Studies of the Motivational Effects of Chronic Buprenorphine Treatment Using Sucrose Pellet Reward

Suzanne Hood

A Thesis

in

The Department

of

Psychology

Presented in Partial Fulfilment of the Requirements
for the Degree of Master of Arts at
Concordia University
Montreal, Quebec, Canada

September 2005

Your file Votre référence
ISBN: 0-494-10181-4
Our file Notre référence
ISBN: 0-494-10181-4

Abstract

Studies of the Motivational Effects of Chronic Buprenorphine Treatment Using Sucrose Pellet Reward

Suzanne Hood

Buprenorphine (BUP), a mixed opioid agonist-antagonist, is currently used as a treatment for opioid addiction. Previous research has shown that chronic BUP treatment decreases self-administration of drugs such as heroin and cocaine and drug-seeking behaviour in monkeys, rats, and humans. Furthermore, BUP attenuates the increase in nucleus accumbens (NAc) dopamine (DA) levels in response to an acute injection of heroin in naïve rats, whereas BUP potentiates the DA response to acute cocaine.

To establish whether the suppression of drug seeking by BUP stems from an effect on motivated behaviour in general, four experiments were conducted to determine the effect of chronic BUP (3.0mg/kg/day) on responding for sucrose pellet reward. Chronic exposure to BUP was achieved with the use of subcutaneous osmotic minipumps. Results from Experiments 1 and 2 showed that rats exposed to chronic BUP took fewer sucrose pellets and reduced their active lever responding on a fixed ratio (FR) 1 schedule of self-administration; however, no such effect of BUP was observed on FR5 or progressive ratio (PR) schedules. In Experiment 3, chronic BUP slightly reduced sucrose seeking during extinction of self-administration and suppressed reinstatement of responding after sucrose priming. In Experiment 4, it was found using *in vivo* microdialysis that chronic BUP had no effect on the sucrose- or lab chow-induced rise in NAc DA, but did significantly increase basal DA tone. Overall, these results indicate that chronic BUP decreases motivation induced by sucrose reward and suggest that BUP reduces drug seeking and drug taking by blunting motivation in general.

Acknowledgements

I would like to thank my supervisor, Dr. Jane Stewart, for providing me with tremendous support, guidance, and inspiration throughout my first two years of graduate studies. I would also like to thank Robert Sorge for his expertise and kindness in helping me to conduct these experiments, as well as Heshmat Rajabi and Demetra Rodaros for their excellent technical assistance.

Many thanks to Dr. Shimon Amir and Dr. Uri Shalev for their comments on this thesis and for serving as members of my examining committee. Finally, I would like to express my gratitude towards my mother Flora, my sister Kate, my beloved friend Nick, and many other friends for their love, patience, and support.

Table of Contents

List of Figures

GENERAL INTRODUCTION

Buprenorphine (BUP) is a mixed opioid agonist-antagonist drug used in the treatment of opioid addiction (Tzschentke, 2002). At the mu opioid receptor, BUP has high affinity but low intrinsic activity once bound and is therefore considered a partial agonist. BUP also binds to kappa and delta opioid receptors but has no intrinsic activity at these receptors and acts as an antagonist when bound (Woods, Charleson, Lane, & Hudgin, 1981).

Because of these mixed agonist-antagonist features, BUP has become an attractive alternative to methadone treatment for opioid dependence (Walsh & Eissenberg, 2003; Davids & Gastpar, 2004). Unlike methadone, an overdose of BUP carries minimal risk of potentially fatal respiratory depression and this effect is attributed to its low intrinsic activity at the mu receptor (e.g., Walsh, Preston, Stitzer, Cone, & Bigelow, 1994; Cowan, Doxey, & Harry, 1977). Furthermore, minimal withdrawal symptoms arise upon discontinuation of treatment indicating that physical dependence on BUP does not develop over prolonged exposure (Jasinski, Pevnick, & Griffith, 1978; Mello, Mendelson, & Kuehnle, 1981).

Pre-clinical and clinical studies confirm that BUP reduces opioid use. In monkeys trained to self-administer abused opioids such as heroin, morphine, alfentanil, and hydromorphone, daily BUP injections reduce operant responding for drug reward (Mello, Bree, & Mendelson, 1983; Mello & Negus, 1998; Winger & Woods, 1996). This reduction persists with continued injections and tolerance does not appear to develop to this effect. For example, Mello and colleagues (1983) found that daily BUP injections in monkeys decreased responding for heroin over a period of six to eight months without

affecting responding for food over the same period. BUP also reduces opioid use in human drug users. Maintenance doses between 8 and 16 mg/day have been found to significantly reduce self-administration of heroin by addicts in clinical trials in which heroin is made available to participants (Mello & Mendelson, 1980; Mello et al., 1981). Similar doses decrease the rate of opioid-positive urine samples in addicts receiving outpatient treatment (Strain, Stitzer, Liebson, & Bigelow, 1994).

Additional studies indicate that BUP decreases drug seeking in animal models of opioid self-administration and reduces self-reports of craving for opioids in human users. Sorge and colleagues (2005) reported that a dose of 3 mg/kg/day of BUP in rats trained to self-administer heroin significantly suppressed operant responding during the early stages of extinction, during which time responses on the previously drug-paired lever led to heroin-associated cues but no drug. Furthermore, the same dose of BUP blocked the reinstatement of responding on the drug-associated lever induced by a priming injection of heroin. Consistent with these findings, clinical reports indicate that a 16 mg/day dose of BUP decreases the number of times opioid-experienced humans will self-administer hydromorphone when given a choice between the drug and a monetary reward (Greenwald, Schuh, Hopper, Schuster, & Johanson, 2002). In this same study, BUP also reduced reported cravings for heroin among human drug users (Greenwald et al., 2002).

New evidence suggests that the effects of BUP on drug seeking and drug taking are not limited to opioid addiction. A number of studies have indicated that BUP decreases intake of other abused drugs, such as cocaine, phencyclidine (PCP), and ethanol in animals and in human drug users. For example, doses of BUP that reduce opioid intake have been found to decrease intake of cocaine in monkeys (Mello, Lukas, Kamien,

Mendelson, Drieze, & Cone, 1992), rats (Carroll, Carmona, May, Buzalsky, & Larson, 1992; Carroll & Lac, 1992; Comer, Lac, Wyvell, & Carroll, 1996; Sorge et al., in preparation), and humans (Foltin & Fischman, 1996; Kosten, Kleber, & Morgan, 1989; Strain et al., 1994). Rats trained to self-administer cocaine show reduced responding on the drug-associated lever during extinction and in tests of cocaine-induced reinstatement if they are exposed to 3 mg/kg/day of BUP (Sorge, Rajabi, & Stewart, 2005). Humans injected with cocaine report significantly less craving for more drug when maintained on 8 mg/day of BUP (Foltin & Fischman, 1996). Similar reductions in drug taking occur following BUP treatment in monkeys trained to self-administer PCP and ethanol (Carroll et al., 1992; June, Cason, Chen, & Lewis, 1998).

Hypothesized mechanisms of buprenorphine

The mechanisms by which BUP reduces drug seeking and drug taking are not yet understood, although several hypotheses have been proposed (Tzschentke, 2002). The most parsimonious account of BUP'S actions – that BUP reduces operant responding by a general suppression of activity – can be discounted (Tzschentke, 2002; Carroll et al., 1992). Sorge and colleagues (2005) showed that rats chronically maintained on BUP alone or BUP paired with an acute injection of cocaine were more active in locomotor tests than were controls. Similarly, Mello and colleagues (1983) have reported that BUP treatment reduces responding for drugs in monkeys without changing responding for food.

Another hypothesis suggests that BUP decreases the rewarding value of abused drugs and thus decreases motivation to respond for drugs (Tzschentke, 2002; Mello & Mendelson, 1980). Evidence that appears to be consistent with this view includes those

findings noted above that BUP reduces drug taking in human addicts and in animals trained to self-administer drugs of abuse (Sorge et al., 2005; Greenwald et al., 2002; Foltin & Fischman, 1996). Additional examples stem from clinical reports indicating that BUP decreases cocaine abuse in humans by diminishing the experience of euphoria following drug administration and increasing dysphoric symptoms (Kosten, Kleber, & Morgan, 1989).

This hypothesis is contradicted by other evidence that suggests instead that BUP reduces motivated responding by augmenting the rewarding value of drugs (Tszchentke, 2002). That is, BUP positively interacts with the effects of abused drugs in the nervous system so that a small quantity of drug paired with BUP is rendered equally reinforcing as is a larger quantity of drug alone. As such, responding for drug decreases proportionally when an animal is treated with BUP. This type of effect was reported by Brown and colleagues (1991), who found that a low dose of cocaine (1.5 mg/kg), which did not support a conditioned place preference (CPP) in rats, did elicit a CPP when combined with a low dose of BUP (0.01mg/kg). This result suggests that the combination of cocaine and BUP was significantly more rewarding than the dose of cocaine alone. Furthermore, BUP (0.03 – 0.3 mg/kg) alone elicited a significant CPP, which implies that certain doses of BUP itself are rewarding. Indeed, BUP is self-administered by monkeys (Mello, Bree, & Mendelson, 1981), which is consistent with the view that BUP is rewarding to some degree.

It must be noted here that an important feature of the experiments reporting a synergistic effect of BUP with other drugs is that BUP is administered acutely at the time of testing rather than chronically before testing is begun. The timing of exposure to BUP

is thought to be an influential factor in determining how the drug interacts with abused drugs such as cocaine (Tzschentke, 2002). For example, Kosten and colleagues (1991) found that a CPP for high-dose cocaine (15 mg/kg) was reduced by BUP (0.5mg/kg), but these authors injected BUP twice daily for a period of two weeks before conditioning was begun and continued injections throughout conditioning.

A number of additional findings, however, cannot be accounted for by either a reward-enhancement or a reward-suppression hypothesis of the actions of BUP. These data have prompted the development of a fourth hypothesis, which proposes that BUP interacts directly with internal motivational states to reduce drug seeking and drug taking (Sorge et al., 2005). These data come from studies in which researchers have examined the behaviour of animals in response to stimuli associated with drug reward, rather than in response to the drug itself. For example, Sorge and colleagues (in preparation) examined the behaviour of rats trained to self-administer heroin and cocaine that were subsequently treated with chronic BUP (3.0mg/kg/day). They observed that BUP-treated rats failed to respond to drug-associated cues in the self-administration environment at the start of the session, whereas control rats made several responses on the drug-associated lever as soon as it and other cues were presented. Similarly, rats treated with chronic BUP showed significantly reduced drug seeking during extinction of heroin and cocaine self-administration (Sorge et al., 2005). Since the effect of BUP in these studies cannot be attributed to a direct interaction with the rewarding properties of abused drugs, these results suggest that BUP decreases drug seeking by blunting incentive motivation induced by drug-associated stimuli.

Buprenorphine and non-drug reward-induced motivation

An important step in determining how BUP reduces motivation induced by drugs and related stimuli is to examine its effects on behaviour towards non-drug rewards. If behavioural responses towards rewards such as food or saccharin are reduced in the presence of BUP, this would suggest that BUP interacts with motivation in a more general fashion – that is, buprenorphine decreases the incentive salience of all types of stimuli instead of drug-related stimuli exclusively (Carroll et al., 1992; Comer, Evans, Pudiak, & Foltin, 2002).

The degree to which BUP interacts with food reward is particularly pertinent because of the extensive literature documenting the role of opioids in feeding. Studies examining the effects of opioid agonists and antagonists on schedule-controlled feeding and *ad libitum* consumption indicate a role for opioid activity in determining the rewarding properties of food and in moderating satiety signals during a meal (for reviews see: Cooper, Jackson, Kirkham & Turkish, 1988; Levine, Morley, Gosnell, Billington, & Bartness, 1985). For example, repeated injections of the pure mu receptor agonists morphine and methadone enhance *ad libitum* feeding in food-deprived and non-deprived rats (Martin, Wikler, Eades, & Pescor, 1963; Rudski, Schaal, Thompson, Cleary, Billington, & Levine, 1992, 1994). Note, however, that operant responding for food is often reduced with repeated injections of mu agonists (Bigelow & Thompson, 1971; MacMillan, Wolf, & Carchman, 1970; Lukas, Mello, Bree, & Mendelson, 1988).

Stimulation of feeding is also observed following injections of agonists for other opioid receptors, such as kappa (Morley & Levine, 1983) and delta (Tepperman & Hirst, 1983). Conversely, injections of naloxone, a potent mu antagonist (Holtzman, 1974;

Cooper, 1983; Yeomans & Gray, 1997), as well as kappa and delta antagonists (Negus & Mello, 2002) can reduce feeding in both deprived and non-deprived animals. Since the effects of opioids on feeding are mediated by more than one type of opioid receptor, it is not immediately clear how the mixed agonist-antagonist actions of BUP would influence feeding behaviour.

Several studies have been conducted to investigate the effects of BUP on responding for food and saccharin reward, but their results are not entirely consistent. A number of reports suggest that BUP treatment has a minimal effect on operant responding for non-drug rewards, such as food pellets (Mello et al., 1983; Negus & Mello, 2002) or saccharin fluid (Comer et al., 1996; Carroll et al., 1992). For example, a study by Mello and colleagues (1983) mentioned above found no effect of daily BUP injections (0.3 – 0.8 mg/kg) on responding for food pellets in non food-deprived monkeys over a period of about 200 testing days. The dose administered, however, significantly reduced responding for intravenous heroin and hydromorphone that were available during alternate sessions.

Other studies indicate that BUP suppresses responding for food and sweet rewards such as saccharin fluid when administered acutely, but that this effect diminishes with prolonged exposure. For example, Comer and colleagues (2002) found that a single injection of BUP (0.03 – 0.3 mg/kg) in non-deprived monkeys significantly reduced responding for sweetened fluid over three days. Lukas and colleagues (1988) reported a decrease in responding for food in non-deprived monkeys during the first four days of BUP administration (1.0 mg/kg) that was followed by a recovery of responding to baseline levels. It should also be noted that responding over the remaining 20 days of

testing in this study actually increased significantly above baseline levels with continued BUP administration. Similarly, Mello and colleagues (1992) reported a 25 – 30 day reduction of responding for food pellets in non-deprived monkeys given daily buprenorphine injections (0.32 mg/kg), which was followed by a significant increase in responding above baseline thereafter. These findings suggest that tolerance to any suppressant effects of BUP on food-maintained responding develops with time and that a stimulatory effect emerges with prolonged administration.

A study by Dykstra (1983), however, is not consistent with these findings. She reported that acute BUP administration (0.003 – 1.0 mg/kg) in squirrel monkeys decreased responding for food pellets over three days and that this decrease in responding persisted over a period of 17 days with continued daily injections (0.01 mg/kg/day). It is important to note, however, that the subjects in this study were maintained at 80 % of their free-feeding weight, unlike the other studies above. Also in contrast are the findings of Rudski and colleagues (1995). These authors observed an increase in responding for food pellets as well as *ad libitum* home cage feeding in non-deprived rats administered daily injections of BUP (0.01 – 0.3 mg/kg) over three days.

One approach to understanding the discrepancies in the results of the above studies is to consider the role of motivational state in determining the effects of BUP on responding for food reward. Note that in the studies in which no effect or a transient effect of BUP was found the animals were not food deprived. Presumably, animals were less motivated to work for food reward in this condition because *ad libitum* food could be obtained outside of the testing situation. In the case of Dykstra (1983), however, food-deprived monkeys showed a long-lasting reduction in operant responding when treated

with BUP. The fact that this study differs from the others in terms of the deprivation state of its subjects suggests that the degree to which an animal is motivated to obtain food is an important factor in determining the effect of BUP on food-maintained behaviour. This interpretation is consistent with the hypothesis that BUP reduces incentive motivation and suggests that the effect of BUP extends to non-drug incentive stimuli.

Rationale of the present experiments

The present experiments were conducted to investigate further how BUP administration affects motivated responding for sucrose pellets, a highly palatable non-drug reinforcer, in *ad libitum* fed rats. The principal objective in the design of these experiments was to determine the influence of chronic exposure to BUP on motivated behaviour. The dose of the drug (3.0 mg/kg/day) was chosen for these experiments because it is known to reduce heroin- and cocaine-seeking behaviour in rats and to moderate the rise in nucleus accumbens (NAc) dopamine (DA) levels stimulated by acute heroin injection or cocaine injection (Sorge et al., 2005). Moreover, this dose does not impair locomotor functioning. In Experiments 1 and 2, the effect of chronic BUP treatment on rats' self-administration of sucrose pellets was examined using different schedules of work requirements. Experiment 3 was conducted to investigate the influence of BUP on extinction of sucrose-reinforced responding and on the reinstatement of responding following a sucrose prime. Experiment 4 was conducted to determine whether BUP treatment moderates the rise in NAc DA levels that is induced by sucrose pellets and by ordinary lab chow pellets.

EXPERIMENTS 1, 2 AND 3

General Methods

Subjects

A total of 35 male Long Evans rats (Charles River, St. Constant, QC) weighing about 350 g at the beginning of the experiments were used. 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 duration of 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.

Surgery

Rats were exposed to a continuous level of BUP with 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 haemostat 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 then closed using stainless steel wound clips. Pumps were removed in a similar fashion under isofluorane anaesthesia. Those animals assigned to the sham condition (SHAM) underwent the same surgical procedure as did BUP-exposed animals but did not receive a pump.

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 behaviour in rats trained to self-administer heroin and cocaine (Sorge et al., 2005) and also significantly suppresses the rise in NAc DA levels following an acute injection of heroin (Sorge et al., 2005). Moreover, this dose is the most concentrated solution of BUP that can be prepared without the addition of alcohols (Hutchings, Zmitrovich, Hamowy, & Liu, 1995).

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 attached to the outside of the operant chamber (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, which was positioned 5 cm above the active lever. The sound-attenuating plywood chambers were equipped with a red

houselight that illuminated at the beginning of every session and remained on throughout each session.

Procedures

Before self-administration training began, rats were given about 100 sucrose pellets in heavy ceramic containers per day in their home cages for three days (in the case of Experiment 1) or four days (in the case of Experiments 2 and 3) to reduce the novelty associated with this food and thereby to 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 illuminated to signal the start of the session. Ten s 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 the illumination of the cue light for 5 s and one sucrose pellet being dispensed into the magazine. A 5 s timeout period followed the dispensing of each pellet and any additional responses on the active lever during this time had no consequences; however, additional responses were recorded by the computer. Responses on the inactive lever had no programmed consequences, but were recorded to provide a measure of general activity within the chamber during the self-administration sessions. At the end of the 180 min session, the active lever retracted and the houselight was extinguished.

Statistical analyses

Data from Experiments 1, 2, and 3 were analysed using analyses of variance (ANOVAs) for groups by session. Follow-up comparisons were made using two-tailed independent samples t-tests and paired samples t-tests, where appropriate, and an alpha level of 0.05.

EXPERIMENT 1: EFFECT OF CHRONIC BUPRENORPHINE ON SELF-ADMINISTRATION OF SUCROSE PELLETS ON AN FR1 SCHEDULE

Introduction

BUP reduces drug taking and drug seeking in animal models of drug addiction and in human drug users (Tzschentke, 2002; Mello et al., 1980, 1982; Carroll et al., 1992). The mechanisms by which BUP exerts these effects are not known. One possible explanation is that BUP acts to blunt motivation induced by all types of incentive stimuli, drug- and non-drug related. In line with this idea, a number of studies have found that BUP reduces operant responding for non-drug rewards such as food or saccharin (e.g., Comer et al., 2002). Additional studies, however, have reported that responding for food or saccharin reward decreases only after acute administration of BUP; over long-term administration, responding returns to control levels (Mello et al., 1992; Lukas et al., 1988). Thus, the effect of BUP on motivation elicited by non-drug rewards is not entirely understood.

The purpose of this experiment was to determine the effect of chronic BUP exposure on self-administration of sucrose pellets by non-food-deprived rats on an FR1 schedule. Chronic administration of BUP was achieved with the use of osmotic minipumps, so that any potential effects of a daily bolus injection of the drug on activity

would be avoided. Rats were exposed to a dose of BUP (3.0 mg/kg/day) that has been shown to significantly reduce both cocaine and heroin seeking and to attenuate the rise in NAc DA levels following an acute injection of heroin (Sorge et al., 2005). Importantly, this dose does not interfere with the rat's ability to make operant responses.

Procedures

Subjects

Seven rats were used in this experiment. All testing was conducted between the hours of 0830 h and 1200 h.

Self-Administration of Sucrose Pellets on a Fixed-Ratio 1 (FR1) Schedule

Rats were trained to self-administer sucrose for eight days on an FR1 schedule. Immediately after completing the final day of training, rats underwent either sham surgery (SHAM) or surgery to implant BUP pumps using the procedure described above. Group assignment was based on rats' active lever responding during the final two training sessions so that the two groups were matched in this respect. Rats were returned to the operant chambers after a 24-h recovery period and continued self-administration on the FR1 schedule for nine days. Immediately after the ninth session, rats underwent another surgery to remove the BUP pumps (or underwent sham surgery again) and then resumed self-administration on the FR1 schedule 24 h later for an additional 10 days.

