The Effects of Chronic Administration of Buprenorphine on Intake of Heroin and Cocaine in Rats: Behavioral and Neurochemical Interactions

Robert E. Sorge

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ABSTRACT

The Effects of Chronic Administration of Buprenorphine on Intake of Heroin and Cocaine in Rats: Behavioral and Neurochemical Interactions

Robert E. Sorge, Ph.D.
Concordia University, 2006

Buprenorphine is a mu opioid receptor agonist used in the treatment of opioid abuse. Buprenorphine effectively reduces opioid intake in opioid abuse patients, but there is evidence that it is effective in reducing cocaine intake in a subset of individuals as well. Chronic administration of buprenorphine was achieved via the use of subcutaneously-implanted, buprenorphine-filled, osmotic minipumps in male rats. Chronic buprenorphine reduced heroin and cocaine seeking under extinction conditions and in tests of drug-induced reinstatement in rats trained to self-administer both drugs. The reduction in responding for drug in extinction and in tests for reinstatement was not due to locomotor sedation, as chronic buprenorphine slightly elevated locomotor activity. Furthermore, this common reduction cannot be explained via unique interactions with the abused drugs. Chronic buprenorphine had no effect on locomotor activity following acute injections of heroin, in spite of a blockade in the nucleus accumbens (NAc) dopamine (DA) response to this drug. On the other hand, chronic buprenorphine potentiated the locomotor and NAc DA responses to acute injections of cocaine. The interactions between buprenorphine and heroin and cocaine on NAc DA were further replicated during self-administration of heroin and cocaine. Buprenorphine had no effect on heroin self-administration at any dose or under any schedule of reinforcement

although the NAc DA response to infusions was completely blocked. In contrast, cocaine self-administration was reduced under all schedules and doses in spite of a potentiated NAc DA response to infusions of cocaine. In all cases, buprenorphine levels in plasma and basal NAc DA levels were increased throughout chronic treatment, suggesting continuous receptor occupation. Although the mechanisms for the variable effects of buprenorphine on self-administration are unclear, a mechanism for the common reduction in responsiveness to drug-associated cues is proposed. The elevated basal levels of DA may have reduced the impact of the firing of DA neurons in response to cues; alternatively buprenorphine may have had its effect by reducing glutamatergic activity. There is reason to believe that buprenorphine could reduce glutamatergic activity and it is known that a reduction in glutamate transmission can disrupt responding for cues associated with drugs of abuse.

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CONTRIBUTION OF AUTHORS

Dr. Jane Stewart, the Ph.D. supervisor, was involved in the design of the experiments, analysis of the data and contributed in great part to the writing of the manuscripts for publication (all chapters).

Mr. Heshmat Rajabi is a laboratory technician in Dr. Jane Stewart's lab and is responsible for the maintenance and utter perfection of the *in vivo* microdialysis protocols and equipment used for these experiments. He made the probes and assisted with data collection and analysis in Chapter 1.

I, Robert E Sorge, was critically involved in the design of the experiments, data collection, analysis and presentation, and the writing and editing of the manuscripts for publication.

