the first 15 min than did SHAM rats ($F(1, 12)=5.97$, $p<0.05$, Fig. 4b). Fig. 4c and d show that the BUP group continued to make fewer active lever responses in the fifth and tenth sessions after pump removal, but the differences between groups were no longer significant.

4.2. Experiment 2

4.2.1. Study of extinction and reinstatement

4.2.1.1. Training. By the end of training, the BUP and SHAM groups were earning comparable numbers of sucrose pellets ($F(1, 12)=0.01$, ns) and did not differ in active lever responding ($F(1, 12)=0.91$, ns). Responding on the inactive lever was minimal and did not differ between the BUP and SHAM groups across the sessions.

4.2.1.2. Extinction. Fig. 5a shows the number of active lever responses made by the BUP and SHAM groups over the six sessions of extinction. Responding decreased rapidly across sessions in both groups. Notably, the BUP group made fewer responses on the first day of extinction (57.9±12.6 (SEM)) than did the SHAM group (92.9±23.1 (SEM)), although this difference was not significant. By the final day of extinction, all rats had reached the criterion of 15 or fewer active lever presses in 60 min.

4.2.1.3. Reinstatement. As shown in Fig. 5b, the reinstatement procedure led to high rates of responding in the SHAM

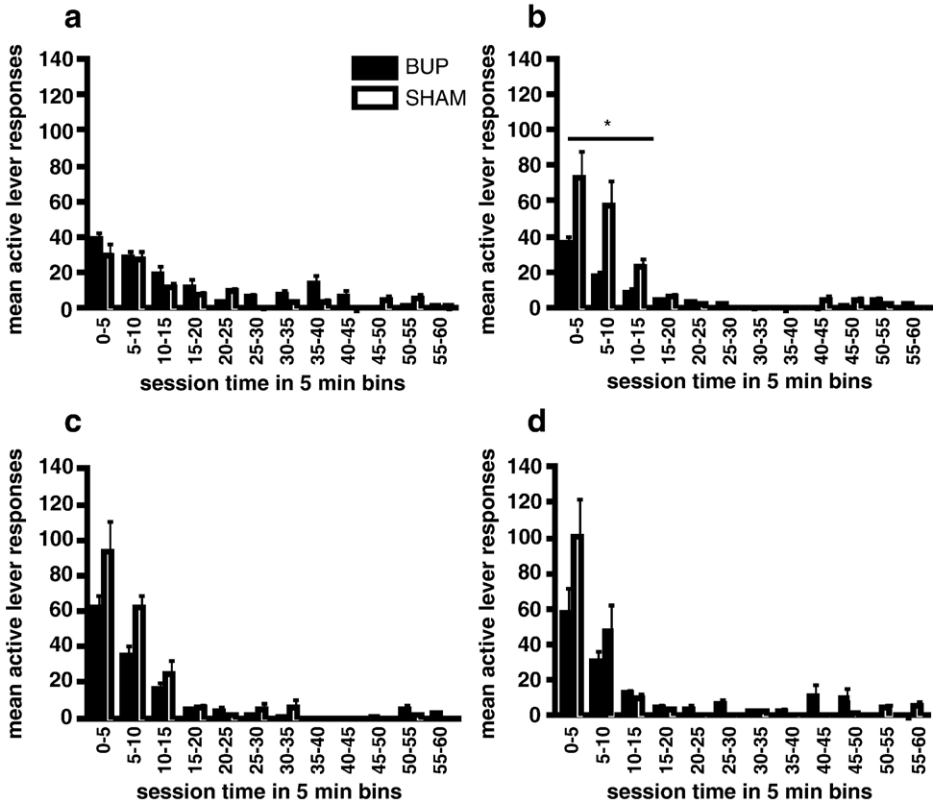

Fig. 4. Number of active lever responses made in 5-min intervals during the first hour of four FR1 schedule sessions. (a) Session #8 of training, before BUP minipumps were implanted. (b) Session #5 during chronic exposure to BUP. (c) Session #5 following the removal of BUP minipumps. (d) Session #10 following the removal of BUP minipumps. *$p<0.05$; #$p=0.06$.