Results

Figure 1A shows the mean number of pellets obtained during each 180 min session across testing days. In the leftmost panel of the three panels, it can be seen that rats developed a steady level of sucrose intake over eight days of training. The mean

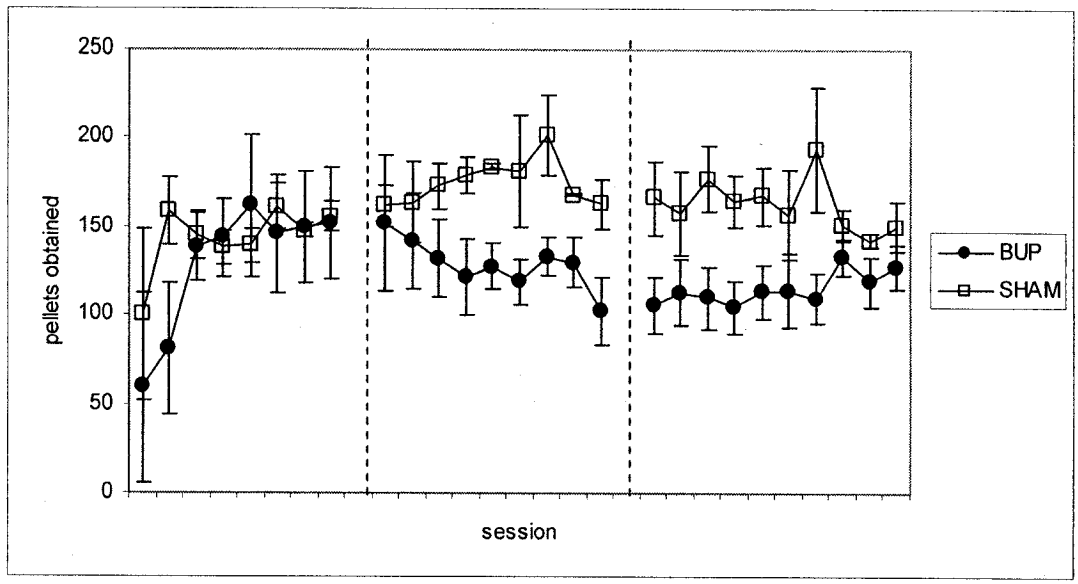
numbers of pellets obtained by each group (BUP +/- SEM vs. SHAM +/- SEM) on the last two days of training were 149.5 +/- 27.5 vs. 148.0 +/- 2.9 and 152.3 +/-27.2 vs. 155.7 +/- 6.9. A group by session ANOVA indicated that there was no difference between the BUP and SHAM groups before pumps were implanted (effect of group: $F(1, 5) = 0.14$, $p = 0.73$).

The middle panel of Figure 1A shows that chronic BUP reduced the number of pellets obtained by rats over testing sessions. A group by session ANOVA, however, did not indicate a significant effect of group ($F(1, 5) = 4.02$, $p = 0.10$) or a significant interaction between group and session ($F(8, 40) = 1.11$, $p = 0.38$). Rats in the BUP group continued to obtain fewer pellets than SHAM rats did for several days after the pumps were removed; it can be seen from the right panel of Figure 1A that responding became similar in the two groups only after seven days. A group by session ANOVA indicated a trend towards a main effect of group ($F(1, 5) = 5.89$, $p = 0.06$), but the interaction of group and session was not significant ($F(9, 45)$ 1.81, $p = 0.09$).

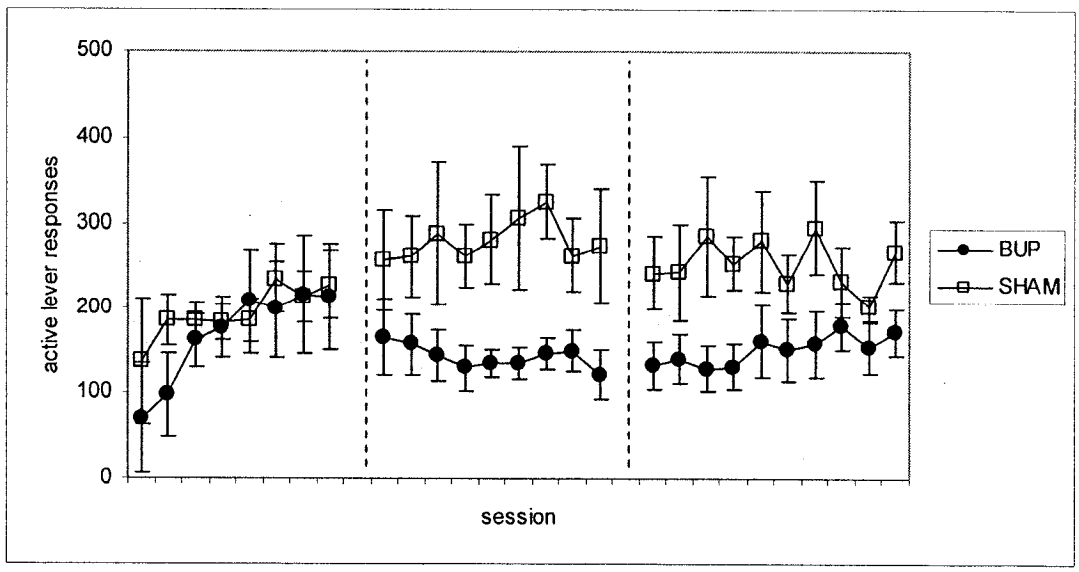
Figure 1B shows the mean number of active lever responses made across sessions. Rats developed reliable levels of responding across eight days of training and there were no differences between the BUP and SHAM groups before pumps were implanted (group by session ANOVA; effect of group: $F(1, 5) = 0.16$, $p = 0.71$). The mean numbers of responses on the active lever made by each group (BUP +/- SEM vs SHAM +/- SEM) on the last two days of training were 216.3 +/- 59.6 vs 213.7 +/-24.8 and 213.0 +/- 54.2 vs 229.0 +/- 32.7. Rats also responded preferentially on the active lever instead of the inactive lever across the training sessions. On the final day of training, the number of

Figure 1. Experiment 1. Effects of chronic BUP on self-administration of sucrose pellets on an FR1 schedule. Leftmost dashed line on graphs indicates point at which BUP pumps inserted; rightmost dashed line on graphs indicates when BUP pumps removed. A. Mean (+/- SEM) pellets obtained across sessions. B. Mean (+/-SEM) active lever responses made across sessions.

A.



B.



inactive lever responses made by each group (BUP +/- SEM vs SHAM +/- SEM) was 0.5 +/- 0.5 vs 9.3 +/- 6.8. The groups did not differ significantly in their amount of inactive lever pressing during training (group by session ANOVA; effect of group: $F(1, 5) = 1.26$, $p = 0.31$).

After the pumps were implanted, BUP rats responded less on the active lever over testing sessions. Although this effect did not reach statistical significance, the group by session ANOVA indicated a trend towards a main effect of group ($F(1, 5) = 5.99$, $p = 0.06$) but not an interaction of group and session ($F(8, 40) = 1.57$, $p = 0.17$). BUP rats continued to respond less on the active lever than SHAM rats did for several days after the pumps were removed. A group by session ANOVA indicated a trend towards an interaction (group by session; $F(9, 45) = 2.02$, $p = 0.06$) and, as can be seen from the right panel of Figure 1B, responding in the two groups became similar after about seven days.

Discussion

The results of this experiment indicate that chronic exposure to BUP reduces self-administration of sucrose by *ad libitum* fed rats on an FR1 schedule. Although the effect of BUP was not statistically significant, there was a clear tendency for rats to respond less and obtain fewer sucrose pellets while they were exposed to BUP. These findings are consistent with a study by Dykstra (1983) in which it was reported that daily BUP injections suppressed operant responding for food in food-deprived monkeys over a 17-day period. The present findings also suggest support for the hypothesis that BUP decreases drug seeking and drug taking by dampening the incentive salience of all types of appetitive stimuli.

It could be argued that the present findings do not rule out the possibility that tolerance develops with time to the effects of BUP on operant responding for sucrose, as has been reported to occur in previous research (e.g., Lukas et al., 1988; Mello et al., 1992). The period of BUP administration in the present experiment was less than two weeks, so it is possible that rats could have adapted to the suppressing effects of BUP if exposure to the drug had continued. It was not possible to prolong the exposure to BUP in this study because the osmotic minipumps used to deliver BUP could be filled with only a 14 day supply of the drug.

It is important to note here, however, that BUP rats in the present study continued to take fewer pellets and make fewer responses for sucrose for about seven days after the pumps were removed. It is known that the actions of BUP last for several days even after a single injection, due to the high affinity of the drug for opioid receptors (e.g. Walsh et al., 1994). The fact that this reduction of self-administration persisted for an additional week after the source of drug delivery was removed argues against the likelihood of tolerance developing to the effect of BUP on sucrose-reinforced responding. Furthermore, the prolonged decrease cannot be attributed to a BUP-induced impairment in motor functioning, since the dose of BUP used in this study has been found previously to enhance locomotor activity slightly in rats (Sorge et al., 2005).

A question that arises from the present results concerns the action by which BUP reduces sucrose self-administration. Since an FR1 schedule does not strongly challenge an animal's motivation to work for a reward, it is unclear from this study whether BUP decreases responding by interacting directly with motivational state or by some other mechanism. In lieu of resorting to food deprivation, it was thought that this question

would be better addressed with the use of more demanding reinforcement schedules than FR1. This strategy was adopted in Experiment 2.

EXPERIMENT 2: EFFECT OF CHRONIC BUP ON SELF-ADMINISTRATION OF SUCROSE ON FR1, FR5, AND PR SCHEDULES

Introduction

An important approach to assessing the strength of motivation for a given reward is through the use of high work to low reward schedules of delivery in self-administration studies. When animals are challenged to make many responses for a single reward, they will persist in responding for much longer if a manipulation is performed to enhance motivation for the reward. For example, rats will respond more for food pellets on a progressive ratio (PR) schedule, which incrementally increases the amount of work required to obtain each successive reward, if their blood glucose levels are reduced by an injection of 2-deoxyglucose before the session (Jewett, Cleary, Levine, Schaal, & Thompson, 1995). Labour-intensive schedules such as a PR can provide a sensitive index of changes in motivation to obtain a given reward.

Sorge and colleagues (in preparation) made use of this type of schedule in their investigation of the effect of chronic BUP on motivation for drug reward. These authors gave rats access to cocaine and to heroin on an FR5 schedule and a PR schedule while exposing them to chronic BUP (3.0 mg/kg/day). Their results showed that BUP decreased responding for cocaine on an FR5 schedule and decreased the mean breakpoint, or the maximum number of responses made for a single drug infusion, for cocaine on the PR schedule. Interestingly, BUP did not affect responding for heroin on either schedule. From these findings, it can be suggested that BUP reduces motivation for cocaine reward.

In the present experiment, the effect of chronic BUP on the motivation of *ad libitum* fed rats for sucrose reward was examined by using two schedules of sucrose self-administration having greater work requirements than an FR1 schedule. An FR1 schedule was first used to determine whether the effect of BUP on sucrose self-administration observed in Experiment 1 could be replicated in a larger group of animals. Rats were then switched to an FR5 schedule and then to a PR schedule to identify whether motivation to work for sucrose is reduced in the presence of chronic BUP.

Procedures

Subjects

Fourteen rats were used in this experiment. All testing was conducted between the hours of 0830 h and 1500 h.

Self Administration on FR1, FR5 and PR schedules

A timeline of the procedure for this experiment is depicted in Figure 2. Rats were trained to self-administer sucrose for eight days on an FR1 schedule. On day 9, all rats were switched to an FR5 schedule for one session. This FR5 session was conducted in the same manner as were the FR1 sessions with the only difference being 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. On day 10, all rats were switched to a PR schedule for one session. This PR session was conducted in the same manner as were the FR1 sessions with the only difference being 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 (see Appendix A for the schedule of response requirements; PR equation taken from Fletcher, Korth, & Chambers, 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 using the procedure described in the general method.

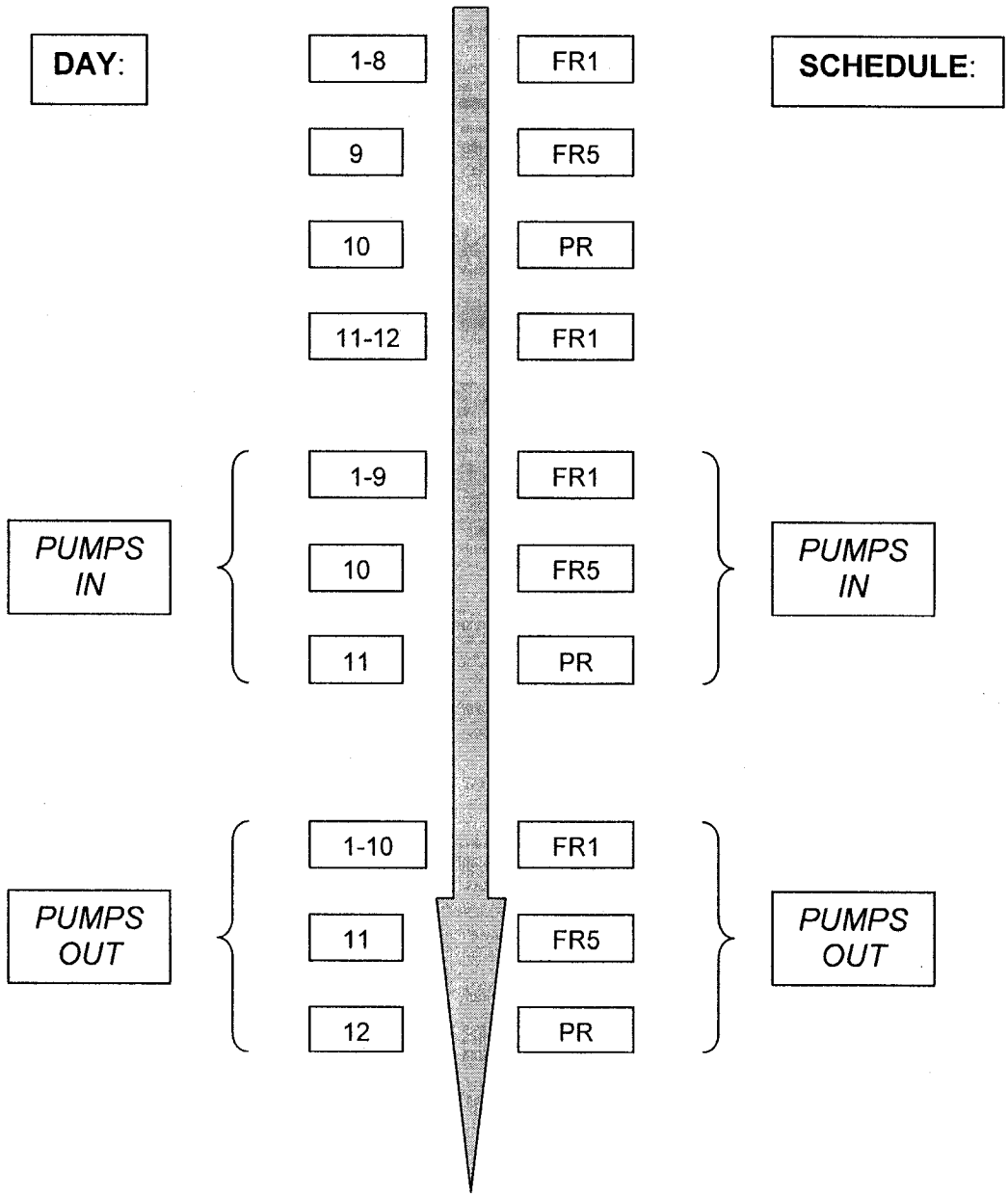
Rats were returned to the operant chambers after a 24 h recovery period and continued self-administration on the FR1 schedule for nine days. On day 10, rats were switched to the FR5 schedule for one session. On day 11, rats were switched to the PR schedule for one session. Immediately after completing 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.

Results

Self-administration on an FR1 schedule

The mean numbers of pellets obtained by BUP and SHAM rats during each training session are shown in Figure 3A. By the end of training, BUP and SHAM rats took a steady number of pellets across the sessions (see leftmost panel of Figure 3A)

Figure 2. Experiment 2. Diagram of the timeline followed for testing sucrose self-administration on the FR1, FR5, and PR schedules.



and the groups did not differ significantly before the pumps were implanted (group by session ANOVA: effect of group: $F(1, 12) = 0.97$, $p = 0.34$). During the last two days of training, the mean numbers of pellets obtained by each group (BUP +/- SEM vs. SHAM +/- SEM) were 164.0 +/- 21.0 vs. 145.6 +/- 19.0 and 176.3 +/- 16.2 vs. 156.4 +/- 16.1.

It can be seen from the middle panel of Figure 3A that BUP reduced slightly the number of pellets obtained by rats. A group by session ANOVA, however, revealed that neither the effect of group ($F(1, 12) = 1.16$, $p = 0.30$) nor the interaction of group and session ($F(8, 96) = 0.98$, $p = 0.44$) was significant. The effect of session was significant ($F(8, 96) = 2.78$, $p < 0.01$) and this was attributable to a gradual increase in the number of pellets obtained by both groups combined over the nine sessions.

BUP rats continued to take fewer pellets than SHAM rats did for several sessions after pumps were removed (shown in the right panel of Figure 3A). A group by session ANOVA revealed a significant interaction (group by session; $F(9, 108) = 2.63$, $p < 0.01$) and subsequent repeated measures ANOVAs for each group indicated a significant effect of session within the BUP group ($F(9, 54) = 2.52$, $p < 0.05$). This effect was not significant within the SHAM group ($F(9, 54) = 1.44$, $p = 0.20$). From Figure 3A, it can be seen that BUP rats obtained fewer pellets than SHAM rats did within the first four sessions after the pumps were removed and then responding in the two groups became similar (independent samples t-test; first session: $t(12) = 2.21$, $p < 0.05$; second session: $t(12) = 2.63$, $p < 0.05$; third session: $t(12) = 1.32$, $p = 0.21$; fourth session: $t(12) = 2.01$, $p = 0.05$).

Figure 3B shows the number of active lever responses made by BUP and SHAM rats across sessions. The mean numbers of responses on the active lever made by each

group (BUP +/- SEM vs. SHAM +/- SEM) on the last two days of training were 224.6 +/- 37.2 vs 179.0 +/- 28.1 and 268.9 +/- 45.1 vs 216.9 +/- 28.4, indicating that both groups were responding reliably by the end of training. Moreover, a group by session ANOVA revealed no difference between the BUP and SHAM groups before the pumps were implanted (effect of group ($F(1, 12) = 0.56$, $p = 0.47$).

Rats also responded preferentially on the active lever instead of the inactive lever by the end of training. On the final training session, the number of inactive lever responses made by each group (BUP +/- SEM vs. SHAM +/- SEM) was 3.4 +/- 1.6 vs 5.7 +/- 2.9 and there was no effect of group on the amount of inactive lever pressing during training (group by session ANOVA; $F(1, 12) = 0.10$, $p = 0.76$).

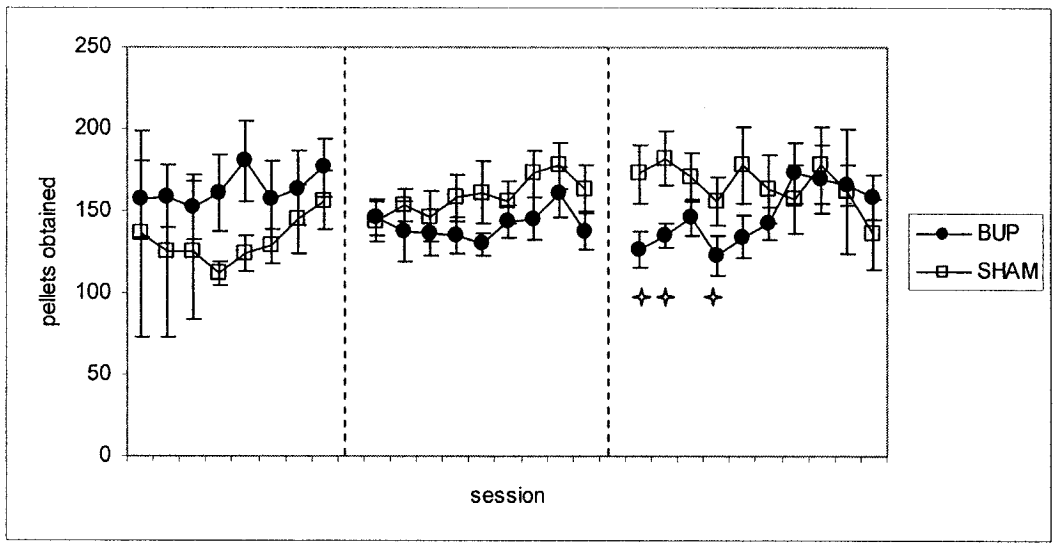
It can be seen from the middle panel of Figure 3B that active lever responding was reduced in the presence of BUP, similar to the effect observed in Experiment 1. This effect of BUP, however, was not significant (group by session ANOVA: effect of group: ($F(1, 12) = 4.11$, $p = 0.07$)). There was a significant main effect of session ($F(8, 96) = 2.30$, $p < 0.05$) that was attributable to an increase in active lever responding over sessions by both groups combined.

The effect of BUP on active lever responding persisted for several days after the pumps were removed (shown in the right panel of Figure 3B), which is also consistent with the results of Experiment 1. A group by session ANOVA revealed a significant interaction (group by session; $F(9, 108) = 3.11$, $p < 0.01$), as well as a significant effect of session ($F(9, 108) = 3.48$, $p < 0.05$). Separate repeated measures ANOVAs for each

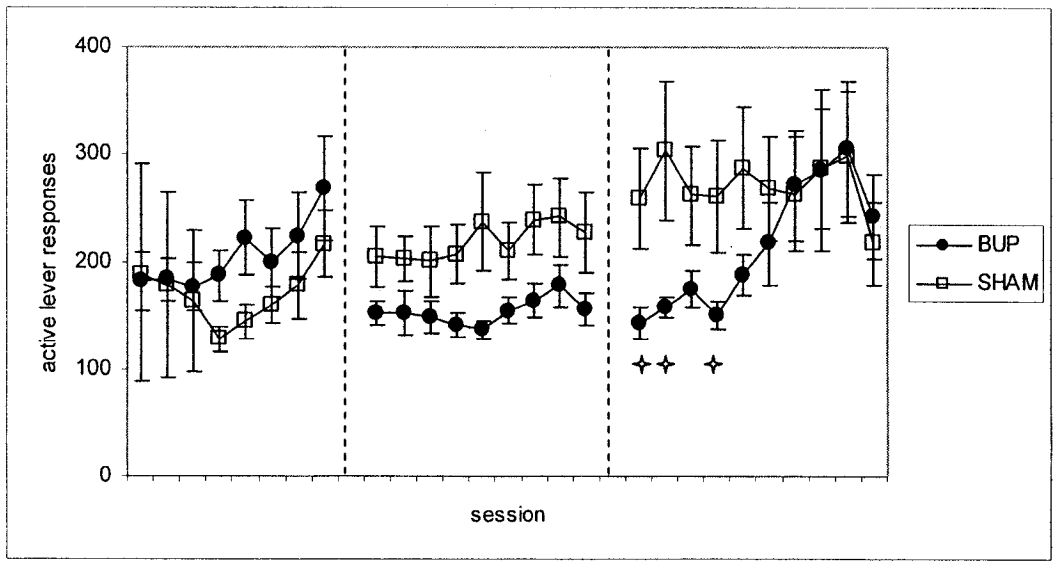
Figure 3. Experiment 2. Effects of chronic BUP on self-administration of sucrose on an FR1 schedule. Leftmost dashed line on graphs indicates point at which BUP pumps inserted; rightmost dashed line on graphs indicates when BUP pumps removed. A. Mean (+/- SEM) pellets obtained across sessions. B. Mean (+/-SEM) active lever responses made across sessions.