LIST OF ABBREVIATIONS

aCSF: artificial cerebrospinal fluid

ANOVA: analysis of variance

BLA: basolateral amygdala

CPP: conditioned place preferences

CS: conditioned stimulus

DA: dopamine

DAMGO: [D-Ala^2, N-Me-Phe^4-Gly^5-ol]-enkephalin

DAT: dopamine transporter

DOPAC: dihydroxyphenylacetic acid

DPDPE: [D-Pen^2, D-Pen^5]-enkephalin

ELISA: enzyme linked immunosorbant assay

FR: fixed ratio

GABA: gamma aminobutyric acid

GluR1: glutamate receptor 1 subunit

HCl: hydrochloride

HIAA: 5-hydroxyindole acetic acid

HPLC-EC: high performance liquid chromatography with electrochemical detection

HVA: homovannilic acid

im: intramuscular

ip: intraperitoneal

LSD: least square difference

mGluR: metabotropic glutamate receptor

MW: molecular weight

NAc: nucleus accumbens

OD: outer diameter

PFC: prefrontal cortex

PR: progressive ratio

sc: subcutaneous

SEM: standard error of the mean

SNc: substantia nigra pars compacta

VTA: ventral tegmental area

GENERAL INTRODUCTION

Psychoactive compounds have been used by cultures around the globe for centuries for both recreational and medicinal purposes. In fact, a metabolite of cocaine has been found in the mummified remains of Andean natives dating from around 1500 AD (Cartmell, Aufderhide, & Weems, 1991) and tetrahydrocannibinol has been found in Egyptian mummies (Balabanova, Parsche, & Pirsig, 1992; Nerlich, Parsche, Wiest, Schramel, & Lohrs, 1995). The medicinal properties of the opium poppy were well known in ancient populations (Hamilton & Baskett, 2000) and, although the use of these compounds was not regulated, it is likely that a small portion of those using the drug would lose control over their intake (Lundberg, Garriott, Reynolds, Cravey, & Shaw, 1977).

This loss of control has been termed "substance abuse" and refers to a pattern of use that leads to significant impairment in functioning. Substance abuse is recognized by the American Psychiatric Association as a treatable disorder and has been described in the Diagnostics and Statistical Manual (DSM) as such since the DSM-III in 1980. This disorder is characterized by recurrent drug use that (a) interferes with social obligations (i.e. family, work), (b) results in legal problems (i.e. incarceration), (c) is done in physically hazardous situations (i.e. driving while intoxicated), and/or (d) is continued despite significant social or interpersonal problems caused by the use (i.e. divorce) (American Psychiatric Association, 2000). Frequent drug use can lead to substance dependence characterized by the presence of tolerance to the effects of the drug and withdrawal symptoms upon termination of drug taking.

Substance Abuse Research

It is known that a minority of individuals who use an illicit substance become addicts and meet the diagnosis for clinical substance abuse. In the case of cocaine, it has been estimated that only 15-16% of users develop cocaine dependence within 10 years of the first episode (Wagner & Anthony, 2002). Although seemingly low, consider that there are approximately 13.7 million cocaine users worldwide with an equally startling 10.6 million heroin users (United Nations Office on Drugs and Crime, 2005). Of course, some individuals may be represented in both figures and not all of these users meet the criterion for substance abuse or dependence, but the number that do each year is sizable. For this reason a research field has emerged with the aim of understanding the development, maintenance and treatment of substance abuse. Research in this field is being done in both human patients and in laboratory animals. The mutual exchange of ideas and findings between animal and clinical researchers is one that has resulted in a number of advances in the development of therapies and our understanding of the mechanisms underlying effective treatments.

Many approaches are used to reduce abuse of psychoactive compounds. Some drugs are criminalized to reduce their availability to the general population and others are regulated by governments and health warnings are provided. Despite these attempts to reduce availability and dissuade the use of drugs, some individuals persist in their drug taking and become addicted. At this point there are many outlets for the treatment of substance abuse, involving counseling, support groups and many forms of cognitive and behavioral therapies. Primarily for illicit substance abuse, some specific behavioral therapies involve relapse prevention, motivational enhancement and contingency

management. There are, however, pharmacological approaches to the treatment for substance abuse that are generally referred to as substitution therapy. The focus of this thesis is on pharmacological therapy for substance abuse and, in particular, the use of buprenorphine for the treatment of heroin (and/or cocaine) abuse.