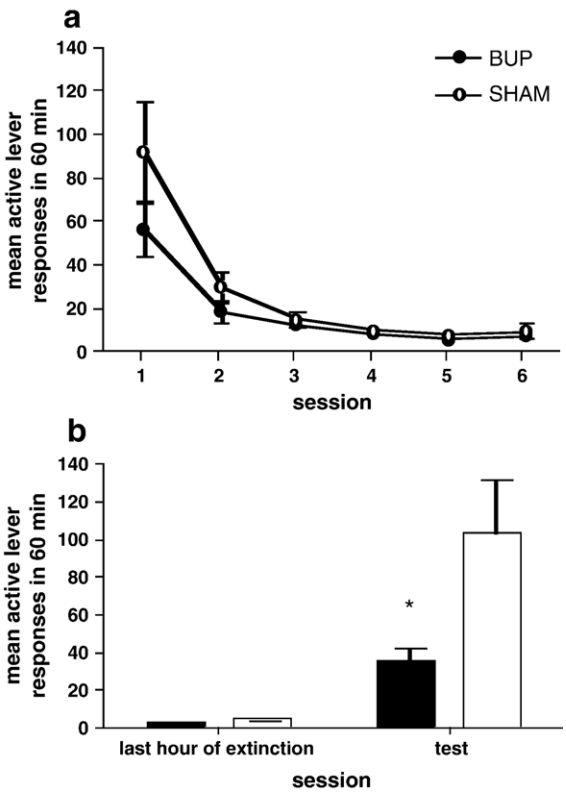

Fig. 5. Mean (±SEM) number of active lever responses during (a) extinction with cue light and (b) reinstatement of sucrose seeking during priming with sucrose pellets and cue light presentations. *group effect, $p<0.05$.

group. BUP-treated rats made significantly fewer responses than did SHAM rats ($t(12)=2.24$, $p<0.05$). It should be noted, however, that both BUP and SHAM rats consumed all the sucrose pellets delivered during the test for reinstatement.

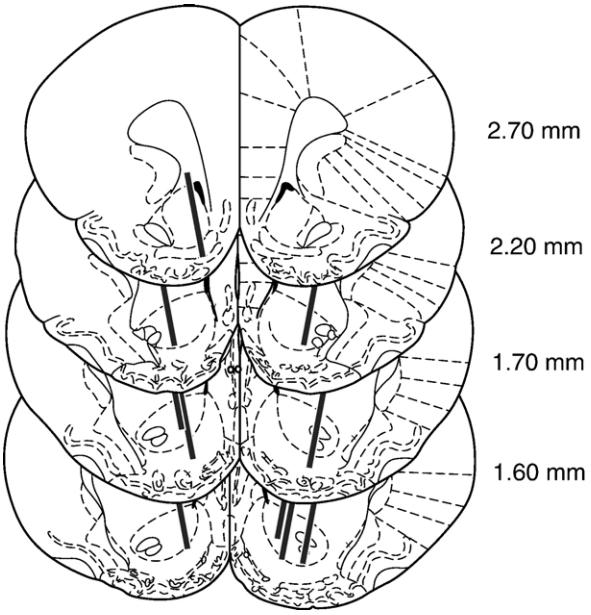

Fig. 6. Histologically verified position of the microdialysis probes of 10 rats used in Experiment 3. Black lines indicate the probable area covered by the probe.

4.3. Experiment 3

4.3.1. Dialysis probe placements

Microdialysis probe placements for 10 rats are shown in Fig. 6. Three rats from the SHAM group were excluded from statistical analyses: one rat was not tested due to illness after the probe was implanted and two were eliminated because of probe malfunction. Probe placements in the remaining 10 rats ranged from +2.7 mm to +1.6 mm from bregma. Solid black lines indicate the area (2.7 mm) covered by each probe including the sampling area (2 mm) and the inactive 0.7 tip of the probe.