Star = significant difference between groups, $p < 0.05$

A.



B.



group revealed a significant effect of session within the BUP group ($F(9, 54) = 4.19$, $p < 0.01$) but not within the SHAM group ($F(9, 54) = 1.47$, $p = 0.19$). Inspection of Figure 3B reveals that BUP rats continued to make fewer active lever responses within the first four days after the pumps were removed (independent samples t-tests; first session: $t(12) = 2.38$, $p < 0.05$; second session: $t(12) = 2.18$, $p = 0.05$; third session: $t(12) = 1.79$, $p = 0.10$; fourth session: $t(12) = 2.47$, $p < 0.05$).

Self-administration on an FR1 schedule: Experiments 1 and 2 combined

The results of Experiments 1 and 2 show that BUP reduces self-administration of sucrose on an FR1 schedule. Given the consistency of this effect over two experiments, it seemed reasonable to combine the data from Experiment 1 and 2 to create a larger sample for analysis. Before these data were combined, however, comparisons were made between like groups from each of the experiments (i.e., Experiment 1 BUP vs. Experiment 2 BUP; Experiment 1 SHAM vs. Experiment 2 SHAM) to determine if there were any significant differences in the number of pellets obtained or in active lever responding.

An analysis of the number of pellets obtained and the active lever responses made during training revealed no significant differences between rats in Experiment 1 and Experiment 2. An experiment by session ANOVA on these data from the last three sessions of training indicated no significant effect of experiment ($F(1, 19) = 0.02$, $p = 0.89$). Similarly, no effect of experiment was found between rats in Experiments 1 and 2 for active lever responding during the last three sessions of training ($F(1, 8) = 0.88$, $p = 0.38$).

No significant differences emerged between like groups from Experiments 1 and 2 after pumps were implanted or after sham surgery. An experiment by session ANOVA for the number of pellets taken across sessions did not indicate any effect of experiment between the SHAM groups ($F(1, 8) = 0.67$, $p = 0.44$) or between the BUP groups ($F(1, 9) = 0.41$, $p = 0.54$). Similarly, no effect of experiment was found for active lever responses across sessions between the SHAM groups ($F(1, 8) = 1.13$, $p = 0.32$) or the BUP groups ($F(1, 9) = 0.23$, $p = 0.64$).

During the sessions after the second sham surgery, no significant differences were found between SHAM groups from Experiment 1 and Experiment 2 in terms of the number of pellets obtained (experiment by session ANOVA: $F(1, 8) = 0.01$, $p = 0.92$) or in active lever responding ($F(1, 8) = 0.05$, $p = 0.83$). For the BUP groups, however, a significant effect of experiment was found for the number of pellets obtained after pumps were removed ($F(1, 9) = 5.61$, $p < 0.05$). Independent samples t-tests were performed on these data and revealed that the BUP group from Experiment 1 obtained fewer pellets than did the BUP group from Experiment 2 during the seventh session ($t(9) = 2.41$, $p < 0.05$) and ninth session ($t(9) = 2.47$, $p < 0.05$) after the pumps were removed. No effect of experiment was found between the BUP groups for active lever responding ($F(1, 9) = 2.02$, $p = 0.19$).

Because no critical differences were found between the BUP groups and SHAM groups from each experiment, the data from Experiments 1 and 2 were pooled. Figure 4A displays the mean number of pellets obtained by the combined BUP and SHAM groups across sessions. As shown in the left panel of Figure 4B, BUP and SHAM groups did not differ in the number of pellets taken during training (group by session ANOVA; effect of

group: $F(1, 19) = 0.41$, $p = 0.53$). After pumps were implanted, however, BUP rats took fewer pellets than did SHAM rats (see middle panel of Figure 4A). A group by session ANOVA indicated a significant effect of session ($F(8, 152) = 2.05$, $p < 0.05$), group ($F(1, 19) = 4.36$, $p = 0.05$), and a trend towards a group by session interaction ($F(8, 152) = 1.93$, $p = 0.06$).

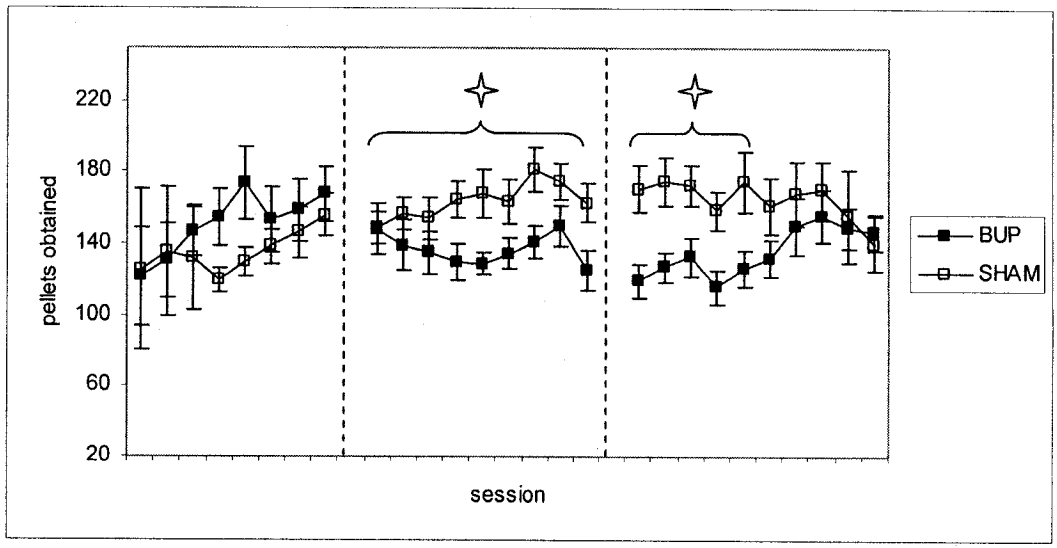
The right panel of Figure 4A shows that BUP rats continued to take fewer pellets than SHAM rats did for several sessions after the pumps were removed. A group by session ANOVA on these data indicated a significant interaction of group by session ($F(9, 171) = 2.89$, $p < 0.01$). Separate repeated measures ANOVAs for each group across sessions revealed a significant effect of session within the BUP group $F(9, 90) = 2.96$, $p < 0.01$) but no effect of session within the SHAM group ($F(9, 81) = 145$, $p = 0.18$). Further inspection of Figure 4A indicates that BUP rats took significantly fewer pellets for another five sessions after pumps were removed (independent samples t-test; first session: $t(19) = 3.27$, $p < 0.01$; second session: $t(19) = 3.12$, $p < 0.01$; third session: $t(19) = 2.57$, $p < 0.05$; fourth session: $t(19) = 3.35$, $p < 0.01$; fifth session: $t(19) = 2.57$, $p < 0.05$).

Active lever responding is shown in Figure 4B for the combined BUP and SHAM groups across sessions. It can be seen in the left panel of the figure that there was no difference between the groups in responding before the pumps were implanted (group by session ANOVA; effect of group: $F(1, 19) = 0.15$, $p = 0.70$). After BUP pumps were implanted, rats made significantly fewer responses (see middle panel of Figure 4B) and this is revealed in a group by session ANOVA (effect of session: $F(8, 152) = 2.22$, $p < 0.05$; effect of group: $F(1, 19) = 9.73$, $p < 0.01$).

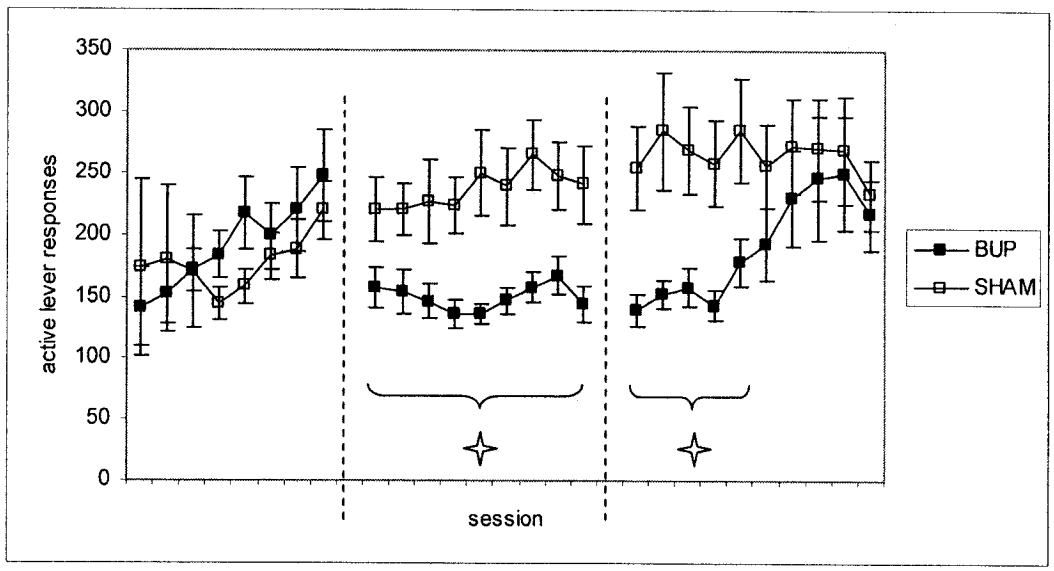
Figure 4. Experiments 1 & 2 combined. Effects of chronic BUP on self-administration of sucrose on an FR1 schedule. Leftmost dashed line on graphs indicates point at which BUP pumps were inserted; rightmost dashed line on graphs indicates when BUP pumps removed. A. Mean (+/- SEM) pellets obtained across sessions. B. Mean (+/-SEM) active lever responses made across sessions.

Star = significant difference between groups, $p < 0.05$

A.



B.



BUP rats continued to respond less on the active lever for several sessions after the pumps were removed (group by session ANOVA; effect of session: $F(9, 171) = 2.89$, $p < 0.01$; group by session interaction ($F(9, 171) = 3.18$, $p < 0.01$). Repeated measures ANOVAs for each of the groups revealed a significant effect of session within the BUP group ($F(9, 90) = 4.59$, $p < 0.01$) but not within the SHAM group ($F(9, 81) = 0.90$, $p = 0.53$). BUP rats responded less on the active lever for five sessions after the pumps were removed (independent samples t-tests; first session: $t(19) = 3.31$, $p < 0.01$; second session: $t(19) = 2.82$, $p < 0.05$; third session: $t(19) = 2.93$, $p < 0.01$, fourth session: $t(19) = 3.65$, $p < 0.01$; fifth session: $t(19) = 2.40$, $p < 0.05$), an effect that is evident from inspection of the right panel of Figure 4B.

Self-administration on an FR5 schedule

The mean number of pellets obtained by BUP and SHAM groups across the three FR5 sessions is shown in Figure 5A. As can be seen from the figure, BUP and SHAM rats obtained approximately equal amounts of pellets during the first FR5 session before the pumps were implanted (independent samples t-test: $t(12) = 1.22$, $p = 0.25$). An analysis of the data across all three sessions, however, indicated a significant effect of session (group by session ANOVA; $F(2, 22) = 7.22$, $p < 0.01$) and a group by session interaction ($F(2, 22) = 4.16$, $p < 0.05$). To analyse the interaction, repeated measures ANOVAs were run for each group. A significant effect of session was identified within the SHAM group ($F(2, 10) = 13.19$, $p < 0.01$) but not within the BUP group ($F(2, 12) = 2.20$, $p = 0.15$). Paired t-tests revealed that the SHAM group took significantly more pellets during the final FR5 session compared to the first ($t(5) = 7.59$, $p < 0.01$) and second ($t(5) = 3.46$, $p < 0.05$) FR5 sessions. Additional independent t-tests indicated that

the BUP group did not differ significantly from the SHAM group while the pumps were in ($t(12) = 0.15$, $p = 0.88$) or after the pumps were removed ($t(11) = 1.38$, $p = 0.20$).

In Figure 5B, it can be seen that BUP and SHAM rats made a similar number of active lever responses during the first FR5 session ($t(12) = 1.23$, $p = 0.24$). A group by session ANOVA on the data from all three FR5 sessions indicated a significant effect of session ($F(2, 22) = 7.62$, $p < 0.01$) and a significant interaction of group by session ($F(2, 22) = 3.48$, $p < 0.05$). Separate repeated measures ANOVAs were run for each group and revealed a significant effect of session within the SHAM group ($F(2, 10) = 13.54$, $p < 0.01$) but not within the BUP group ($F(2, 12) = 2.41$, $p = 0.13$). Upon further analysis, it was found that the SHAM group made significantly more responses on the active lever during the third FR5 session in comparison to the first (paired t-test: $t(5) = 6.95$, $p < 0.01$ and the second ($t(5) = 3.55$, $p < 0.05$) FR5 sessions. Notably, the BUP group did not differ from the SHAM group in active lever pressing during any of the three sessions (second session: $t(12) = 0.22$, $p = 0.83$; third session: $t(11) = 1.17$, $p = 0.27$).

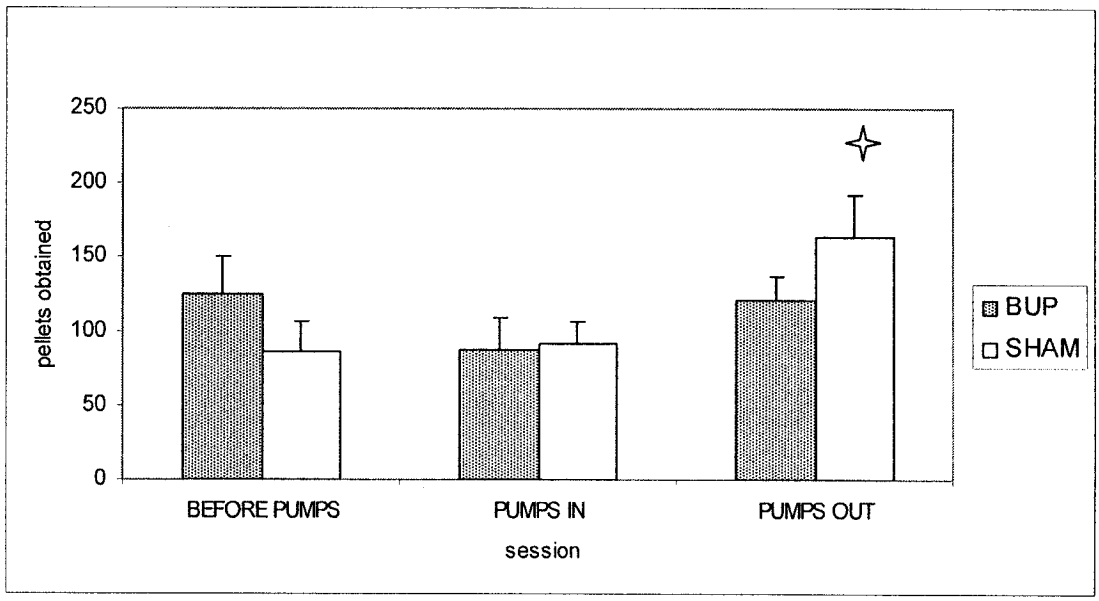
Self-administration on a PR schedule

There was no significant difference between BUP and SHAM rats in the number of pellets obtained across the three PR sessions (Figure 6A; group by session ANOVA; effect of group: $F(1, 12) = 0.55$, $p = 0.47$). Similarly, BUP and SHAM rats did not differ in the amount of active lever responding made over the three PR sessions (Figure 6B; effect of group: $F(1, 12) = 0.04$, $p = 0.84$).

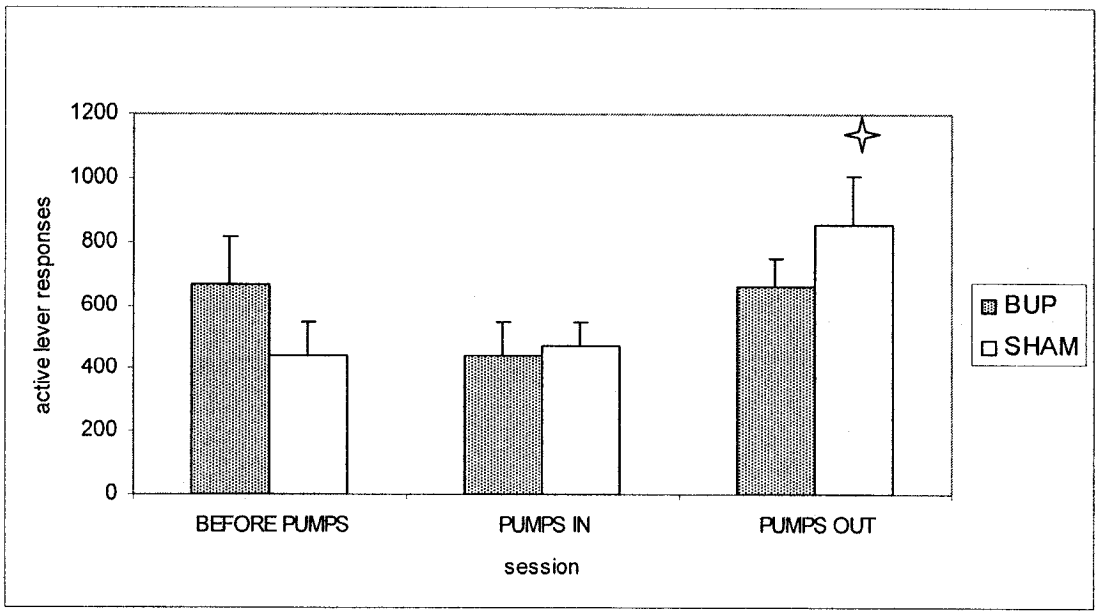
Figure 5. Experiment 2. Effects of chronic BUP on self-administration of sucrose on an FR5 schedule. A. Mean (+/- SEM) pellets obtained across sessions. B. Mean (+/-SEM) active lever responses made across sessions.

Star = significantly different from SHAM group in 'before pumps' and 'pumps in' sessions, $p < 0.05$

A.



B.



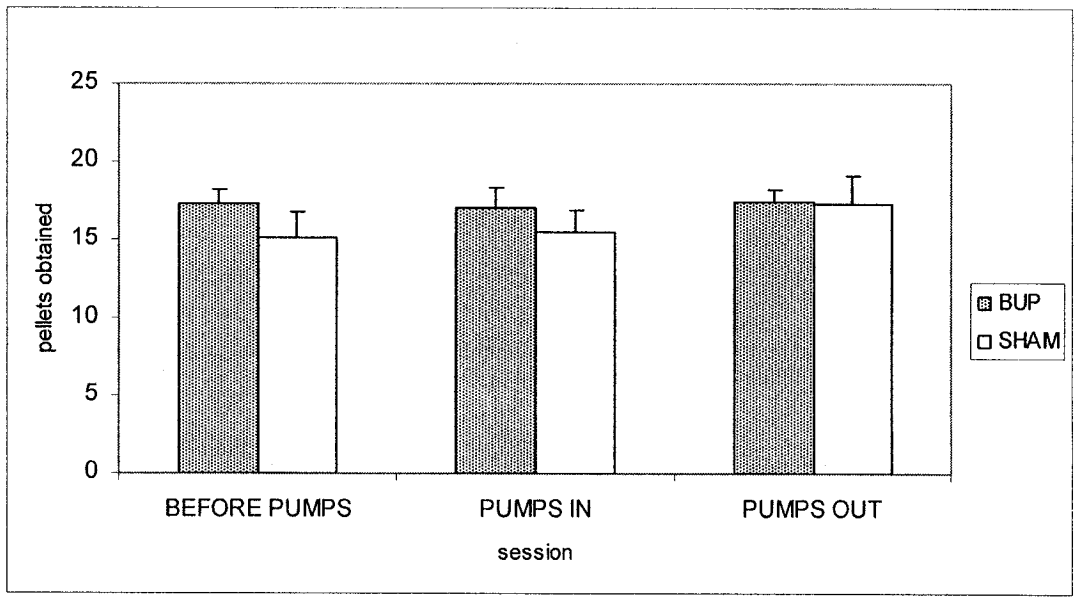
Ratio of active lever responses made to active lever responses required

The results of the FR1 schedule show that BUP reduces self-administration of sucrose; however, this finding is not supported by the results of the FR5 and PR schedules. Rather, the findings from these schedules suggest that BUP has no effect on sucrose self-administration. To explore this discrepancy further, an analysis was performed on the ratio of active lever responses made to the active lever responses required for the mean quantity of sucrose pellets obtained per session by each group on each schedule. This approach to examining the data was adopted because it was noted that the effect of BUP on sucrose self-administration emerged only on the schedule (FR1) during which rats made a substantial number of active lever responses in excess of what was needed to obtain sucrose pellets. In contrast, both BUP and SHAM rats appeared to make about the minimum number of responses needed to obtain the mean amount of sucrose pellets taken on the FR5 and PR schedules.