The pharmacological agents for the treatment of substance abuse and dependence often provide a constant/steady level of drug to a dependent nervous system and thus allow individuals to avoid, or alleviate, symptoms that accompany the withdrawal from drug use. Opioid abuse treatment drugs like methadone and buprenorphine are effective when used alone; however, a combination of drugs and behavioral therapy often results in the greatest probability of success for the treatment of opioid abuse and dependence (McLellan, Arndt, Metzger, Woody, & O'Brien, 1993; Rawson et al., 2002; Kakko, Svanborg, Kreek, & Heilig, 2003). To date, however, there are no substitution treatment drugs for cocaine addiction. Thus, individuals who are dependent on cocaine or other psychostimulants must rely on behavioral therapies for treatment. Interestingly, however, there is evidence that opioid abuse treatment drugs are effective in reducing concurrent cocaine intake as well as opioid intake in a subset of patients (Montoya et al., 2004).

Research Approaches to the Study of Substance Abuse and Ultimate Treatment

The study of the development and treatment of substance abuse can be separated into three primary approaches reflecting the distinct aims of the research. One approach seeks to characterize the changes in morphology and/or intracellular mechanisms that arise from drug exposure. This approach is not directly linked to treatment, but is aimed at understanding the alterations involved in addiction, perhaps to provide insight for

future treatments. Another approach is to try to understand the development and control of drug taking behavior through learning and conditioned stimuli associated with drugs. For example, drugs such as 'cognitive enhancers' are being studied for their potential to enhance the extinction of responses to drug related cues whereas protein synthesis inhibitors are being used to understand the maintenance of drug-related memories with the hope of finding pharmacological agents that might induce forgetting. These findings provide insight into possible treatments, but are not likely to be utilized in the immediate future. The final approach is to investigate currently used pharmacological treatments for substance abuse. Research is focused on the examination of effective treatments such as substitution therapy in human abusers with the aim of understanding the mechanisms involved such that further improvements can be made in the future. This approach is the focus of this thesis; however, a brief overview of each approach will follow to provide perspective on the state of the substance abuse research field.

Characterization and Identification of Changes Resulting from Drug Exposure

In the 1970s it was found that acute administration of morphine to cultured locus ceruleus cells initially decreased the expression of adenylate cyclase (AC), but this decrease could be reversed by administration of the opioid receptor antagonist naloxone (Sharma, Klee, & Nirenberg, 1975). Adenylate cyclase is the enzyme that synthesizes the intracellular "second messenger" cyclic adenosine monophosphate (cAMP) from adenosine triphosphate (ATP) and, thus, this was one of the earliest demonstrations of a drug that could reverse the alterations seen following exposure to a drug of abuse. More recently researchers have been investigating the role of factors that are mediated by the

effects of cAMP, with particular attention spent on cAMP response element binding protein (CREB). CREB proteins are transcription factors that modulate transcription of certain genes and are activated by protein kinases, as a result of cAMP activation. It has been found that CREB is upregulated in the nucleus accumbens (NAc) of mice and rats treated with various stimulants (Cole, Konradi, Douglass, & Hyman, 1995; Shaw-Lutchman, Impey, Storm, & Nestler, 2003) and that artificial alterations in CREB in the NAc can increase (via overexpression of dominant-negative mutant CREB) or decrease (via overexpression of CREB) the reinforcing effects of cocaine as measured in the place conditioning paradigm in rats (Carlezon, Jr. et al., 1998).

In addition to the intracellular changes discussed above, exposure to drugs of abuse leads to morphological changes in many areas of the brain. For instance, chronic exposure to morphine reduces the size, but not the number, of dopamine (DA) neurons in the ventral tegmental area (VTA) (Sklair-Tavron et al., 1996) and reduces dendritic branching and the density of the spines (Robinson & Kolb, 1999b; Robinson, Gorny, Savage, & Kolb, 2002). Repeated injections of stimulant drugs, such as amphetamine, *increase* dendritic arborization and spine density on medium spiny neurons in the NAc (Robinson & Kolb, 1997; Robinson & Kolb, 1999a). Much more recently exposure to amphetamine has been found to increase dendritic length of DA neurons in the VTA as well (Mueller, Chapman, & Stewart, 2006). Although tempting to speculate that an increase in spines is likely to lead to an increase in the number of synaptic connections explaining the persistence of abuse-related behaviors, there is a paradox. Opioids and stimulants are both abused, but exposure to these distinct classes of drugs results in

opposite changes in morphology, suggesting that these changes are not directly related to the persistence of addiction.