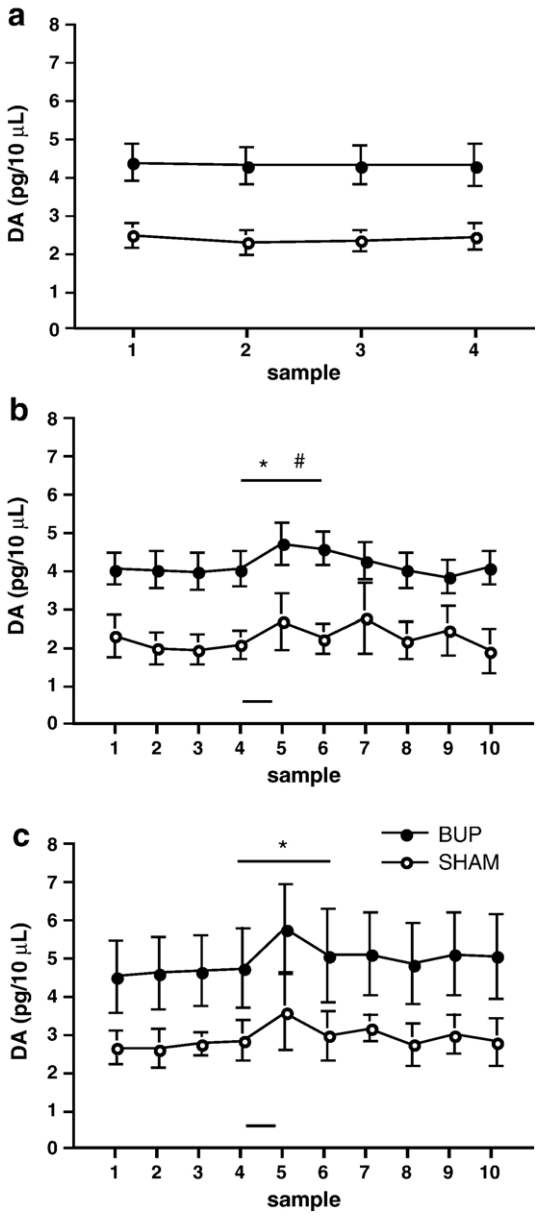

Fig. 7. (a) Mean basal DA levels in NAc (pg/10 µl) for samples 1–4 on each of the test days. DA levels after the presentation of (b) lab chow pellets and (c) sucrose pellets (Experiment 3). Black bars indicate the 20-min period during which food was available. * sample effect, $p<0.05$; # group effect, $p<0.05$.

4.3.2. Amount of lab chow and sucrose consumed

To determine the amount of lab chow consumed by each rat during dialysis, the approximate size of the pellets remaining was judged in proportion to the original amount presented. In the case of sucrose, the amount consumed was determined by counting the number of pellets remaining from the original amount (100). The BUP and SHAM groups consumed the same amount of lab chow during the dialysis session (approximately one 4 g chow pellet). The SHAM group, however, ate significantly more of the sucrose pellets (100%) than did the BUP group (approx. 64%; $t(5)=3.35$, $p<0.05$).

4.3.3. Basal level of DA

An analysis of basal DA levels in the NAc was conducted by averaging the amounts of DA in each of the four baseline samples (in pg/10 µl) over the two days of testing for 10 rats (BUP $n=7$, SHAM $n=3$). As seen in Fig. 7a, the basal levels of DA in the BUP group were nearly twice those of the SHAM group ($F(1, 18)=5.89$, $p<0.05$).

4.3.4. Lab chow-and sucrose-induced changes in NAc DA

Fig. 7b and c show changes in the amount of DA (pg/10 µl) in the NAc induced by presentation and consumption of lab chow and sucrose pellets, respectively. During the first 20-min sampling period after the presentation of lab chow, DA levels rose by about 1 pg/10 µl in both the BUP and SHAM groups (about 25–30% above baseline; sample: $F(2, 16)=4.64$, $p<0.05$; group: $F(1, 8)=6.74$, $p<0.05$). Similarly, levels of NAc DA rose by about 1 pg/10 µl in both the BUP and SHAM groups immediately after the presentation of sucrose (sample: $F(2, 16)=4.27$, $p<0.05$; group: $F(1, 8)=1.3$, ns).