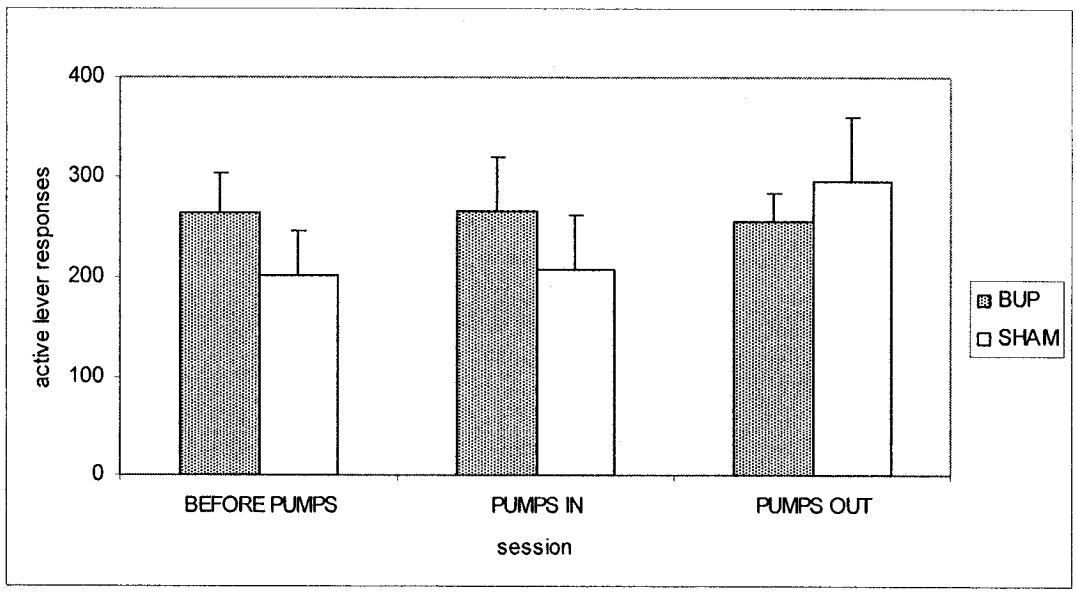
To calculate the ratios using the FR1 schedule data, the mean number of active lever responses made by each rat in a given session was divided by the mean number of pellets taken by the rat in that session (since the number of pellets obtained is equal to the number of responses required on the FR1 schedule). Statistical comparisons were then carried out on these ratios. Figure 7 shows the mean ratios of the BUP and SHAM groups over FR1 sessions. A group by session ANOVA on the ratios derived from the final two training sessions indicated that there was no difference between the BUP and SHAM groups before the pumps were implanted ($F(1, 19) = 0.27$, $p = 0.61$). After the pumps were implanted, ratios of the BUP group became significantly lower than those of SHAM rats (effect of group: $F(1, 19) = 9.46$, $p < 0.01$). This effect persisted after the

Figure 6. Experiment 2. Effects of chronic BUP on self-administration of sucrose on a PR schedule. A. Mean (+/- SEM) pellets obtained across sessions. B. Mean (+/-SEM) active lever responses made across sessions.

A.



B.



pumps were removed; a group by session ANOVA for the ratios over these sessions indicated a significant effect of session ($F(9, 153) = 4.06$, $p < 0.01$) and a significant interaction between session and group ($F(9, 153) = 2.67$, $p < 0.01$). From separate repeated measures ANOVAs run for each group across sessions, a significant effect of session was found within the BUP group ($F(9, 90) = 7.15$, $p < 0.01$) but not within the SHAM group ($F(9, 81) = 0.87$, $p = 0.56$). Inspection of Figure 7 reveals that BUP ratios remained lower than SHAM ratios for another four days after pumps were removed (independent samples t-test; first session: $t(19) = 2.64$, $p < 0.05$; second session: $t(19) = 2.32$, $p < 0.05$; third session: $t(19) = 2.81$, $p < 0.05$; fourth session: $t(19) = 2.73$, $p < 0.05$).

To calculate the ratios using the FR5 schedule data, the mean number of pellets obtained by each group in a given session was multiplied by five to provide the minimum number of responses needed to obtain the quantity of pellets. In turn, this value served as the denominator for the ratio for that session. The ratios for each group over the three FR5 sessions are presented in Figure 8. A group by session ANOVA indicated a significant effect of session ($F(2, 22) = 4.62$, $p < 0.05$) and additional paired t-tests on the combined BUP and SHAM data revealed a significant increase in the mean ratio of the third FR5 session compared to the mean of the second FR5 session ($t(12) = 3.20$, $p < 0.01$).

For the PR schedule data, the total number of active lever presses needed to obtain the quantity of pellets taken by each group in a given session was determined from the PR schedule equation (see Appendix A). This value was then used as the denominator for

Figure 7. Experiment 2. Mean ratios (+/-SEM) of active lever responses made to the number of active lever responses required to obtain the amount of pellets taken across FR1 sessions. Leftmost vertical dashed line indicates point at which BUP pumps inserted; rightmost dashed line indicates when BUP pumps removed.

Star = significant difference between groups, $p < 0.05$

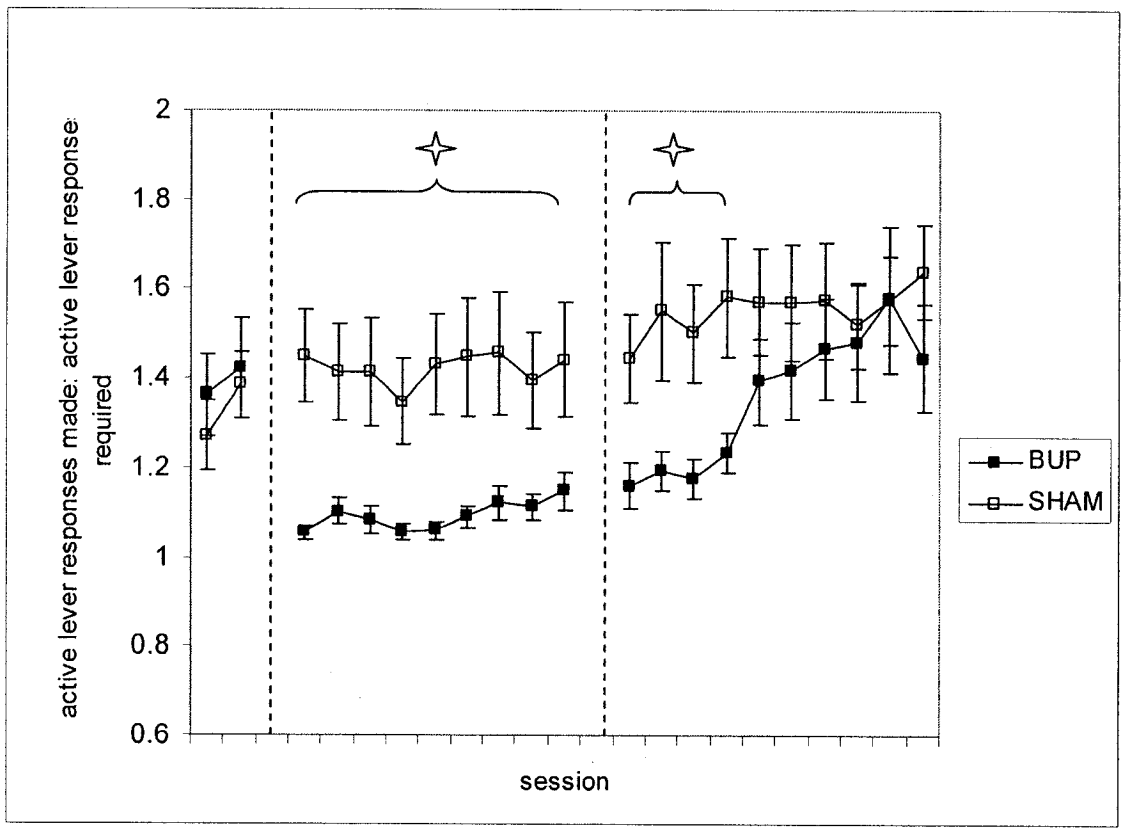


Figure 8. Experiment 2. Mean ratios (+/-SEM) of active lever responses made to the number of active lever responses required for the amount of pellets taken across FR5 sessions

Star = significantly different from 'pumps in session', $p < 0.01$

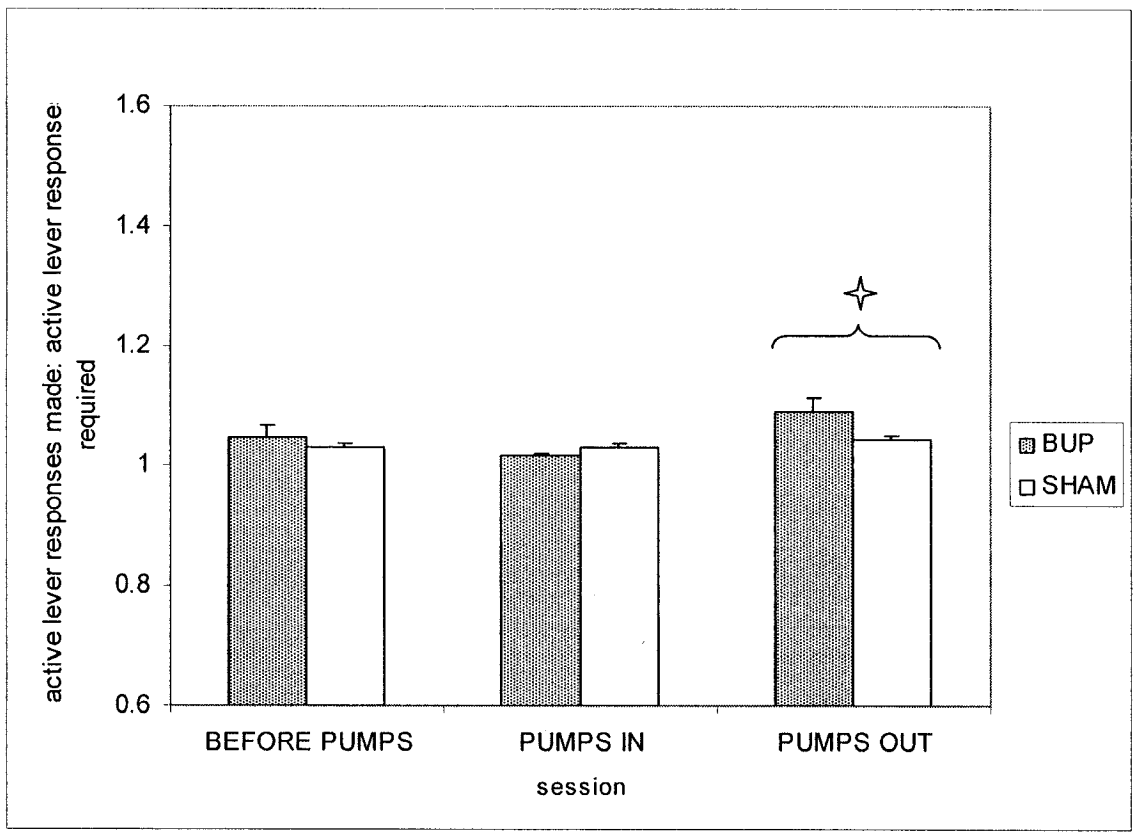
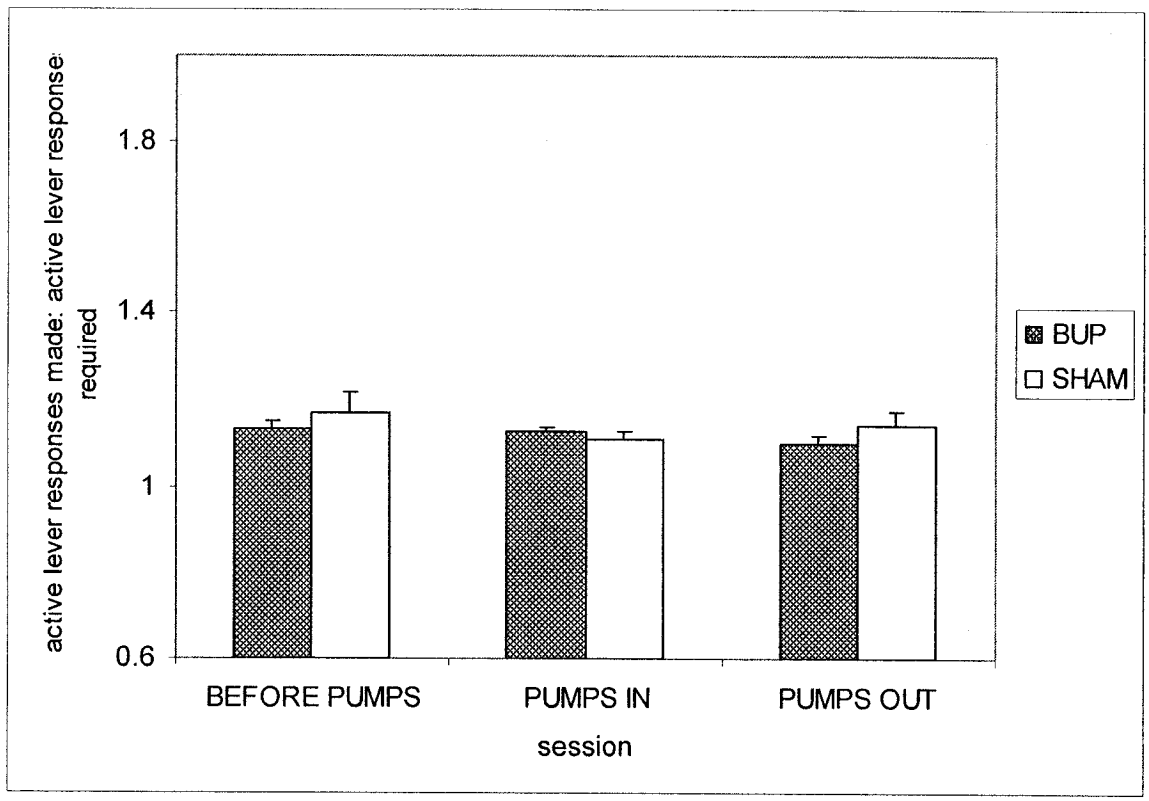


Figure 9. Experiment 2. Mean ratios (+/-SEM) of active lever responses made to the number of active lever responses required to obtain the amount of pellets taken across PR sessions



the ratio for that session. The mean ratios for each group over the three PR sessions are presented in Figure 9. From a group by session ANOVA, no significant difference between the groups across the three sessions was found (effect of group: ($F(1, 12) = 1.23$, $p = 0.29$; effect of session: $F(2, 24) = 0.63$, $p = 0.54$; group by session interaction: $F(2, 24) = 0.44$, $p = 0.65$).

Discussion

The results of Experiment 2 show that chronic BUP reduces self-administration of sucrose on an FR1 schedule, which replicates the results of Experiment 1. From the combined data of Experiments 1 and 2, BUP rats pressed for and ate on average 140 pellets compared to the 165 pellets consumed on average by SHAM rats. Moreover, BUP rats made an average of 150 active lever responses whereas SHAM rats averaged 240. Chronic BUP, however, did not reduce responding on an FR5 schedule or decrease break points on a PR schedule.

It is somewhat surprising that chronic BUP had no effect on sucrose self-administration when the more demanding schedules of reinforcement were used, since there appears to be a clear effect of BUP on FR1 responding. These results from the FR5 and PR schedules suggest that BUP does not affect how hard rats will work for sucrose and thus imply that BUP does not decrease motivation for this reward. As such, the nature of the effect of BUP on FR1 responding for sucrose is open to interpretation.

It is possible, however, that the lack of difference between the BUP and SHAM groups on the FR5 and PR schedules is attributable to the *ad libitum* feeding conditions of the rats in this experiment. Rats might not have been as motivated to work for sucrose under these conditions as they could have been had their access to food been restricted.

As such, these schedules might not have sufficiently challenged their willingness to work and thus might not have provided an adequate test of whether or not chronic BUP dampens motivation induced by sucrose reward. From this perspective, however, it is difficult to explain why chronic BUP decreased sucrose self-administration on the FR1 schedule.

Additional evidence in support of the idea that FR5 and PR schedules do not completely address the issue of whether BUP alters incentive motivation comes from a recent study by Sorge and colleagues (in preparation). As noted previously, these authors found that BUP treatment reduced rats' responding on FR1, FR5, and PR schedules for cocaine infusions but did not affect responding for heroin. These results, which suggest that BUP reduces motivation for cocaine and not heroin, differ from the findings of Mello and colleagues (1980; 1983; 1992) that BUP treatment decreases both opioid and cocaine self-administration in monkeys. These results are also difficult to reconcile with other findings reported by Sorge et al. (2005; in preparation) showing that BUP reduces both heroin and cocaine seeking in rats during extinction of self-administration and during priming-induced reinstatement.

It could also be argued that the data from the FR5 and PR schedules do not clearly reflect whether chronic BUP dampens motivation for sucrose if these data are analyzed for a different feature of self-administration behaviour. This feature is the amount of unreinforced lever responses made during self-administration sessions. According to this argument, responses made during the timeout periods following sucrose pellet delivery reflect the incentive motivation of an animal to approach reinforcing stimuli (i.e., the

sucrose-associated lever and cue light). As such, increases or decreases in the number of these responses may serve as an index of incentive motivation.

An analysis of this index of motivation was undertaken by calculating the ratios of the active lever responses made to the active lever responses required to obtain the quantity of pellets taken on each schedule. From these results, it was found that rats made a large number of unreinforced responses on the FR1 schedule and that these responses were significantly reduced by chronic BUP exposure. On the FR5 and PR schedules, however, the amount of work expended by BUP and SHAM rats was essentially the minimum needed to obtain the mean number of sucrose pellets consumed per session (that is, ratios were very close to 1.0 in both BUP and SHAM groups on both schedules). In effect, the high work demands of these schedules could have imposed a floor effect on unreinforced responding that prevented any dampening action of BUP from being manifested in this way.

Because of the discrepancies observed in the effects of chronic BUP on the FR1, FR5, and PR schedules in the present experiment and in previous research, it seemed important to test the effect of BUP on motivation for non-drug reward using another approach. One strategy that has been used to gauge motivation is through monitoring the vigour of operant responding when a reward is no longer available (i.e., extinction). Responding during extinction occurs independently of any effects that the reward itself may have on behaviour. As such, responding in extinction can be considered to reflect the incentive salience ascribed to stimuli associated with the reward.

Another way to assess motivation for reward is through the presentation of a previously worked-for reward after responding has been extinguished, known as a test of

priming-induced reinstatement. Reinstatement of responding for both drugs of abuse and for food has been found to occur reliably following a non-response contingent delivery of the reward (e.g., Stretch & Gerber, 1973; de Wit & Stewart, 1983; Sun, Akins, Mattingly, & Rebec, 2005). These two approaches were adopted in Experiment 3.

EXPERIMENT 3: EFFECT OF CHRONIC BUP ON SUCROSE SEEKING IN EXTINCTION AND FOLLOWING SUCROSE PRIMING

Introduction

The results of Experiments 1 and 2 indicate that chronic BUP decreases self-administration of sucrose pellets on an FR1 schedule, but not on an FR5 schedule or a PR schedule. It is not clear what these results signify about the effect of BUP on motivation induced by non-drug rewards. The lack of change in responding on the FR5 schedule or in breakpoint on the PR schedule suggests that BUP does not decrease motivation for sucrose; however, the reduction in unreinforced responding on the FR1 schedule suggests that BUP does affect motivation for sucrose.

Experiments by Sorge and colleagues (2005; in preparation) have revealed seemingly discrepant effects of chronic BUP on drug-induced motivation. As previously mentioned, they found that chronic BUP does not reduce self-administration of heroin on an FR1, FR5, or PR schedule. Conversely, chronic BUP retards responding for heroin or cocaine in the presence of drug-associated cues during self-administration and during extinction of self-administration. These latter findings suggest that chronic BUP blunts the incentive salience of these cues and this interpretation is supported by additional findings that chronic BUP blocks priming-induced reinstatement of heroin and cocaine seeking.

In an attempt to clarify the effect of BUP on motivation induced by non-drug reward, the impact of chronic BUP on responding for sucrose in extinction was investigated in Experiment 3. Rats were exposed to chronic BUP at the start of extinction training after having learned to self-administer sucrose in a drug-naïve state. Changes in motivation were also assessed by examining the effect of chronic BUP on the reinstatement of operant responding induced by sucrose priming after extinction. Inasmuch as Experiment 2 showed that chronic BUP treatment did not reduce responding for sucrose on the FR5 and PR schedules, it was hypothesized that chronic BUP would not reduce responding for sucrose in extinction or after the presentation of a sucrose prime.

Procedures

Subjects

Fourteen rats were used in this experiment. All testing was conducted between the hours of 1000 h and 1300 h.

Study of Reinstatement

Training. Rats were trained to self-administer sucrose for eight sessions on an FR1 schedule. These sessions were conducted in the same manner described in the general method 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 Experiments 1 and 2 that rats obtained the majority of the total sucrose pellets normally taken in a 180 min session during the first 60 min. Immediately following the final training session, rats were assigned to either the BUP or SHAM condition and underwent surgery as described in the general method. Group assignment was made on the basis of

active lever responding during the final training session so that the two groups were matched in this respect.

Extinction. After a 24 h recovery period, rats were returned to the operant chambers and began extinction training. Extinction sessions were conducted in the same manner as were the FR1 sessions with the exception that the hoppers did not contain any sucrose pellets. Responses on the active lever resulted only in the illumination of the cue light. Extinction training continued for six days.

Reinstatement. On the seventh day, rats were returned to the operant chambers for another extinction session. This session was conducted to verify that the rats' responses on the active lever met an extinction criterion of fewer than 15 responses in 60 min. If this criterion was not met, then additional extinction sessions were run until rats reached this criterion. A 15 min time-out period elapsed between the end of an extinction session and the beginning of a subsequent session.