In addition to morphological changes, pretreatment with amphetamine increases the expression of basic fibroblast growth factor (BFGF) in the VTA and substantia nigra pars compacta (SNc) and this increase can be blocked by administration of a glutamate antagonist (Flores, Rodaros, & Stewart, 1998). The increase in BFGF, as a result of amphetamine exposure, can also be attenuated by administration of antibodies to BFGF, resulting in attenuation of both behavioral sensitization (Flores, Samaha, & Stewart, 2000) and the increase in dendritic length in the VTA (Mueller et al., 2006). Rats pretreated with amphetamine or cocaine do not show increased dendritic branching or spine density on NAc neurons following exposure to an enriched environment, suggesting that exposure to these drugs prevents a form of synaptic plasticity (Kolb, Gorny, Li, Samaha, & Robinson, 2003).

The findings discussed above provide insight into the changes that arise following exposure to drugs on a cellular and morphological level. What remains unknown, however, is how or whether treatment for substance abuse could arise from these findings. It remains unclear how changes in intracellular signaling may result in morphological changes and whether these changes are the cause or the result of addiction to drugs. It may be possible, however, to modulate learning processes involved in substance abuse and the following section outlines this potential approach to treatment.

Identification of Learning Processes Controlling Drug Seeking

Early in the study of learning and memory it was discovered that, although N-methyl-D-aspartate (NMDA) receptors are not necessary for synaptic transmission, they are critical for long-term potentiation (LTP) (Harris, Ganong, & Cotman, 1984; Coan & Collingridge, 1987; Coan, Saywood, & Collingridge, 1987; Brown, Chapman, Kairiss, & Keenan, 1988). LTP is a process whereby synaptic connections between two neurons are strengthened and is critical to learning. Later, it was found that antagonism of NMDA receptors impairs both acquisition (Miserendino, Sananes, Melia, & Davis, 1990; Flood, Baker, & Davis, 1990; Kim & McGaugh, 1992; Fanselow & Kim, 1994) and extinction (Falls, Miserendino, & Davis, 1992; Santini, Muller, & Quirk, 2001) of conditioned fear and avoidance, suggesting that NMDA receptor activation is critical to promoting learning. Thus, it follows that the partial NMDA agonist, D-cycloserine (DCS), enhances acquisition of water maze learning (Riekkinen & Riekkinen, Jr., 1997; Riekkinen, Jr., Ikonen, & Riekkinen, 1998), reduces errors and enhances retention in the delayed non-match to sample radial arm maze task (Pussinen & Sirvio, 1999) and enhances extinction of fear-potentiated startle responses (Walker, Ressler, Lu, & Davis, 2002). This drug has been recently used to treat phobic patients to facilitate the extinction of fear-related emotions (Ressler et al., 2004) and has potential for the treatment of substance abuse as well. In fact, research in our laboratory has found that administration of DCS immediately following an extinction trial enhanced the extinction of cocaine CPP that persisted for weeks following treatment (Botreau, Paolone, & Stewart, 2006). The possibility of enhancing the extinction of drug-associated behaviors is intriguing, but the effects of DCS on learning processes are relatively short-lived and so treatment would necessarily involve multiple administrations and pairings with the plethora of stimuli that

become associated with substance abuse. In this case, although the treatments may be successful and long-lasting, it is likely that they would be therapist intensive and thus costly, and appropriate only for highly motivated patients.