5. Discussion

Taken together, the results of these experiments show that rats treated with chronic BUP responded less on the active lever in the presence of sucrose-associated cues during the FR1 schedule. The BUP rats responded less frequently at the start of the FR1 sessions than SHAM rats and made fewer responses during extinction. Furthermore, they made significantly fewer responses in the presence of sucrose-associated cues during reinstatement in spite of consuming all the sucrose pellets. These effects of BUP appear to be consistent with the results of Sorge and Stewart (2006) who reported that rats treated chronically with BUP showed longer latencies to begin responding at the outset of self-administration sessions for both heroin and cocaine (at the time of lever entry and when the cue light previously paired with drug delivery was illuminated). They are also consistent with those of Sorge et al. (2005), who showed that chronic BUP treatment in rats trained to self-administer cocaine or heroin reduces responding in extinction and blocks the reinstatement of drug seeking following priming injections of either drug. Overall, these findings seem to reflect a reduction in the salience and behavioral impact of reward-associated cues by chronic BUP treatment.

The findings that chronic BUP treatment nearly eliminated 'extra' or unrewarded lever presses made on the FR1 schedule during the cue light presentation and reduced the frequency of responses at the outset of sessions are interesting in light of previous research indicating that an increase in unrewarded responding reflects a strengthening of the incentive value of conditioned stimuli. For example, Ghitza et al. (2006) reported that, in food-deprived rats given daily access to palatable food pellets on an FR1 schedule, unrewarded active lever responding during a 20-s presentation of a tone-light stimulus after the delivery of a food pellet increased by more than 10-fold over 12 daily sessions. In contrast, the number of pellets consumed per session increased by only 3 to 4 times over the same period. Similarly, Di Ciano and Everitt (2004) found in food-deprived rats that responding on a lever for a conditioned stimulus previously paired with a 10% sucrose solution significantly increased over a 60-day period following the final sucrose-stimulus pairing, during which time rats received intermittent exposures to the stimulus. Our results suggest that one effect of chronic BUP treatment may be to halt this progressive increase in the incentive salience of reward-paired cues across sessions.

At first glance, the lack of an effect of BUP treatment on behavior during the FR5 and PR schedules is surprising and seems problematic for this interpretation of the present findings. If BUP treatment reduces the efficacy of sucrose-associated cues in initiating and maintaining responding, it would seem reasonable to expect that this effect would be manifested in lower responding on these more demanding schedules of reinforcement. It should be noted, however, that both BUP-treated and SHAM rats consumed all of the pellets they obtained on the FR1, FR5, and PR schedules and ate most of the food presented in during microdialysis, which suggests that chronic BUP treatment has no impact on the rewarding value of sucrose itself. Instead, our results point towards a selective effect of BUP on the ability of cues to invigorate motivated behavior, as noted above. Support for this dissociation between the effects of BUP on the salience of cues and on the rewarding value of stimuli can be found in other experiments; for example, a previous study found that chronic BUP treatment did not reduce the number of heroin infusions taken by rats on FR1, FR5, and PR schedules, but did reduce the response to cues during self-administration sessions, extinction, and reinstatement of heroin seeking induced by priming injection (Sorge and Stewart, 2006). In the present experiments, this selective effect of BUP on cue-maintained behavior may have been obscured on the FR5 and PR schedules because both BUP and SHAM groups made very few 'extra' or unrewarded responses during the cue light presentation on these schedules.

The results of Experiment 3 show that chronic BUP did not affect the small but significant increase in extracellular DA levels in the NAc in response to sucrose pellets or to lab chow in non food-deprived rats. However, perhaps more important for the interpretation of the findings of Experiments 1 and 2, BUP treatment significantly increased basal levels of DA, confirming the findings in a previous paper (Sorge and Stewart, 2006) where it was suggested that these higher baseline levels of DA might reduce the signal to noise ratio of the increase in DA induced by conditioned cues. Although the present experiment does not address this issue directly, previous research indicates

that the presentation of stimuli associated with palatable foods does elicit modest increases in DA levels in the NAc (e.g., Ahn and Phillips, 1999; Cheng et al., 2003). It would be of interest, therefore, to determine whether chronic BUP treatment would reduce the proportional increase in NAc DA by sucrose-associated cues, and thus reduce the ability of cues to promote appetitive behaviors.

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