Once the extinction criterion was met, the reinstatement procedure was initiated. 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 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, three sucrose pellets were dispensed into the magazine, each spaced by 10 s intervals. The cue light illuminated for 10 s coincident with the delivery of each pellet. This train of three pellets and three 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.

Results

Training

By the end of training, BUP and SHAM rats were taking a steady amount of sucrose and were responding reliably on the active lever. The two groups did not differ significantly in terms of sucrose intake (group by session ANOVA; effect of group: $F(1, 12) = 0.01$, $p = 0.92$) or active lever responding ($F(1, 12) = 0.91$, $p = 0.77$). During the last two sessions of training, the mean numbers of pellets obtained by each group (BUP +/- SEM vs. SHAM +/- SEM) were 152.9 +/- 18.1 vs. 137.3 +/- 26.4 and 156.1 +/- 23.2 vs. 138.9 +/- 24.4. The mean responses on the active lever per session during the last two days of training were 214.3 +/-33.9 vs 183.9 +/- 51.1 and 238.0 +/- 53.6 vs 197.7 +/- 53.2. Furthermo re, both groups responded preferentially on the active lever instead of the inactive lever by the end of training and did not differ significantly in this respect (group by session ANOVA; effect of group: $F(1, 12) = 0.01$, $p = 0.94$). On the final training session, the mean number of inactive lever responses (BUP +/- SEM vs. SHAM +/- SEM) was 2.0 +/- 0.7 vs. 2.7 +/- 1.2.

Extinction

Figure 10 shows each group's active lever responding over six days of extinction. Both groups decreased responding rapidly over extinction sessions but BUP reduced the amount of responses made on the first day of extinction. On the first day, BUP rats made 57.9 +/- 12.6 (SEM) responses on the active lever, whereas SHAM rats made 92.9 +/- 23.1 (SEM) responses. This effect, however, was not found to be significant in a group by session ANOVA (effect of group: $F(1, 12) = 1.91$, $p = 0.19$; group by session

interaction: $F(6, 72) = 1.53$, $p = 0.18$). By the final day of extinction, all rats had reached the criterion of 15 or fewer active lever presses in 60 min.

Reinstatement

Before the reinstatement procedure began on day 7, all rats underwent one additional 60 min session of extinction to verify that the level of active lever responding in each group did not exceed the criterion for extinction. This session was followed by a 15 min timeout period and then the test for reinstatement was initiated. BUP significantly reduced responding on the active lever during the test of reinstatement ($t(12) = 2.24$, $p < 0.05$) and this effect is depicted in Figure 11.

Discussion

The results of Experiment 3 show that chronic BUP does not significantly reduce responding for sucrose during the first few days of extinction, although BUP rats made fewer responses on average. Chronic BUP significantly reduced responding on the sucrose-associated lever following non-contingent presentation of sucrose. These findings suggest that chronic BUP blunts motivation for sucrose in *ad libitum* fed rats, which contrasts with the absence of an effect of BUP on the FR5 and PR schedules seen in Experiment 2. These findings also parallel the observations reported by Sorge and colleagues (2005; in preparation) that chronic BUP blocks reinstatement of responding for cocaine and heroin in rats following a priming injection of the previously self-administered drug.

When the results of Sorge and colleagues (2005; in preparation) and those of the present experiments are reviewed together, it appears that chronic BUP reduces motivation for drug and sucrose reward. This is clearly demonstrated by the decrease in

Figure 10. Experiment 3. Mean (+/- SEM) active lever responses across extinction training sessions.

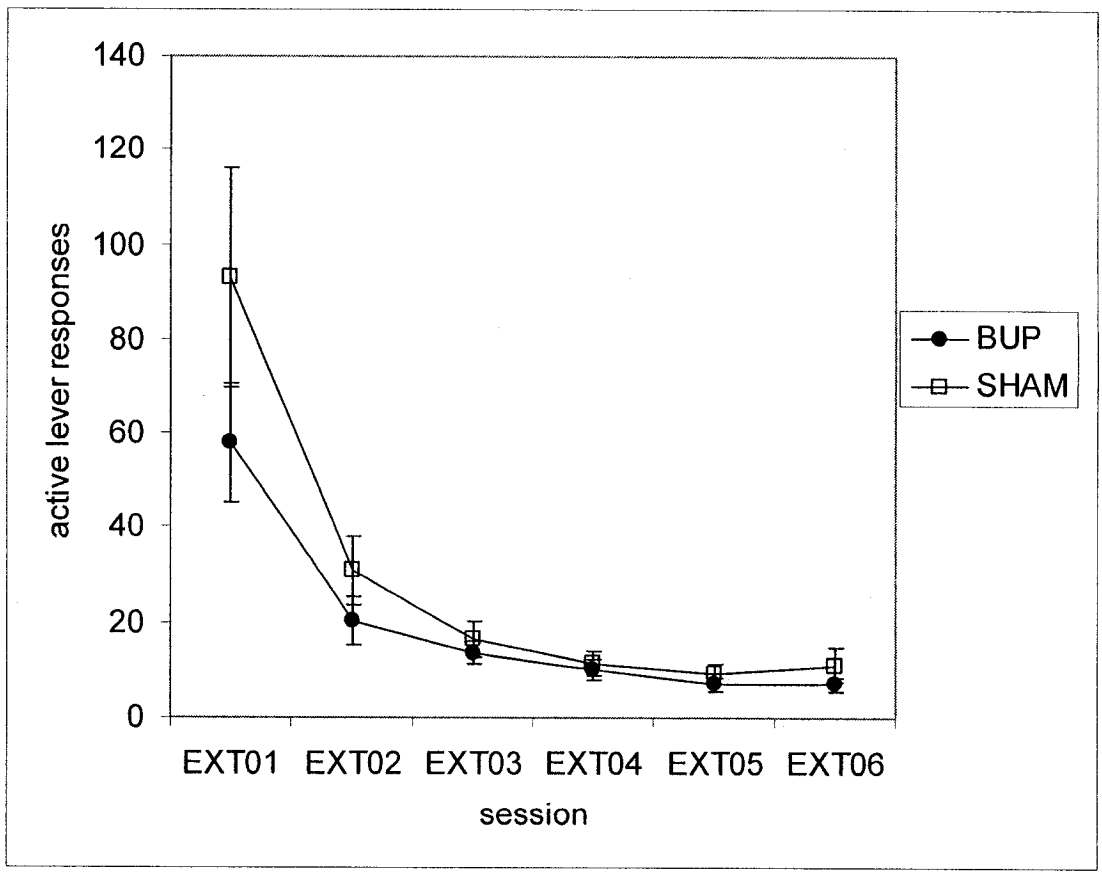
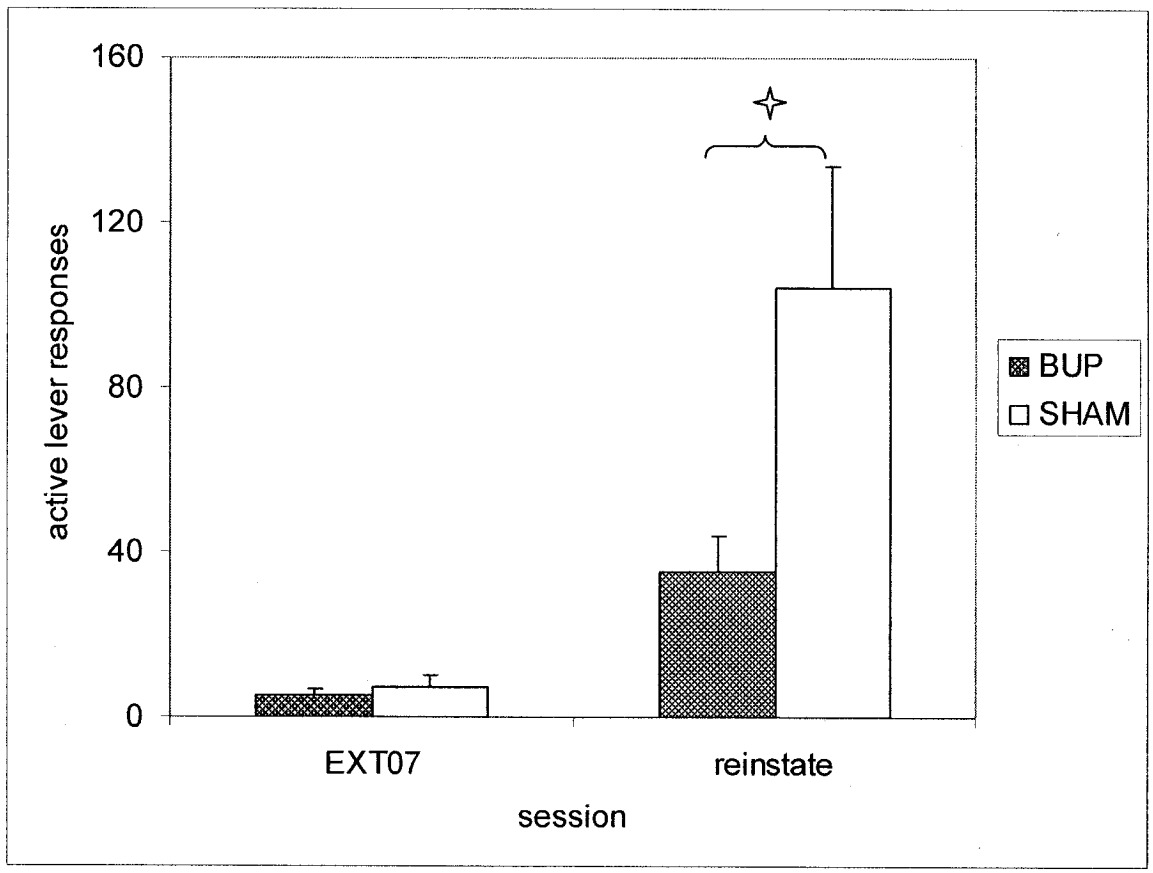


Figure 11. Experiment 3. Mean (+/- SEM) active lever responses made during the final extinction session (EXT 07) and during the test of reinstatement (reinstate).

Star = significant difference between groups, $p < 0.05$



responding for reward-associated cues in extinction and during prime-induced reinstatement. The effect of chronic BUP on the self-administration of drugs and sucrose rewards, however, is less consistent. Results from these studies vary according to the reward used, the schedule under which reinforcement is administered, and the motivational state of the animal. Specifically, chronic BUP reduces administration of cocaine on an FR1, an FR5, and a PR schedule. It reduces FR1 administration of sucrose in *ad libitum* fed rats without affecting responding on an FR5 or PR schedule, although this effect of BUP might be subject to whether or not animals are food-deprived. On the other hand, chronic BUP does not alter heroin intake on any of the schedules tested. It is important to remember, however, that other studies have reported that opioid self-administration is reduced in the presence of BUP (Mello et al., 1983; Mello & Negus, 1998; Winger & Woods, 1996).

In view of the findings that chronic BUP blunts motivation induced by both drug and non-drug rewards, it became of interest to investigate the neural mechanisms involved in this effect. One approach to addressing this issue is to examine the influence of chronic BUP on the mesolimbic DA system, a region of the brain known to be activated by many types of reinforcing events. Activity within the mesolimbic DA system is strongly associated with approach behaviour towards appetitive stimuli (e.g., Robbins & Everitt, 1996; Berridge & Robinson, 1993). Furthermore, many drugs of abuse and food increase levels of DA within terminal regions of the mesolimbic system, including the NAc (Di Chiara & Imperato, 1988; Phillips, Atkinson, Blackburn, & Blaha, 1993). Since BUP attenuates the motivation elicited by both drugs of abuse and palatable

food reward, it is possible that this effect is associated with an influence of BUP on extracellular levels of DA in the NAc. This issue was investigated in experiment 4.

EXPERIMENT 4: EFFECT OF CHRONIC BUP ON FOOD- AND SUCROSE-INDUCED CHANGES IN NAC DA LEVELS

Introduction

Activation of the mesolimbic DA system is strongly associated with motivated behaviour, such as approach toward salient stimuli in the environment (Robbins & Everitt, 1996; Berridge & Robinson, 1998). One example of this association occurs in feeding behaviour. In general, levels of extracellular DA increase in terminal regions of the mesolimbic system, such as the NAc, when animals perform operant responses to obtain food and when they consume food (Hernandez & Hoebel, 1988; Salamone, Cousins, McCullough, Carriero, & Berkowitz, 1994; Phillips et al., 1993; Smith, 2004). Most drugs of abuse also lead to increased levels of extracellular DA in terminal regions, including the NAc (Di Chiara & Imperato, 1988). It has been hypothesized that this increase in DA underlies the incentive salience of drugs and drug-associated stimuli (Robinson & Berridge, 1998). In support of these findings, a decrease in DA levels within the NAc has been shown to decrease both drug seeking and food seeking (e.g., Caine & Koob, 1994; Beninger et al., 1987; Salamone & Correa, 2005).

Research indicates that both acute and chronic administration of BUP increase basal levels of extracellular DA within the NAc (Brown et al., 1991; Sorge et al., 2005). BUP treatment also appears to affect the increase in extracellular DA levels in the NAc that follow acute injections of drugs of abuse. For example, Sorge and colleagues (2005; in preparation) reported that chronic BUP blocks the rise in NAc DA level induced by an

injection of heroin whereas BUP potentiates the rise in NAc DA induced by cocaine. At present, these findings appear contradictory and difficult to interpret; however, it seems worthwhile to examine the effect of chronic BUP on the change in NAc DA elicited by a non-drug reward, such as sucrose.

In the current experiment, the effect of chronic BUP on DA levels in the NAc was examined in rats given small amounts of sucrose pellets and regular lab chow pellets. Consistent with the conditions of the first three experiments, the rats used in this study were not food deprived. It was hypothesized that BUP-treated rats would not show the same degree of increase in NAc DA levels following sucrose pellets that SHAM rats would, since the results of Experiment 3 suggests that chronic BUP reduces the incentive salience of sucrose and sucrose-associated stimuli.

Methods

Subjects

Fourteen male Long-Evans rats (about 350 g; Charles River, St. Constant, QC.) were used in this experiment. Rats were housed in the university colony according to the conditions described in the general methods for experiments 1, 2, and 3. Microdialysis testing was conducted between 0900 h and 1700 h.

Surgery

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. Cannulae were angled at 10° laterally to avoid puncturing the ventricle 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.

Osmotic minipumps. Rats were exposed to a continuous level of BUP (3.0 mg/kg/day) with the use of osmotic minipumps, which were implanted according to the procedure described in the general method for Experiments 1, 2, and 3.

Apparatus

Testing chambers. The dialysis experiment was conducted using four custom-made hexagonal chambers (42 x 39 x 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.

Microdialysis probes. Probes were 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. Three cm from the join between the PE tubing and the stainless steel tubing, an incision was made in the PE tubing to allow the insertion of small diameter fused silica tubing (HRS Scientific,

Montreal, QC). The fused silica tubing extended internally through the PE tubing and into the probe with one end positioned 0.5 mm from the tip of the probe. The incision site was glued with epoxy to prevent leakage. The opposite end of the silica tubing was attached to the other end of the PE tubing with masking tape and small vials were fastened to the masking tape to collect dialysate samples from the silica tubing. The probe assembly was connected to a liquid swivel, positioned directly above the testing chamber, by the PE tubing. The swivel itself was connected to a variable speed syringe infusion pump (Harvard Apparatus Canada, Montreal, QC) located outside of the chamber. During dialysis testing, the full lengths of the PE tubing and fused silica tubing were covered by a steel spring to protect against chewing by the rat. The dialysis probe was secured within the rat's head by screwing a stainless steel collar at the end of the steel spring onto the guide cannula (HRS Scientific, Montreal, QC). Probes were inserted into the animals' heads the day before testing and artificial cerebrospinal fluid (145 mM Na^{+}, 2.7 mM K^{+}, 1.2 mM Ca^{2+}, 1.0mM Mg^{2+}+, 150 mM Cl^{-}, 0.2mM ascorbate, 2mM $Na^{2}HPO^{4}$, pH 7.4+0.1) was perfused overnight at a rate of 0.5 uL/min to prevent the probes from blocking.

High-performance liquid chromatography. Ten µl of dialysate were withdrawn from each sample and analysed immediately using an HPLC system with electrochemical detection (HPLC-EC). The samples were loaded onto C-18 reverse-phase columns (5um, 15 cm) through manual injection ports (Reodyn 7125; 20 uL loop). Dual-channel ESA coulometric detectors (Coulochem III, with model 5011 analytical cell) were used to measure oxidation and reduction currents for DA and its metabolites (dihidroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 5-hydroxyindole

acetic acid (HIAA)). Currents for DA were measured on a separate channel of the Coulochem detector than that used for DOPAC, HVA, and HIAA. 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 peaks obtained for DA, DOPAC, HVA, and HIAA were integrated and quantified by the EZChrom Chromatography Data System (Scientific Software Inc., San Ramon, CA). The mobile phase was adjusted to allow for the separation of DA, DOPAC, HVA, and HIAA in a single run.

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 uL/min. At about 0900 h the next day, food was removed from the chambers and the dialysate flow rate was increased to 0.7uL/min. Baseline samples (of about 14 uL total volume) and locomotor activity scores were taken every 20 min. A baseline of DA and metabolite levels was said to be

established once there was less than 10 % variation in these readings across three consecutive samples.

Once baselines were established, 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 uL. 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 pellets 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.

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 um 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.

Statistical Analyses

For each rat, baseline samples were averaged to give a mean value for DA, DOPAC, HVA, and HIAA. All samples taken subsequent to the last baseline sample

from each rat were transformed into a percentage of the rat's respective mean baseline value. Group by sample ANOVAs were carried out on these transformed data.

An additional ANOVA was carried out on the untransformed baseline levels of DA (in picograms) for BUP and SHAM groups. This analysis was conducted because previous findings from this laboratory indicate that buprenorphine increases basal DA levels in the NAc (Sorge et al., 2005).

Results

Amount of lab chow and sucrose consumed

The amount of lab chow and sucrose pellets eaten by each rat was measured after the food was removed from the dialysis chambers. In the case of lab chow, the approximate size of the pellets remaining was judged in proportion to the original amount presented. In the case of sucrose, this amount was determined by counting the number of pellets remaining from the original quantity (100). The amounts of each food eaten were then converted into percentages of the whole amounts made available to the rats.

The quantity of lab chow pellets eaten was not found to differ significantly between the BUP and SHAM groups (independent samples t-test: $t(10) = 1.22$, $p = 0.25$). BUP rats consumed about 39.3 % +/- 7.4 of their chow pellets whereas SHAM rats consumed about 53.2 % +/- 8.5 (i.e., rats in each group consumed about one lab chow pellet). In the case of sucrose, however, the SHAM group ate significantly more pellets than did the BUP group ($t(5) = 3.35$, $p < 0.05$). All SHAM rats consumed 100 % of the sucrose available whereas BUP rats consumed about 64.2 % +/- 10.7.

Lab chow-induced changes in NAc DA and metabolites

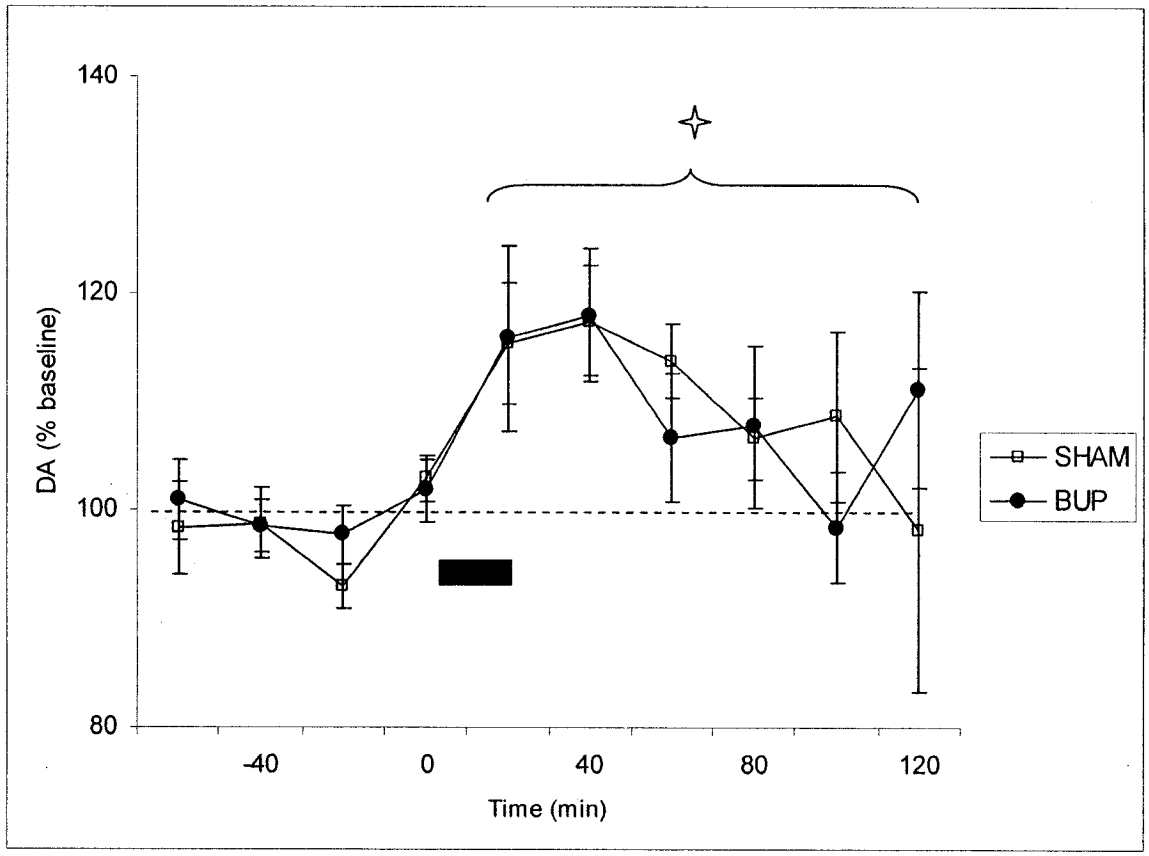
An analysis of the effect of lab chow on DA and metabolites levels was carried out using data from seven rats in the BUP group and five rats in the SHAM group. Two rats from the SHAM group were excluded from analysis: one rat was not tested due to illness after the probe was implanted and another was excluded because of abnormally high readings of DA. In the case of the second rat, the level of DA observed was too high to be attributed to an incorrect probe placement (e.g., in the dorsal striatum) and was attributed instead to an equipment malfunction.