As opposed to enhancing new learning, it is possible to suppress, or eliminate, established memories. Memories return to their initially labile state when they are retrieved before being reconsolidated into long-term memory; a process requiring protein synthesis. Administration of the protein synthesis inhibitor, anisomycin, following retrieval of a fear memory results in less freezing to a tone previously paired with footshock, suggesting amnesia for the memory (Nader, Schafe, & Le Doux, 2000). Very recently it has been reported that administration of the protein synthesis inhibitors, anisomycin or cycloheximide, persistently inhibited the expression of an established morphine conditioned place preference when administered following a reconditioning trial in rats (Milekic, Brown, Castellini, & Alberini, 2006). This research provides insights into the mechanisms whereby drug-associated memories are reinforced over time, but the use of protein synthesis inhibitors is highly unlikely to be a viable treatment. These findings may enable more effective approaches to treatment of substance abuse, however, in the interim, the following approaches are successful treatments for substance abuse.

Current Pharmacological Treatment Approaches

The most traditional approach to substance abuse treatment is to develop drugs based on the pharmacology of the drug of abuse. The development of such drugs has followed three basic strategies. One strategy relies on developing antibodies to bind to

and break down the abused drug prior to crossing the blood-brain barrier, thereby reducing the drug's impact on the central nervous system. Similarly, another strategy involves the use of antagonists to compete at the receptor with the drug of abuse to reduce available binding sites and allow metabolism of the abused drug. Finally, the most common strategy is to administer a controlled dose of an agonist to replace the drug of abuse in a system that has been chronically exposed to the abused drug and thereby reduce the need to administer the abused drug. These treatment drugs have low efficacy, high affinity and are slow to dissociate from their receptors. Administration of these drugs is meant to maintain a constant/steady amount of receptor occupation in order to avoid withdrawal effects and craving.

Antibody Development. In the early 1970s researchers investigated the use of vaccines to attenuate the effects of abused drugs. It was shown that monkeys reduced their intake of heroin following immunization to the primary metabolite of morphine (Bonese, Wainer, Fitch, Rothberg, & Schuster, 1974). Although promising, this line of research was discontinued due to potential complications with pain management drugs in these immunized subjects. Vaccines have been developed, however, to counter the effects of cocaine (Carrera et al., 1995) and, more recently, nicotine (Pentel et al., 2000). When rats or mice are either actively or passively immunized against cocaine they show a reduction in cocaine intake (Carrera et al., 2000), in locomotion induced by cocaine (Carrera et al., 1995; Carrera, Ashley, Wirsching, Koob, & Janda, 2001) and in levels of cocaine itself in the striatum (Carrera et al., 1995; Fox et al., 1996). In addition, these vaccinations have been found to be effective in human cocaine abusers, with inoculated

patients having fewer cocaine-positive drug tests following treatment (Kosten et al., 2002; Martell, Mitchell, Poling, Gonsai, & Kosten, 2005). The advantage of this method, if it were to come into practice, is that few inoculations are required to induce a significant amount of antibodies in the system to reduce cocaine-positive urine tests and the subjective effects of cocaine for up to 6 months following treatment (Martell et al., 2005). Though promising, this approach is apt to be costly and effective only in highly dedicated patients.

Receptor Antagonists. A direct strategy for combating or reducing substance abuse would be to block at least some of the reinforcing effects of the abused drug. This blockade should, in time, extinguish the effectiveness of stimuli associated with the drug to elicit responding. The current state of antagonist treatment is, however, not so simple. Antagonists developed to reduce intake of a specific drug are often found to be problematic, in that there is a risk of overdose in patients attempting to overcome the receptor blockade induced by the antagonist treatment. Surprisingly, however, these treatments are sometimes more effective as treatments for another class of drugs altogether. For example, the opioid antagonists naltrexone and naloxone successfully prevent opioid overdose (O'Brien, Testa, O'Brien, & Greenstein, 1976; O'Brien, Testa, O'Brien, Brady, & Wells, 1977), but there is little evidence that naltrexone is an effective treatment strategy for all, but highly motivated and detoxified, opioid abusers (Crabtree, 1984; San, Pomarol, Peri, Olle, & Cami, 1991; Kirchmayer et al., 2002). Naltrexone is, however, very effective in reducing alcohol intake in alcoholics (Volpicelli, Volpicelli, & O'Brien, 1995; Volpicelli, Clay, Watson, & O'Brien, 1995; Volpicelli, Watson, King,

Sherman, & O'Brien, 1995) and may have utility as a smoking cessation aid as it has been shown to reduce subjective effects of nicotine in smokers (Brauer, Behm, Westman, Patel, & Rose, 1999; Rukstalis et al., 2005). Similarly, the cannabinoid receptor 1 (CB1) antagonist, rimonabant, though ineffective in altering tetrahydrocannabinol pharmacokinetics, is effective in reducing nicotine self-administration and nicotine seeking in rats (Cohen, Kodas, & Griebel, 2005).

Replacement or Substitution. As mentioned, the most widely used strategy for pharmacological treatment of substance abuse is to develop drugs that are pharmacologically related to the particular drug of abuse and can be delivered in low, controlled doses. This method has been used successfully for the treatment of nicotine (as in the nicotine patch, which allows for slow release into the blood stream) and for opioid abuse (e.g. methadone or buprenorphine). These latter two drugs have long half-lives and, due to their particular pharmacokinetics, have slow dissociation from, low efficacy at, and high affinity for the mu-opioid receptor (Lee, Akil, Woods, & Traynor, 1999). This form of treatment was developed based on the assumption that chronic drug use leads to alterations that require the presence of the drug to maintain a 'normalized' system and avoid withdrawal effects. Therefore, administration of these drugs can be used to alleviate the patients' need for abused substances. The goal of this treatment strategy is to slowly wean the patient off of the abused drug, though, in the case of opioid abuse, most patients continue substitution therapy for many years.

Aim of the Research Presented in this Thesis

The aim of the studies in this thesis is to determine the mechanism via which the opioid-treatment drug buprenorphine acts to reduce the intake of the abused drugs, heroin and cocaine. Buprenorphine is effective in reducing opioid intake in human patients (Johnson, Jaffe, & Fudala, 1992; Ling et al., 1998) and, from the time buprenorphine was first administered as a substitution treatment to heroin addicts (Mello & Mendelson, 1980), it has been shown to be as effective as methadone (Strain, Stitzer, Liebson, & Bigelow, 1994a; Strain, Stitzer, Liebson, & Bigelow, 1994b; Strain, Stitzer, Liebson, & Bigelow, 1996; Johnson et al., 2000). The advantage of buprenorphine over methadone for opioid abuse treatment comes primarily from the lower overdose liability (Walsh, Preston, Stitzer, Cone, & Bigelow, 1994; Umbricht, Huestis, Cone, & Preston, 2004). In addition it has a more practical window of treatment, in that it can be administered at intervals of up to 96 hours with few withdrawal effects (Petry, Bickel, & Badger, 2000). There is also evidence in human patients (Montoya et al., 2004) and in monkeys (Mello, Mendelson, Bree, & Lukas, 1990; Mello et al., 1992; Mello et al., 1993) that buprenorphine reduces cocaine intake as well, but the mechanism of this action is unknown. The experiments reported in this thesis were carried out to examine the effects of chronic administration of buprenorphine on heroin and cocaine self-administration behavior, drug seeking, and reinstatement in the hope of determining precisely the aspects of the behavior that are affected and the neurochemical events underlying these behavioral changes. In the following sections I will explore the procedures included in this thesis, with particular attention on the intravenous self-administration procedure, and

the relevance of the related measures to substance abuse treatment. This thesis is primarily focused on the interaction of buprenorphine with both heroin and cocaine and, thus, the majority of the research presented in the upcoming portions will reflect opioid abuse treatments, heroin and/or cocaine, and buprenorphine itself.