DA. Figure 12 shows the effect of lab chow on NAc DA level. Immediately after the presentation of lab chow, DA levels rose by about 17 % above baseline in both the BUP group and the SHAM group and then returned to baseline over the remaining samples. A group by sample ANOVA over the six samples collected after the presentation of chow pellets indicated a significant effect of sample ($F(5, 50) = 2.49$, $p < 0.05$), but not of group ($F(1, 10) = 0.09$, $p = 0.77$) or an interaction of group and sample ($F(5, 50) = 2.11$, $p = 0.08$).

DOPAC, HVA, HIAA. There were no significant changes in the levels of DA metabolites in either the BUP group or the SHAM group over the six samples collected after chow presentation (DOPAC: effect of sample: $F(5, 50) = 1.79$, $p = 0.13$; DOPAC: effect of group: $F(1, 10) = 0.41$, $p = 0.54$; HVA: effect of sample $F(5, 50) = 0.90$, $p = 0.49$, HVA: effect of group: $F(1, 10) = 0.01$, $p = 0.93$; HIAA: effect of sample: $F(5, 50) = 1.29$, $p = 0.28$; HIAA: effect of group: $F(1, 10) = 1.02$, $p = 0.34$).

Figure 12. Experiment 4. Mean (+/- SEM) percent increase in the level of extracellular DA in the NAc in response to lab chow pellets. Black bar indicates period of time during which chow pellets were available to the animal.

Star = significantly different from baseline, $p < 0.05$



Sucrose-induced changes in DA and metabolites

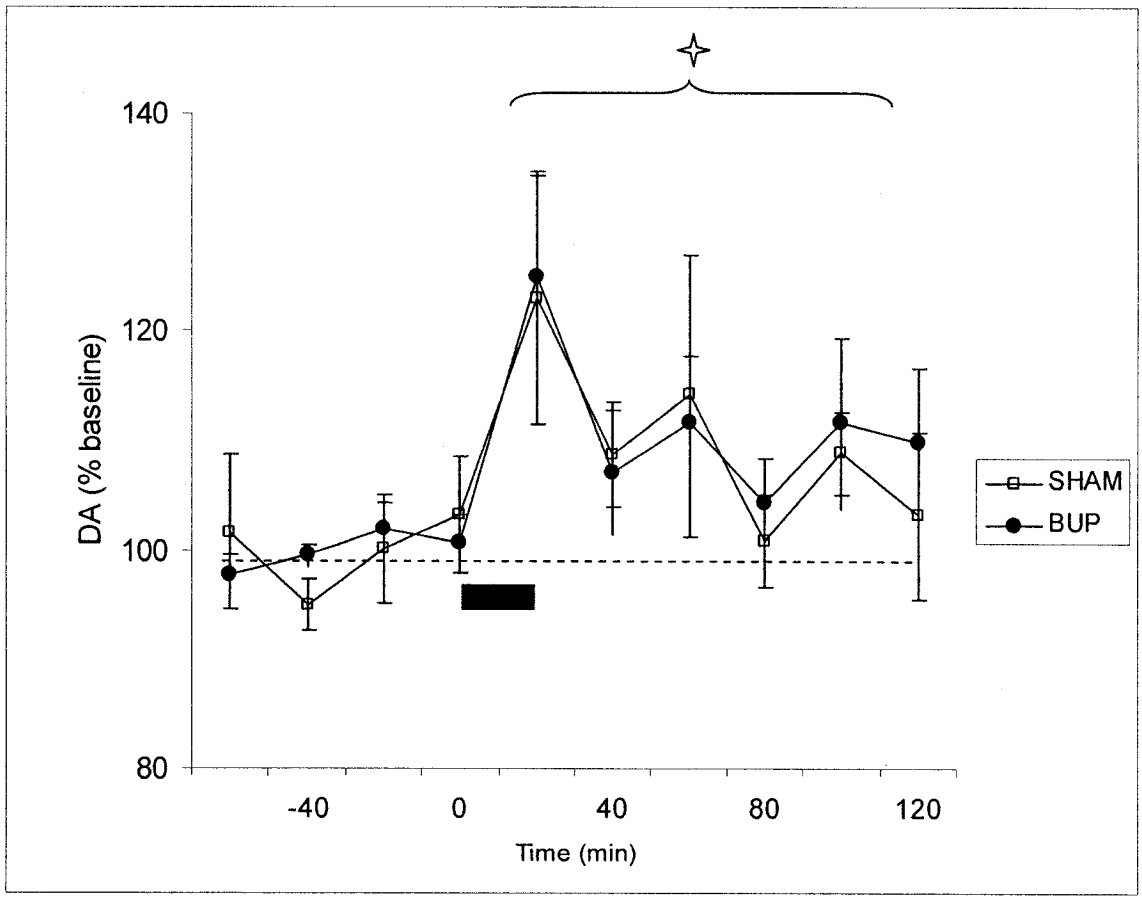
Data from seven rats in the BUP group and four rats in the SHAM group were used in the analysis of the effect of sucrose on DA and metabolites levels. Three rats in the SHAM group were excluded: the first SHAM rat was not tested due to illness, the second was excluded on the basis of abnormally high levels of DA (as noted in the analysis of the lab chow data), and the third was excluded because of greater than 10 % variability in the level of DA within baseline on the day of sucrose testing.

DA. Immediately after the presentation of sucrose, DA rose by about 25 % in both BUP rats and SHAM rats and then returned to baseline over the remaining samples. These results are shown in Figure 13. A group by sample ANOVA over the six samples collected after sucrose presentation indicated a significant effect of sample ($F(5, 45) = 2.73$, $p < 0.05$) but not of group ($F(1, 9) = 0.06$, $p = 0.82$) or an interaction of group and sample ($F(5, 45) = 0.14$, $p = 0.98$).

DOPAC, HVA, HIAA. Over the six samples collected after sucrose presentation, the level of DOPAC in both BUP and SHAM groups gradually but significantly decreased from baseline. A group by sample ANOVA for DOPAC levels indicated a significant effect of sample ($F(5, 45) = 4.64$, $p < 0.01$) but not of group ($F(1, 9) = 0.14$, $p = 0.91$). The final reading of DOPAC in each group (BUP +/- SEM vs. SHAM +/- SEM) was 91.3 % +/- 6.2 vs. 89.3 % +/- 5.2 of baseline. In contrast, HVA and HIAA levels did not change significantly over the six samples collected after sucrose presentation (HVA: effect of sample: $F(5, 45) = 1.07$, $p = 0.39$; HVA: effect of group: $F(1, 9) = 0.17$, $p = 0.69$; HIAA: effect of sample: $F(5, 45) = 1.71$, $p = 0.15$; HIAA: effect of group: $F(1, 9) = 0.03$, $p = 0.86$).

Figure 13. Experiment 4. Mean (+/- SEM) percent increase in the level of extracellular DA in the NAc in response to sucrose pellets. Black bar indicates period of time during which sucrose pellets were available to the animal.

Star = significantly different from baseline, $p < 0.05$



Basal level of DA

An analysis of basal DA levels in the NAc was conducted using the baseline samples from 12 rats (BUP n = 7, SHAM n = 5). Basal levels of DA were established by averaging the amounts (in pg) of DA in each baseline sample over the two days of testing for each rat. The values for one SHAM rat were determined from only one testing day, however, because the data from the second testing day were highly variable and were excluded from all other analyses.

When all 12 animals were included in the group by sample ANOVA, no significant difference in the mean levels of DA over baseline samples was found between the BUP group and SHAM group ($F(1, 10) = 0.23$, $p = 0.65$). It was observed, however, that the mean level of DA in one SHAM rat exceeded the mean of the other four SHAM rats by more than three standard deviations. When this rat was removed from the SHAM data, a group by sample ANOVA yielded a significant effect of group ($F(1, 9) = 7.16$, $p < 0.05$). As shown in Figure 14, the basal level of DA in the BUP group was about twice as high as it was in the SHAM group.

Dialysis probe placements

Microdialysis probe placements for 13 rats are shown in Figure 15. Placements ranged from + 2.7 mm to + 1.6 mm from bregma. Solid black lines indicate the probable sampling area (2 mm) covered by each probe.

Figure 14. Experiment 4. Mean (+/- SEM) basal extracellular levels of DA in the NAc (pg/10uL).

Star = significant difference between groups, $p < 0.05$

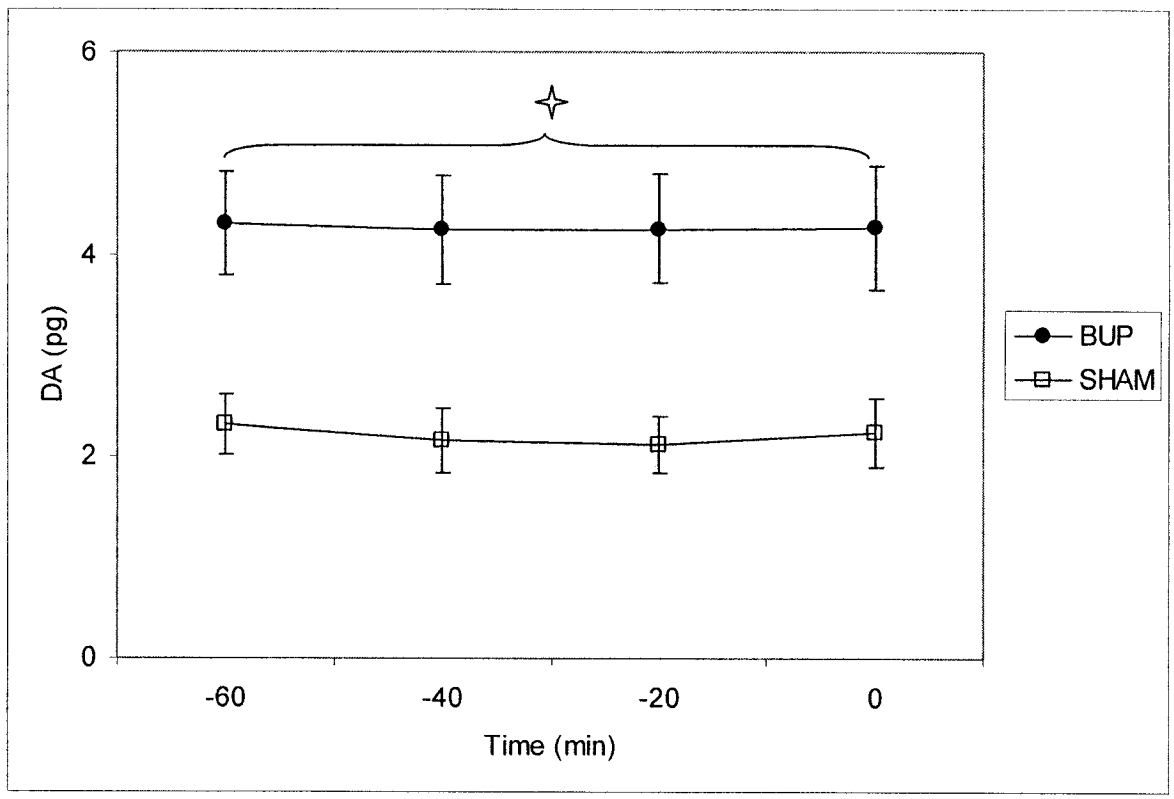
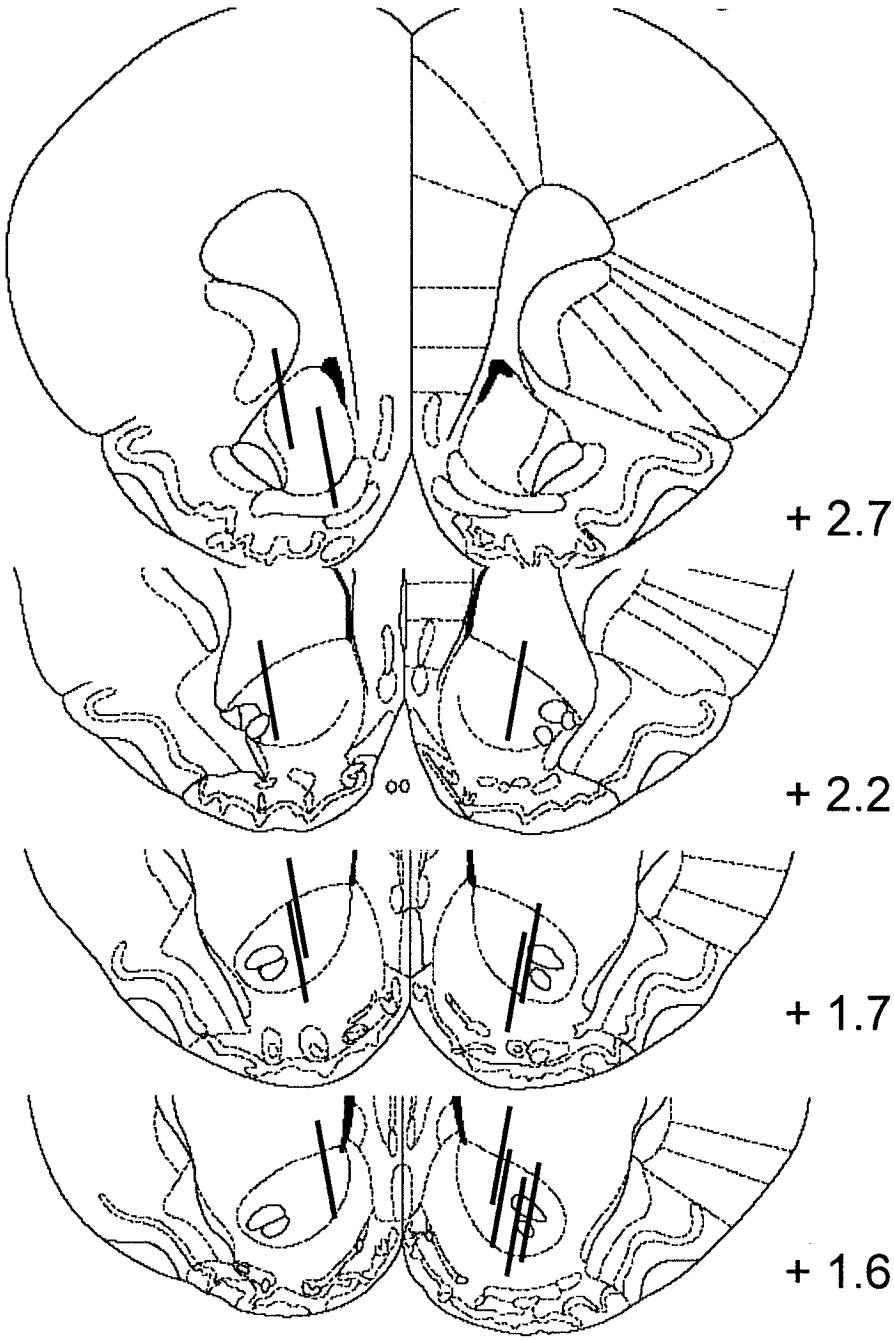


Figure 15. Experiment 4. Microdialysis probe placements for the 13 rats tested. Solid lines indicate the probable probe sampling area (2 mm); co-ordinates are relative to bregma.



Discussion

The results of Experiment 4 show that in *ad libitum* fed rats chronic BUP does not alter the rise in NAc DA levels induced by either lab chow pellets or sucrose pellets. Basal DA level, however, was significantly increased by chronic BUP, which is consistent with other reports in the literature (Brown et al., 1991; Sorge et al., 2005). These findings suggest that the dampening effect of chronic BUP on incentive motivation induced by sucrose and sucrose-associated stimuli is not associated with a decrease in NAc DA levels in response to sucrose.

In interpreting the present findings, however, it is important to bear in mind that the deprivation state of an animal can significantly alter both basal and food-induced changes in extracellular DA within the NAc. Previous research has shown that basal DA levels in the NAc are reduced in rats restricted to 80 % of their free-feeding weight (Pothos, Creese, & Hoebel, 1995; Pothos, Hernandez, & Hoebel, 1995). Furthermore, the increase in NAc DA levels that occurs following food is greatly enhanced in rats that have been food deprived for a period of 20 hours (Wilson, Nomikos, Collu, & Fibiger, 1995). Although correlative, these results suggest an association between motivational state and increase in DA levels in the NAc in response to food. Thus, it seems unlikely that the findings observed in Experiment 4 would necessarily remain consistent if rats were food deprived.

Given the significant impact of food deprivation on NAc DA levels, one could speculate that the effect of chronic BUP on NAc DA in food-deprived rats would be twofold: chronic BUP would elevate basal DA levels otherwise reduced during deprivation and it would reduce the increase in DA levels following sucrose. This

speculation is based in part on evidence from the present experiments as well as previous research (Sorge et al., 2005) indicating that chronic BUP significantly increases basal levels of extracellular DA in the NAc. This speculation is also supported by research by Fibiger and colleagues (1998) showing that the deprivation-induced enhancement in extracellular DA levels following food is contingent upon opioid activity. A systemic injection of naloxone, for example, will block the rise in NAc DA induced by food in deprived animals and it will reduce the overall amount of food consumed (Taber, Zernig, & Fibiger, 1998). Given the mixed agonist-antagonist profile of BUP, chronic BUP exposure could antagonize the action of endogenous opioids released in response to food presentation and prevent the subsequent increase in DA within the NAc.

It would be of interest to determine whether an effect of chronic BUP could be associated with changes in DA levels in other terminal region of the mesolimbic DA system, such as the medial prefrontal cortex (mPFC). Both food and drug rewards increase levels of DA within the mPFC (Bassareo & Di Chiara, 1999; Ahn & Phillips, 1999; Robinson & Berridge, 1993). Importantly, the rise in mPFC DA levels that occurs in the presence of food and food-associated stimuli does not habituate with repeated presentations; this effect has been suggested to reflect a role for DA levels in the mPFC in coding for the incentive salience of stimuli associated with food reward (Bassareo & Di Chiara, 1997; Ahn & Phillips, 1999). As such, the blunting effect of BUP on incentive motivation observed in Experiments 1, 2, and 3 could be associated with an effect of chronic BUP on changes in DA levels in the mPFC induced by sucrose.

In addition, research has revealed that drug-, stress-, and cue-induced reinstatement of drug seeking all rely on activation of mPFC circuitry and further

indicates a role for this region in incentive motivation (Capriles, Rodaros, Sorge, & Stewart, 2003; McLaughlin & See, 2003; See, 2002; Kalivas & McFarland, 2003; McFarland & Kalivas, 2001). It must be noted, however, that temporary inactivation of the mPFC does not reduce self-administration of sucrose pellets in non-deprived rats (Capriles et al, 2003). Thus, this region might not be critically involved in the motivational effects of chronic BUP observed in the present experiments with sucrose.

GENERAL DISCUSSION

Effect of chronic BUP on motivated behaviour induced by sucrose reward

The results of the present experiments show that chronic BUP reduces self-administration of sucrose pellets on an FR1 schedule, but does not reduce self-administration on an FR5 or a PR schedule (Experiment 1 and Experiment 2). During extinction when sucrose was no longer available, chronic BUP slightly decreased operant responding (Experiment 3). Furthermore, BUP significantly reduced the reinstatement of responding that occurs following priming with sucrose (Experiment 3).

Taken together, these findings indicate that chronic BUP blunts the incentive motivation elicited by sucrose and sucrose-associated stimuli, which supports the hypothesis that the effect of BUP on drug-taking and drug-seeking behaviour stems from a more general dampening influence of BUP on the salience of all incentive stimuli. A clear illustration of this effect is found in Experiment 3, in which BUP rats responded less on average than SHAM rats did during extinction. This decrease in responding in the presence of sucrose-associated stimuli is consistent with the findings of Sorge and

colleagues (2005): the same dose of BUP used in the present experiments significantly reduced responding in the presence of heroin- and cocaine-associated stimuli in extinction. The effect of chronic BUP in reducing responding during the test of sucrose-induced reinstatement also parallels the decrease in heroin and cocaine seeking that has been found in BUP-treated rats after a priming injection of heroin or cocaine (Sorge et al., 2005).

It must be acknowledged, however, that the results obtained from Experiment 2 are not entirely consistent with the interpretation that BUP reduces motivation induced by all incentive stimuli. One discrepancy is the absence of effect of BUP on the FR5 and PR schedules in spite of the reduction in responding observed on the FR1 schedule. If BUP decreases motivation for sucrose, it seems reasonable to expect that this effect would be manifested in reduced willingness to work for the reward. Previous research by Sorge and colleagues concerning drug reinforcement (2005; in preparation), however, has also encountered this type of discrepancy. Although BUP clearly suppresses heroin seeking in response to drug-associated cues during self-administration and extinction, as well as in priming-induced reinstatement, these authors reported no effect of BUP in reducing heroin intake by rats on FR1, FR5, and PR schedules.

One explanation for the lack of effect of BUP on the FR5 and PR schedules is that motivation might not have been sufficiently challenged in this experiment because the rats were not food deprived. The maximum amount of work that rats were willing to exert on the FR5 and PR schedules under *ad libitum* feeding conditions might have been more moderate than it would have been had the rats been hungry. As such, the likelihood of observing an effect of BUP on motivation for sucrose might have been reduced in

these experimental conditions. Future research should explore whether chronic BUP does indeed reduce responding for sucrose on an FR5 schedule and lower breakpoints on a PR schedule when rats are food deprived.

This reasoning, however, does not readily account for the present results from the FR1 schedule on which BUP did significantly reduce responding. That is, it seems unlikely that the effect of BUP on FR1 responding for sucrose was attributable to the rats being significantly more motivated on this schedule. Additional experiments are needed to explore this result in more depth.

Effect of BUP on extracellular levels of DA in the NAc following sucrose

The results of Experiment 4 show that chronic BUP does not affect the increase in extracellular DA levels in the NAc in response to sucrose pellets or to lab chow in non food-deprived rats. This finding suggests that the influence of chronic BUP on incentive motivation for sucrose is not associated with differences in levels of DA in the NAc per se.

It is important to note, however, that any interpretation of the present results must consider the influence of the *ad libitum* feeding conditions on the motivational state of rats. The fact that rats were not food deprived in this experiment suggests that motivation for sucrose was less intense than it would have been under deprivation conditions. Although extracellular DA levels in the NAc in themselves do not equate with motivational state, deprivation affects both basal and food-induced NAc DA activity (Pothos, Creese, & Hoebel 1995; Pothos, Hernandez, & Hoebel 1995b; Wilson et al., 1995). As such, it must be assumed that the effect of chronic BUP on the increase in NAc DA levels in response to sucrose might have been different if rats had been food-

deprived. Such an experiment would be important to conduct in the interest of clearly establishing whether an effect of chronic BUP on sucrose-induced motivation is associated with an influence of chronic BUP on NAc DA levels in response to sucrose rewards.

The results of experiments to date concerning the influence of chronic BUP on NAc DA levels in response to drug and non-drug rewards are not easy to reconcile. In the case of drug reward, chronic BUP enhances the cocaine-induced increase in NAc DA, whereas it suppresses the increase in DA levels in response to acute heroin (Sorge et al., 2005). These results combined with the present findings suggest that, in the presence of chronic BUP, DA levels in the NAc per se are not associated with the reinstatement of drug or sucrose seeking (Sorge et al., 2005). More needs to be learned about the influence of chronic BUP on changes in the effects of NAc DA levels on downstream neural mechanisms.

In future research, it would also be of interest to determine the effects of BUP on changes in DA levels in other terminal regions of the mesolimbic system. As noted in the discussion of Experiment 4, one region of interest is the mPFC. Research clearly indicates a role for the mPFC in mediating responses to incentive stimuli; for example, inactivation of the mPFC blocks reinstatement of drug seeking induced by cues, priming drug injections, and stress (Capriles et al., 2003; See, 2002; Kalivas & McFarland, 2003). Furthermore, the presence of food and food-associated stimuli are associated with increases in DA levels within the mPFC (Bassareo & Di Chiara, 1999; Ahn & Phillips, 1999). It is possible that the blunting of incentive motivation that appears to occur in the presence of chronic BUP is associated with changes in mPFC levels of DA.

Summary

The results of the present experiments show that chronic BUP reduces incentive motivation induced by sucrose and sucrose-associated stimuli in non-food-deprived rats. Chronic BUP decreases self-administration of sucrose on an FR1 schedule and reduces sucrose seeking both in extinction and during a test of sucrose prime-induced reinstatement. The increase in levels of extracellular DA in the NAc induced by sucrose and lab chow is not altered by chronic BUP, although this effect might be influenced by whether or not rats are food deprived. Overall, these findings are consistent the hypothesis that BUP decreases drug seeking and drug taking by reducing motivation elicited by drug and non-drug incentive stimuli. Additional experiments involving food-deprived animals are needed, however, to further support this argument.

Reference List

Ahn, S. & Phillips, A.G. (1999). Dopaminergic correlates of sensory-specific satiety in the medial prefrontal cortex and nucleus accumbens of the rat. *Journal of Neuroscience, 19,* RC29-34.

Bassareo, V. & Di Chiara, G. (1999). Modulation of feeding-induced activation of mesolimbic dopamine transmission by appetitive stimuli and its relation to motivational state. *European Journal of Neuroscience, 11,* 4389-4397.

Beninger, R.J., Cheng, M., Hahn, B.L., Hoffman, D.C., Mazurski, E.J., Morency, M.A., Ramm, P. & Stewart, R.J. (1987). Effects of extinction, pimozide, SCH 23390, and metoclopramide on food-rewarded operant responding of rats. *Psychopharmacology, 92,* 343-349.

Berridge, K.C. & Robinson, T.E. (1998). What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain Research Reviews, 28,* 309-369.

Bigelow, G.E. & Thompson, T. (1971). Behavioral effects of morphine and methadone in rhesus monkeys. *Psychonomic Sciences, 24,* 215-216.

Brown, E.E., Finlay, J.M., Wong, J.T.F., Damsma, G. & Fibiger, H.C. (1991). Behavioral and neurochemical interactions between cocaine and buprenorphine: implications for the pharmacotherapy of cocaine abuse. *Journal of Pharmacology and Experimental Therapeutics, 256,* 119-125.

Caine, S.B. & Koob, G.F. (1994). Effects of dopamine D-1 and D-2 antagonists on cocaine self-administration under different schedules of reinforcement in the rat. *Journal of Pharmacology and Experimental Therapeutics, 270,* 209-217.

Capriles, N., Rodaros, D., Sorge, R.E. & Stewart, J. (2003). A role for the prefrontal cortex in stress- and cocaine-induced reinstatement of cocaine seeking in rats. *Psychopharmacology, 168,* 66-74.

Carroll, M.E., Carmona, G.N., May, S.A., Buzalsky, S. & Larson, C. (1992). Buprenorphine's effects on self-administration of smoked cocaine base and orally delivered phencyclidine, ethanol and saccharin in rhesus monkeys. *Journal of Pharmacology and Experimental Therapeutics, 261,* 26-37.

Carroll, M.E. & Lac, S.T. (1992). Effects of buprenorphine on self-administration of cocaine and a nondrug reinforcer in rats. *Pyschopharmacology, 106,* 439-446.

Comer, S.D., Evans, S.M., Pudiak, C.M. & Foltin, R.W. (2002). Effects of buprenorphine on candy and sweetened fluid self-administration by rhesus monkeys. *Psychopharmacology, 164,* 200-206.

Comer, S.D., Hunt, V.R. & Carroll, M.E. (1994). Effects of concurrent saccharin availability and buprenorphine pretreatment on demand for smoked cocaine base in rhesus monkeys. *Psychopharmacology, 115,* 15-23.

Comer, S.D., Lac, S.T., Wyvell, C.L. & Carroll, M.E. (1996). Combined effects of buprenorphine and a nondrug alternative reinforcer on the I.V. cocaine self-administration in rats maintained under F.R. schedules. *Psychopharmacology, 125,* 355-360.

Cooper, S.J. (1983). Effects of opiate agonists and antagonists on fluid intake and saccharine choice in the rat. *Neuropharmacology, 22,* 323-328.

Cooper, S.J., Jackson, A., Kirkham, T.C. & Turkish, S. (1988). Endorphins, opiates and food intake. In Rodgers, R.J. & Cooper, S.J. (Eds.), *Endorphins, Opiates and Behavioural Processes* (pp.143-186). Toronto: John Wiley & Sons.

Cowan, A., Doxey, J.C. & Harry, E.J.R. (1977). The animal pharmacology of buprenorphine, an oripavine analgesic agent. *British Journal of Pharmacology, 60,* 547-554.

Davids, E. & Gastpar, M. (2004). Buprenorphine in the treatment of opioid dependence. *European Neuropsychopharmacology, 14,* 209-216.

De Wit, H. & Stewart, J. (1983). Drug reinstatement of heroin-reinforced responding in the rat. *Psychopharmacology, 79,* 29-31.

Dykstra, L.A. (1983). Behavioral effects of buprenorphine and diprenorphine under a multiple schedule of food presentation in squirrel monkeys. *Journal of Pharmacology and Experimental Therapeutics, 226,* 317-323.

Fletcher, P.J., Korth, K.M. & Chambers, J.W. (1999). Depletion of brain serotonin following intra-raphe injections of 5,7-dihydroxytryptamine does not alter d-amphetamine self-administration across different schedules and access conditions. *Psychopharmacology, 146,* 185-93.

Foltin, R.W. & Fischman, M.W. (1996). Effects of methadone or buprenorphine maintenance on the subjective and reinforcing effects of intravenous cocaine in humans. *Journal of Pharmacology and Experimental Therapeutics, 278,* 1153-1164.

Greenwald, M.K, Schuh, K.J., Hopper, J.A., Schuster, C.R. & Johanson, C.E. (2002). Effects of buprenorphine sublingual tablet maintenance on opioid drug-seeking behaviour in humans. *Psychopharmacology, 260,* 344-352.

Hernandez, & Hoebel, B.G. (1988). Food reward and cocaine increase extracellular dopamine in the nucleus accumbens as measured by microdialysis. *Life Sciences, 42,* 1705-1712.

Holtzman, S.G. (1974). Behavioral effects of separate and combined administration of naloxone and d-amphetamine. *Journal of Pharmacology and Experimental Therapeutics, 189,* 51-60.

Hutchings,D.E., Zmitrovich,A.C., Hamowy,A.S., Liu,P.Y. (1995). Prenatal administration of buprenorphine using the osmotic minipump: a preliminary study of maternal and offspring toxicity and growth in the rat. *Neurotoxicology &.Teratology, 17,* 419-423.

Jasinski, D.R., Pevnick, J.S. & Griffith, J.D. (1978). Human pharmacology and abuse potential of the analgesic buprenorphine. *Archives of General Psychiatry, 35,* 501-516.

Jewett, D.C., Cleary, J., Levine, A.S., Schaal, D.W., Thompson, T. (1995). Effects of neuropeptide Y, insulin, 2-deoxyglucose, and food deprivation on food-motivated behavior. *Psychopharmacology, 120,* 267-271.

June, H.L., Cason, C.R., Chen, S.H.A. & Lewis, M.J. (1998). Buprenorphine alters ethanol self-administration in rats: dose-response and time-dependent effects. *Psychopharmacology, 140,* 29-37.

Kalivas, P.W. McFarland, K. (2003). Brain circuitry and the reinstatement of cocaine-seeking behavior. *Psychopharmacology, 168,* 44-56.

Kosten, T.R., Kleber, H.D. & Morgan, C. (1989). Treatment of cocaine abuse with buprenorphine. *Biological Psychiatry, 26,* 637-639.

Kosten, T.R., Marby, D.W. & Nestler, E.J. (1991). Cocaine conditioned place preference is attenuated by chronic buprenorphine treatment. *Life Sciences, 49,* PL201-206.

Kosten, T.R., Schottenfeld, R., Ziedonis, D. & Falcioni, J. (1993). Buprenorphine versus methadone maintenance for opioid dependence. *Journal of Nervous and Mental Disease, 181,* 358-364.

Levine, A.S., Morley, J.E., Gosnell, B.A., Billington, C.J. & Bartness, T.J. (1985). Opioids and consummatory behavior. *Brain Research Bulletin, 14,* 663-672.

Lukas, S.E., Mello, N.K., Bree, M.P. & Mendelson, J.H. (1988). Differential tolerance development to buprenorphine-, diprenorphine-, and heroin-induced disruption

of food-maintained responding in macaque monkeys. *Pharmacology, Biochemistry and Behavior, 30,* 977-982.

Martin, W.R., Wikler, A., Eades, C.G., & Pescor, F.T. (1963). Tolerance to and physical dependence on morphine in rats. *Psychopharmacologia, 4,* 247-260.

McFarland, K. & Kalivas, P.W. (2001). The circuitry mediating cocaine-induced reinstatement of drug-seeking behavior. *Journal of Neuroscience, 21,* 8655-8663.

McLaughlin, J. & See, R.E. (2003). Selective inactivation of the medial prefrontal cortex and the basolateral amygdala attenuates conditioned-cue reinstatement of extinguished cocaine-seeking behavior in rats. *Psychopharmacology, 168,* 57-65.

McMillan, D.E., Wolf, P.S. & Carchman, R.A. (1970). Antagonism of the behavioral effects of morphine and methadone by narcotic antagonists in the pigeon. *Journal of Pharmacology and Experimental Therapeutics, 175,* 443-457.

Mello, N.K., Bree, M.P. & Mendelson, J.H. (1983). Comparison of buprenorphine and methadone effects on opiate self-administration in primates. *Journal of Pharmacology and Experimental Therapeutics, 225,* 378-386.

Mello, N.K., Lukas, S.E., Kamien, J.B., Mendelson, J.H., Drieze, J. & Cone, E.J. (1992). The effects of chronic buprenorphine treatment on cocaine and food self-

administration by rhesus monkeys. *Journal of Pharmacology and Experimental Therapeutics, 260,* 1185-1193.

Mello, N.K. & Mendelson, J.H. (1980). Buprenorphine suppresses heroin use by heroin addicts. *Science, 207,* 657-659.

Mello, N.K., Mendelson, J.H. & Kuehnle, J.C. (1982). Buprenorphine effects on human heroin self-administration: an operant analysis. *Journal of Pharmacology and Experimental Therapeutics, 223,* 30-39.

Mello, N.K. & Negus, S.S. (1998). The effects of buprenorphine on self-administration of cocaine and heroin "speedball" combination and heroin alone by rhesus monkeys. *Journal of Pharmacology and Experimental Therapeutics, 285,* 444-456.

Negus, S.S., Bidlack, J.N., Mello, N.K., Furness, M.S., Rice, K.C. & Brandt, M.R. (2002). Delta opioid antagonist effects of buprenorphine in rhesus monkeys. *Behavioural Pharmacology, 13,* 557-570.

Phillips, A.G., Atkinson, L.J., Blackburn, J.R. & Blaha, C.D. (1993). Increased extracellular dopamine in the nucleus accumbens of the rat elicited by a conditional stimulus for food: an electrochemical study. *Canadian Journal of Physiology and Pharmacology, 71,* 387-393.

Pothos, E.N., Creese, I. & Hoebel, B.G. (1995). Restricted eating with weight loss selectively decreases extracellular dopamine in the nucleus accumbens and alters dopamine response to amphetamine, morphine, and food intake. *Journal of Neuroscience, 15,* 6640-6650.

Pothos, E.N., Hernandez, L. & Hoebel, B.G. (1995). Chronic food deprivation decreases extracellular dopamine in the nucleus accumbens: implications for a possible neurochemical link between weight loss and drug abuse. *Obesity Research, suppl.4,* 525S-529S.

Robbins, T.W. & Everitt, B.J. (1996). Neurobehavioural mechanisms of reward and motivation. *Current Opinion in Neurobiology, 6,* 228-236.

Rudski, J.M., Schaal, D.W., Thompson, T., Cleary, J., Billington, C.J. & Levine, A.S. (1992). Effects of methadone on free feeding in satiated rats. *Pharmacology, Biochemistry, and Behavior, 43,* 1033-1037.

Rudski, J.M., Schaal, D.W., Thompson, T., Cleary, J., Billington, C.J. & Levine, A.S. (1994). Methadone and feeding: sources of differences between home cage and operant chamber assessment procedures. *Pharmacology, Biochemistry, and Behavior, 49,* 143-146.

Rudski, J.M., Thomas, D., Billington, C.J. & Levine, A.S. (1995). Buprenorphine increases intake of freely available and operant-contingent food in satiated rats. *Pharmacology, Biochemistry, and Behavior, 50,* 271-276.

Salamone, J.D. & Correa, M. (2002). Motivational views of reinforcement: implications for understanding the behavioral functions of nucleus accumbens dopamine. *Behavioural Brain Research, 137,* 3-25.

Salamone, J.D., Cousins, M.S., McCullough, L.D., Carriero, D.L. & Berkowitz, R.J. (1994). Nucleus accumbens dopamine release increases during instrumental lever pressing for food but not free food consumption. *Pharmacology, Biochemistry and Behavior, 49,* 25-31.

See, R.E. (2002). Neural substrates of conditioned-cue relapse to drug-seeking behavior. *Pharmacology, Biochemistry, and Behavior, 71,* 517-529.

Smith, G.P. (2004). Accumbens dopamine mediates the rewarding effect of orosensory stimulation by sucrose. *Appetite, 43,* 11-13.

Sorge, R.E., Rajabi, H., & Stewart, J. (2005). Rats maintained chronically on buprenorphine show reduced heroin and cocaine seeking in tests of extinction and drug-induced reinstatement. *Neuropsychopharmacology, 30,* 1681-1692.

Sorge, R.E. & Stewart, J. Chronic buprenorphine reduces self-administration of cocaine and potentiates the NAc DA response to cocaine, but does not affect heroin self-administration or the heroin-induced increase in NAc DA. Manuscript in preparation.

Strain, E.C., Stitzer, M.L., Liebson, I.A. & Bigelow, G.E. (1994). Comparison of buprenorphine and methadone in the treatment of opioid dependence. *American Journal of Psychiatry, 151,* 1025-1030.

Stretch, R. & Gerber, G.J. (1973). Drug-induced reinstatement of amphetamine self-administration behaviour in monkeys. *Canadian Journal of Psychology, 27,* 168-177.

Sun, W., Akins, C.K., Mattingly, A.E. & Rebec, G.V. (2005). Ionotropic glutamate receptors in the ventral tegmental area regulate cocaine-seeking behavior in rats. *Neuropsychopharmacology*, on-line publication, April 20.

Taber, M.T., Zernig, G. & Fibiger, H.C. (1998). Opioid receptor modulation of feeding-evoked dopamine release in the rat nucleus accumbens. *Brain Research, 785,* 24-30.

Tepperman, F.S. & Hirst, M. (1983). Effect of intrahypothalamic injection of (D-Ala, D-Leu) enkephalin on feeding and temperature in the rat. *European Journal of Pharmacology, 96,* 243-249.

Tzschentke, T.M. (2002). Behavioral pharmacology of buprenorphine, with a focus on preclinical models of reward and addiction. *Psychopharmacology, 161,* 1-16.

Walsh, S.L. & Eissenberg, T. (2003). The clinical pharmacology of buprenorphine: extrapolating from the laboratory to the clinic. *Drug and Alcohol Dependence, 70,* S13-27.

Walsh, S.L, Preston, K.L., Stitzer, M.L, Cone, E.J. & Bigelow, G.E. (1994). Clinical pharmacology of buprenorphine: ceiling effects at high doses. *Clinical Pharmacology & Therapeutics, 55,* 569-580.

Wilson, C., Nomikos, G.G., Collu, M. & Fibiger, H.C. (1995). Dopaminergic correlates of motivated behavior: importance of drive. *Psychopharmacology, 15,* 5169-5178.

Winger, G. & Woods, J.H. (1996). Effects of buprenorphine on behavior maintained by heroin and alfentanil in rhesus monkeys. *Behavioural Pharmacology, 7,* 155-159.

Wood, P.L., Charleson, S.E., Lane, D. & Hudgin, R.L. (1981). Multiple opiate receptors: differential binding of mu, kappa, and delta agonists. *Neuropharmacology, 20,* 1215-1220.

Yeomans, M.R. & Gray, R.W. (1997). Effects of naltrexone on food intake and changes in subjective appetite during eating: evidence for opioid involvement in the appetizer effect. *Physiology and Behavior, 62,* 15-21.

Appendices

Appendix A

Response requirements on the progressive ratio schedule (Experiment 2)

pellet #	responses required for given pellet	total responses required
1	1	1
2	1	2
3	2	4
4	3	7
5	4	11
6	5	16
7	7	23
8	8	27
9	10	41
10	12	53
11	14	67
12	16	83
13	19	102
14	22	124
15	25	149
16	29	178
17	33	211
18	38	249
19	44	293
20	50	343
21	57	399
22	64	462
23	74	534
24	83	614
25	95	705

Appendix B

Analysis of Variance for the effects of chronic BUP on **number of pellets obtained** on an FR1 schedule during training (Experiment 1)

Tests of Within-Subjects Effects:

Source	Sum of Squares	df	Mean Square	F	Sig.
SESSION	30659.749	7	4379.964	4.268	.002
SESSION * GROUP	11771.177	7	1681.597	1.639	.157
Error(SESSION)	35916.323	35	1026.181		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
GROUP	2780.787	1	2780.787	.137	.727
Error	101649.427	5	20329.885		

Analysis of Variance for effects of chronic BUP on **number of pellets obtained** on an FR1 schedule after pumps implanted (Experiment 1)

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
SESSION	4535.439	8	566.930	.916	.514
SESSION * GROUP	5499.820	8	687.478	1.111	.377
Error(SESSION)	24759.259	40	618.981		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
GROUP	32989.466	1	32989.466	4.024	.101
Error	40991.741	5	8198.348		

Analysis of Variance for effects of chronic BUP on **number of pellets obtained** on an FR1 schedule after pumps removed (Experiment 1)

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
SESSION	2184.524	9	242.725	.557	.824
SESSION * GROUP	7110.124	9	790.014	1.813	.092
Error(SESSION)	19612.133	45	435.825		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
GROUP	39278.019	1	39278.019	5.892	.060
Error	33332.867	5	6666.573		

Analysis of Variance for effects of chronic BUP on **active lever responding** on an FR1 schedule during training (Experiment 1)

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
SESSION	81181.429	7	11597.347	6.339	.000
SESSION * GROUP	16080.286	7	2297.184	1.256	.300
Error(SESSION)	64038.250	35	1829.664		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
GROUP	9661.167	1	9661.167	.160	.706
Error	302074.583	5	60414.917		

Analysis of Variance for effects of chronic BUP on **active lever responding** on an FR1 schedule after pumps implanted (Experiment 1)

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
SESSION	8365.534	8	1045.692	1.060	.410
SESSION * GROUP	12361.090	8	1545.136	1.566	.166
Error(SESSION)	39463.481	40	986.587		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
GROUP	292128.731	1	292128.731	5.987	.058
Error	243949.269	5	48789.854		

Analysis of Variance for effects of chronic BUP on **active lever responding** on an FR1 schedule after pumps removed (Experiment 1)

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
SESSION	17004.929	9	1889.437	1.798	.095
SESSION * GROUP	19125.043	9	2125.005	2.022	.059
Error(SESSION)	47282.100	45	1050.713		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
GROUP	181633.219	1	181633.219	4.360	.091
Error	208311.067	5	41662.213		

Analysis of Variance for effects of chronic BUP on **number of pellets obtained** on an FR1 schedule during training (Experiment 2)

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
SESSION	9495.848	7	1356.550	.543	.800
SESSION * BUP	4575.205	7	653.601	.261	.967
Error(SESSION)	210044.321	84	2500.528		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
BUP	27625.723	1	27625.723	.974	.343
Error	340459.964	12	28371.664		

Analysis of Variance for effects of chronic BUP on **number of pellets obtained** on an FR1 schedule after pumps implanted (Experiment 2)

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
SESSION	8383.778	8	1047.972	2.775	.008
SESSION * BUP	3011.397	8	376.425	.997	.444
Error(SESSION)	36255.048	96	377.657		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
BUP	9603.175	1	9603.175	1.161	.302
Error	99276.095	12	8273.008		

Analysis of Variance for effects of chronic BUP on **number of pellets obtained** on an FR1 schedule after pumps removed (Experiment 2)

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
SESSION	10885.864	9	1209.540	1.344	.223
SESSION * BUP	21303.179	9	2367.020	2.631	.009
Error(SESSION)	97178.057	108	899.797		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
BUP	12164.464	1	12164.464	.819	.383
Error	178168.229	12	14847.352		

Independent samples t-tests on **number of pellets obtained** across sessions on an FR1 schedule after pumps removed (Experiment 2)

Independent Samples Test

	t-test for Equality of Means				
	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
Session1	2.209	12	.047	45.86	20.76
Session2	2.634	12	.022	46.57	17.68
Session3	1.321	12	.211	24.43	18.50
Session4	2.102	12	.057	38.57	18.35
Session5	1.661	12	.123	43.86	26.41
Session6	.898	12	.387	21.00	23.38
Session7	-.594	12	.564	-16.29	27.43
Session8	.272	12	.790	8.43	31.00
Session9	-.098	12	.924	-3.86	39.46
Session10	-.890	12	.391	-22.14	24.89

Analysis of Variance for effects of chronic BUP on **active lever responding** on an FR1 schedule during training (Experiment 2)

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
SESSION	62042.920	7	8863.274	1.232	.295
SESSION * BUP	19516.991	7	2788.142	.387	.907
Error(SESSION)	604447.214	84	7195.800		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
BUP	34968.223	1	34968.223	.555	.471
Error	756156.214	12	63013.018		

Analysis of Variance for effects of chronic BUP on **active lever responding** on an FR1 schedule after pumps implanted (Experiment 2)

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
SESSION	17093.571	8	2136.696	2.300	.027
SESSION * BUP	6741.635	8	842.704	.907	.514
Error(SESSION)	89165.683	96	928.809		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
BUP	131402.865	1	131402.865	4.105	.066
Error	384098.603	12	32008.217		

Analysis of Variance for effects of chronic BUP on **active lever responding** on an FR1 schedule after pumps removed (Experiment 2)

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
SESSION	136454.436	9	15161.604	3.482	.001
SESSION * BUP	121930.721	9	13547.858	3.111	.002
Error(SESSION)	470267.743	108	4354.331		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
BUP	111841.779	1	111841.779	1.006	.336
Error	1333977.114	12	111164.760		

Independent samples t-tests on **active lever responding** on an FR1 schedule after pumps removed (Experiment 2)

Independent Samples Test

	t-test for Equality of Means				
	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
Session1	2.383	12	.035	115.29	48.38
Session2	2.182	12	.050	143.00	65.55
Session3	1.788	12	.099	86.29	48.27
Session4	2.468	12	.030	112.71	45.67
Session5	1.643	12	.126	98.14	59.73
Session6	.816	12	.431	50.14	61.48
Session7	-.115	12	.910	-8.43	73.20
Session8	.009	12	.993	.86	93.09
Session9	-.089	12	.931	-7.71	86.78
Session10	-.456	12	.656	-25.00	54.80

Analysis of Variance for **effect of experiment** (Experiment 1 vs. Experiment 2) on number of pellets obtained on an FR1 schedule during the last three sessions of training (i.e., all subjects in Experiment 1 vs. all subjects in Experiment 2)

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
SESSION	1433.333	2	716.667	1.730	.191
SESSION * EXP#	1186.476	2	593.238	1.432	.251
Error(SESSION)	15739.238	38	414.190		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
EXP#	132.071	1	132.071	.021	.887
Error	120560.690	19	6345.299		

Analysis of Variance for **effect of experiment** (Experiment 1 vs. Experiment 2) on number of pellets obtained on an FR1 schedule after first sham surgery (i.e., SHAM groups only)

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
SESSION	6827.284	8	853.410	1.593	.144
SESSION * EXP#	2855.328	8	356.916	.666	.719
Error(SESSION)	34276.561	64	535.571		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
EXP#	4749.215	1	4749.215	.666	.438
Error	57050.296	8	7131.287		

Analysis of Variance for **effect of experiment** (Experiment 1 vs. Experiment 2) on number of pellets obtained on an FR1 schedule after second sham surgery (i.e., SHAM groups only)

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
SESSION	8114.471	9	901.608	1.141	.346
SESSION * EXP#	7114.391	9	790.488	1.000	.448
Error(SESSION)	56899.219	72	790.267		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
EXP#	251.680	1	251.680	.012	.915
Error	167884.210	8	20985.526		

Analysis of Variance for **effect of experiment** (Experiment 1 vs. Experiment 2) on number of pellets obtained on an FR1 schedule after pumps implanted (i.e., BUP groups only)

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
SESSION	6816.234	8	852.029	2.294	.030
SESSION * EXP#	4635.506	8	579.438	1.560	.152
Error(SESSION)	26737.746	72	371.358		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
EXP#	3829.208	1	3829.208	.414	.536
Error	83217.540	9	9246.393		

Analysis of Variance for **effect of experiment** (Experiment 1 vs. Experiment 2) on number of pellets obtained on an FR1 schedule after pumps removed (i.e., BUP groups only)

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
SESSION	14234.138	9	1581.571	2.139	.035
SESSION * EXP#	4517.992	9	501.999	.679	.726
Error(SESSION)	59890.971	81	739.395		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
EXP#	27194.514	1	27194.514	5.611	.042
Error	43616.886	9	4846.321		

Independent samples t-tests (Experiment 1 vs. Experiment 2) on **number of pellets obtained** after pumps removed (BUP groups only)

Independent Samples Test

	t-test for Equality of Means				
	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
OUT1	-1.130	9	.288	-21.00	18.59
OUT2	-1.371	9	.204	-22.96	16.75
OUT3	-1.827	9	.101	-36.43	19.93
OUT4	-.943	9	.370	-18.50	19.62
OUT5	-1.044	9	.324	-21.18	20.29
OUT6	-1.408	9	.193	-28.43	20.19
OUT7	-2.413	9	.039	-63.68	26.39
OUT8	-1.281	9	.232	-36.93	28.82
OUT9	-2.469	9	.036	-46.71	18.92
OUT10	-1.542	9	.158	-31.04	20.13

Analysis of Variance for effects of chronic BUP on **number of pellets obtained** during training on an FR1 schedule (data from Experiments 1 & 2 combined)

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
SESSION	22077.051	7	3153.864	1.532	.162
SESSION * BUP	10422.575	7	1488.939	.723	.652
Error(SESSION)	273719.068	133	2058.038		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
BUP	10100.121	1	10100.121	.410	.530
Error	468357.950	19	24650.418		

Analysis of Variance for effects of chronic BUP on **number of pellets obtained** on an FR1 schedule after pumps implanted (data from Experiments 1 & 2 combined)

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
SESSION	7394.150	8	924.269	2.051	.044
SESSION * BUP	6963.610	8	870.451	1.931	.059
Error(SESSION)	68505.141	152	450.692		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
BUP	34156.609	1	34156.609	4.360	.050
Error	148846.259	19	7834.014		

Analysis of Variance for effects of chronic BUP on **number of pellets obtained** on an FR1 schedule after pumps removed (data from Experiments 1 & 2 combined)

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
SESSION	9486.169	9	1054.019	1.403	.190
SESSION * BUP	19510.988	9	2167.888	2.887	.003
Error(SESSION)	128422.574	171	751.009		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
BUP	45553.624	1	45553.624	3.622	.072
Error	238947.290	19	12576.173		

Independent samples t-test on **number of pellets obtained** on an FR1 schedule after pumps removed (Experiment 1 & Experiment 2)

Independent Samples Test

	t-test for Equality of Means				
	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
Session1	3.271	19	.004	51.54	15.76
Session2	3.117	19	.006	47.64	15.28
Session3	2.565	19	.019	39.52	15.41
Session4	3.348	19	.003	46.23	13.81
Session5	2.570	19	.019	48.27	18.78
Session6	1.581	19	.130	29.31	18.54
Session7	.767	19	.453	17.93	23.38
Session8	.635	19	.533	13.80	21.74
Session9	.266	19	.793	7.27	27.30
Session10	-.369	19	.716	-6.60	17.90

Analysis of Variance for effects of chronic BUP on **active lever responding** on an FR1 schedule during training (Experiments 1 & 2 combined)

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
SESSION	95569.871	7	13652.839	2.520	.018
SESSION * BUP	37180.395	7	5311.485	.980	.448
Error(SESSION)	720692.891	133	5418.744		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
BUP	8629.133	1	8629.133	.149	.703
Error	1097297.200	19	57752.484		

Analysis of Variance for effects of chronic BUP on **active lever responding** on an FR1 schedule after pumps implanted (Experiments 1 & 2 combined)

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
SESSION	16773.842	8	2096.730	2.217	.029
SESSION * BUP	12112.678	8	1514.085	1.601	.129
Error(SESSION)	143733.259	152	945.614		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
BUP	359064.935	1	359064.935	9.729	.006
Error	701229.414	19	36906.811		

Analysis of Variance for effects of chronic BUP on **active lever responding** on an FR1 schedule after pumps removed (Experiments 1 & 2 combined)

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
SESSION	92487.628	9	10276.403	2.888	.003
SESSION * BUP	101782.371	9	11309.152	3.178	.001
Error(SESSION)	608465.305	171	3558.277		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
BUP	290826.723	1	290826.723	3.344	.083
Error	1652424.458	19	86969.708		

Independent samples t-test for **active lever responding** on an FR1 schedule after pumps removed (Experiments 1 & 2 combined)

Independent Samples Test

		t-test for Equality of Means				
	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
OUT1	.009	3.312	19	.004	114.60	34.60
OUT2	.009	2.820	19	.011	132.19	46.88
OUT3	.028	2.932	19	.009	110.97	37.85
OUT4	.040	3.646	19	.002	117.37	32.19
OUT5	.058	2.400	19	.027	106.51	44.37
OUT6	.510	1.412	19	.174	62.77	44.47
OUT7	.935	.779	19	.446	42.55	54.65
OUT8	.967	.364	19	.720	23.66	64.94
OUT9	.995	.290	19	.775	18.56	63.91
OUT10	.964	.396	19	.697	15.93	40.23

Analysis of Variance for effects of chronic BUP on **number of pellets obtained** on an FR5 schedule (Experiment 2)

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
SESSION	16070.399	2	8035.200	7.721	.003
SESSION * BUP	8649.784	2	4324.892	4.156	.029
Error(SESSION)	22895.857	22	1040.721		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
BUP	601.984	1	601.984	.089	.771
Error	74315.452	11	6755.950		

Analysis of Variance for effects of chronic BUP on **active lever responding** on an FR5 schedule (Experiment 2)

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
SESSION	513570.349	2	256785.175	7.619	.003
SESSION * BUP	234757.426	2	117378.713	3.483	.049
Error(SESSION)	741508.317	22	33704.924		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
BUP	7474.360	1	7474.360	.037	.850
Error	2195939.897	11	199630.900		

Paired samples t-tests for **number of pellets obtained** on an FR5 schedule (SHAM group only; Experiment 2)

Paired Samples Test

		Paired Differences			t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean			
Pair 1	FR5TRAIN - FR5PUMP	-5.43	35.37	13.37	-.406	6	.699
Pair 2	FR5TRAIN - FR5OUT	-69.17	22.34	9.12	-7.585	5	.001
Pair 3	FR5PUMP - FR5OUT	-64.33	45.52	18.58	-3.462	5	.018

Independent samples t-tests for **number of pellets obtained** on an FR5 schedule (BUP vs. SHAM; Experiment 2)

Independent Samples Test

		t-test for Equality of Means				
		t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
FR5TRAIN	Equal variances assumed	-1.221	12	.245	-38.86	31.81
FR5PUMP	Equal variances assumed	.153	12	.881	3.86	25.14
FR5OUT	Equal variances assumed	1.378	11	.196	42.43	30.79

Paired samples t-tests for **active lever responding** on an FR5 schedule (SHAM group only; Experiment 2)

Paired Samples Test

		Paired Differences			t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean			
Pair 1	FR5TRAIN - FR5PUMP	-30.14	182.18	68.86	-.438	6	.677
Pair 2	FR5TRAIN - FR5OUT	-368.00	129.68	52.94	-6.951	5	.001
Pair 3	FR5PUMP - FR5OUT	-340.33	234.79	95.85	-3.551	5	.016

Independent samples t-tests for **number of pellets obtained** on an FR5 schedule (BUP vs. SHAM; Experiment 2)

Independent Samples Test

	t-test for Equality of Means				
	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
FR5TRAIN	-1.226	12	.244	-223.57	182.36
FR5PUMP	.216	12	.832	28.14	130.03
FR5OUT	1.170	11	.267	194.31	166.10

Analysis of Variance for effects of chronic BUP on **number of pellets obtained** on a PR schedule (Experiment 2)

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
SESSION	11.476	2	5.738	1.215	.314
SESSION * BUP	7.190	2	3.595	.761	.478
Error(SESSION)	113.333	24	4.722		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
BUP	16.095	1	16.095	.549	.473
Error	351.810	12	29.317		

Analysis of Variance for effects of chronic BUP on **active lever responding** on a PR schedule (Experiment 2)

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
SESSION	27999.476	2	13999.738	2.023	.154
SESSION * BUP	18264.333	2	9132.167	1.320	.286
Error(SESSION)	166079.524	24	6919.980		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
BUP	1710.095	1	1710.095	.043	.839
Error	475064.190	12	39588.683		

Analysis of Variance for effects of chronic BUP on the **ratio of active lever responses made to the number of active lever responses required** for the amount of pellets taken per session (Experiment 2):

FR1 schedule data
(after pumps implanted)

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
SESSION	.125	8	1.563E-02	1.624	.122
SESSION * GROUP	6.244E-02	8	7.805E-03	.811	.594
Error(SESSION)	1.463	152	9.622E-03		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
GROUP	5.148	1	5.148	9.460	.006
Error	10.340	19	.544		

FR1
(after pumps removed)

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
SESSION	1.285	9	.143	4.062	.000
SESSION * GROUP	.845	9	9.389E-02	2.670	.007
Error(SESSION)	5.380	153	3.516E-02		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
GROUP	2.697	1	2.697	2.799	.113
Error	16.379	17	.963		

Independent samples t-tests for the **ratio of active lever responses made to the number of active lever responses required** for the amount of pellets taken per session (Experiment 2):

(after pumps removed)

Independent Samples Test

	t-test for Equality of Means				
	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
SESS1	2.642	19	.016	.2860	.1082
SESS2	2.320	19	.032	.3555	.1532
SESS3	2.809	19	.011	.3243	.1155
SESS4	2.726	19	.013	.3357	.1232
SESS5	1.169	19	.257	.1775	.1519
SESS6	.902	19	.378	.1510	.1674
SESS7	.629	19	.537	.1078	.1714
SESS8	.235	19	.817	3.862E-02	.1641
SESS9	-.136	19	.893	-2.6059E-02	.1918
SESS10	1.221	19	.237	.1941	.1590

Analysis of Variance for effects of chronic BUP on the **ratio of active lever responses made to the number of active lever responses required** for the amount of pellets taken per session (Experiment 2):

FR5

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
SESSION	1.246E-02	2	6.229E-03	4.618	.021
SESSION * GROUP	6.164E-03	2	3.082E-03	2.285	.125
Error(SESSION)	2.967E-02	22	1.349E-03		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
GROUP	2.593E-03	1	2.593E-03	1.626	.228
Error	1.754E-02	11	1.595E-03		

Paired t-tests for the ratio **of active lever responses made to the number of active lever responses required** across FR5 sessions (Experiment 2):

Paired Samples Test

		Paired Differences			t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean			
Pair 1	FR5SES1 - FR5SES2	1.566E-02	4.252E-02	1.136E-02	1.378	13	.192
Pair 2	FR5SES1 - FR5SES3	-2.9167E-02	6.598E-02	1.830E-02	-1.594	12	.137
Pair 3	FR5SES2 - FR5SES3	-4.5687E-02	5.156E-02	1.430E-02	-3.195	12	.008

Analysis of Variance for effects of chronic BUP on the **ratio of active lever responses made to the number of active lever responses required** for the amount of pellets taken per session (Experiment 2):

PR

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
SESSION	9.490E-03	2	4.745E-03	.631	.541
SESSION * GROUP	6.649E-03	2	3.325E-03	.442	.648
Error(SESSION)	.181	24	7.525E-03		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
GROUP	3.967E-03	1	3.967E-03	1.233	.289
Error	3.861E-02	12	3.217E-03		

Analysis of Variance for effects of chronic BUP on **active lever responding during extinction** (Experiment 3)

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
SESSION	50636.857	6	8439.476	24.173	.000
SESSION * GROUP	3200.286	6	533.381	1.528	.181
Error(SESSION)	25136.857	72	349.123		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
GROUP	1568.000	1	1568.000	1.906	.193
Error	9872.000	12	822.667		

Independent samples t-test for effects of chronic BUP on **active lever responding in test of reinstatement** (Experiment 3)

Independent Samples Test

	t-test for Equality of Means				
	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
Reinstatement responding	2.241	12	.045	68.71	30.66

Independent samples t-test for effects of chronic BUP on the **percentage of lab chow pellets eaten during dialysis testing** (Experiment 4)

Independent Samples Test

	t-test for Equality of Means				
	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
% of lab chow eaten	1.223	10	.249	13.9143	11.3785

Independent samples t-test for effects of chronic BUP on the **percentage of sucrose pellets eaten during dialysis testing** (Experiment 4)

Independent Samples Test

	t-test for Equality of Means				
	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
% of sucrose pellets eaten	2.291	7	.056	35.8333	15.6407

Analysis of Variance for effects of chronic BUP on the **increase in extracellular DA following lab chow pellets** (Experiment 4)

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
SAMPLE	2215.606	5	443.121	2.494	.043
SAMPLE * GROUP	1872.331	5	374.466	2.108	.080
Error(SAMPLE)	8883.975	50	177.680		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
GROUP	103.037	1	103.037	.090	.770
Error	11458.603	10	1145.860		

Analysis of Variance for effects of chronic BUP on the **increase in extracellular DOPAC following lab chow pellets** (Experiment 4)

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
SAMPLE	1088.675	5	217.735	1.794	.131
SAMPLE * GROUP	184.793	5	36.959	.304	.908
Error(SAMPLE)	6069.345	50	121.387		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
GROUP	245.745	1	245.745	.411	.536
Error	5974.459	10	597.446		

Analysis of Variance for effects of chronic BUP on the **increase in extracellular HVA following lab chow pellets** (Experiment 4)

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
SAMPLE	568.111	5	113.622	.895	.492
SAMPLE * GROUP	178.255	5	35.651	.281	.922
Error(SAMPLE)	6348.537	50	126.971		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
GROUP	6.243	1	6.243	.008	.931
Error	7878.220	10	787.822		

Analysis of Variance for effects of chronic BUP on the **increase in extracellular HIAA following lab chow pellets** (Experiment 4)

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
SAMPLE	821.934	5	164.387	1.290	.283
SAMPLE * GROUP	369.713	5	73.943	.580	.715
Error(SAMPLE)	6370.589	50	127.412		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
GROUP	1072.235	1	1072.235	1.018	.337
Error	10536.765	10	1053.677		

Analysis of Variance for effects of chronic BUP on the **increase in extracellular DA following sucrose pellets** (Experiment 4)

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
SAMPLE	2799.330	5	559.866	2.727	.031
SAMPLE * GROUP	144.548	5	28.910	.141	.982
Error(SAMPLE)	9238.020	45	205.289		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
GROUP	48.297	1	48.297	.058	.815
Error	7490.431	9	832.270		

Analysis of Variance for effects of chronic BUP on the **increase in extracellular DOPAC following sucrose pellets** (Experiment 4)

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
SAMPLE	1194.464	5	238.893	4.644	.002
SAMPLE * GROUP	224.062	5	44.812	.871	.508
Error(SAMPLE)	2314.921	45	51.443		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
GROUP	9.362	1	9.362	.014	.907
Error	5820.453	9	646.717		

Analysis of Variance for effects of chronic BUP on the **increase in extracellular HVA following sucrose pellets** (Experiment 4)

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
SAMPLE	264.407	5	52.881	1.069	.390
SAMPLE * GROUP	299.742	5	59.948	1.211	.319
Error(SAMPLE)	2226.744	45	49.483		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
GROUP	125.118	1	125.118	.170	.690
Error	6629.631	9	736.626		

Analysis of Variance for effects of chronic BUP on the **increase in extracellular HIAA following sucrose pellets** (Experiment 4)

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
SAMPLE	813.104	5	162.621	1.714	.151
SAMPLE * GROUP	235.117	5	47.023	.496	.778
Error(SAMPLE)	4270.376	45	94.897		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
GROUP	44.992	1	44.992	.032	.861
Error	12537.916	9	1393.102		

Analysis of Variance for effects of chronic BUP on **basal levels of DA in the NAc** (Experiment 4)

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
SESSION	9.895E-02	3	3.298E-02	.584	.631
SESSION * GROUP	3.497E-02	3	1.166E-02	.206	.891
Error(SESSION)	1.526	27	5.651E-02		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
GROUP	43.745	1	43.745	7.155	.025
Error	55.027	9	6.114		