The Self-Administration Model

The drug self-administration procedure (Weeks, 1962) is considered to be the one to use to model drug taking in humans and it has been used in rats, mice, monkeys and dogs (Pickens & Harris, 1968; Risner & Jones, 1975; Johanson, Balster, & Bonese, 1976). From the early work of Skinner, it has been shown repeatedly that animals will readily perform an operant behavior that results in reinforcement. This has been seen with rats pressing a lever for food (Ts'o, Baker, & Boeckler, 1975), access to a sexually receptive female rat (Everitt, Fray, Kostarczyk, Taylor, & Stacey, 1987), electrical brain stimulation (Olds & Milner, 1954) and for various drugs of abuse (Gardner, 2000).

Support for this procedure as a model of drug taking comes from evidence that animals show remarkably similar patterns of intake of the various drugs used to those of humans (Kramer, Fischman, & Littlefield, 1967; Deneau, Yanagita, & Seevers, 1969; Yokel & Pickens, 1973; Risner & Jones, 1976). Specifically, opioid drugs are taken at longer intervals than stimulant drugs. In rats, opioid drugs are administered in approximately 20-minute intervals, depending on the dose, and are often accompanied by bursts of responding that are non-reinforced (Wise, Leone, Rivest, & Leeb, 1995). On the other hand, stimulants, such as cocaine, are self-administered in a more regimented pattern during daily sessions with shorter inter-infusion intervals and little non-reinforced responding in well-trained animals (Panlilio, Katz, Pickens, & Schindler, 2003). There is

also evidence that animals will self-administer cocaine in bursts followed by periods of quiescence when given access for 24-hour periods (Pickens et al., 1968; Deneau et al., 1969; Yokel et al., 1973; Risner et al., 1975; Johanson et al., 1976; Risner et al., 1976), similar to binging behavior in human patients (Pottieger, Tressell, Surratt, Inciardi, & Chitwood, 1995; Levin, Foltin, & Fischman, 1996; Ward, Haney, Fischman, & Foltin, 1997a; Ward, Haney, Fischman, & Foltin, 1997b). Akin to human drug-taking behavior, animals can learn to self-administer drugs in a variety of ways including intravenously, intraorally, and intranasally. In addition, animals will administer drugs intracerebroventricularly and intracranially, providing important information about the site of action of particular drugs.

The Intravenous Self-Administration Procedure in Rodents

For intravenous self-administration, in-dwelling catheters are secured to a major vein (i.e. jugular or femoral) with the tip of the catheter placed at the entrance to the heart. The catheter is passed subcutaneously to the back of the animal to exit between the scapulae or the top of the skull, where it is fixed in place. Following an operant response, commonly a lever press or nose poke, the animal receives an infusion of a drug directly into the bloodstream. This infusion is often accompanied by an unconditioned stimulus that can be used to elicit responding on its own following sufficient training.

A benefit of the self-administration procedure is the versatility with which interventions can used to explore different aspects of drug taking and substance abuse in animals. Modifications to the schedule of reinforcement can allow a measure of the animal's motivation to work for a specific drug. In addition, treatments can be

administered during the acquisition or maintenance phase of self-administration to determine the effectiveness of the treatment on drug-taking behavior. Once training has been completed and the animal is exposed to extinction conditions, drug treatments can be implemented to reduce the degree of drug seeking during this period. A reduction in seeking is thought to be a reflection of reduced craving for the drug (Markou et al., 1993). Finally, extinguished drug seeking can then be reinstated by exposure to specific stimuli and treatments can be administered prior to these tests to determine the efficacy of the treatment for reducing or preventing relapse.

Measuring the Motivation to Take Drugs

During Self-Administration. By changing the schedule of reinforcement during self-administration from one in which every response is followed by a drug infusion to one in which the animal must make several responses before receiving the infusion, it becomes possible to obtain a measure of how hard the animal will work to obtain the drug. For example, one can change the ratio of responding required for a drug infusion from 1:1 to 5:1 (FR1 or FR5), or use a progressive ratio (PR) schedule of reinforcement in which the ratio increases proportionally within the session (Hodos, 1961). In the latter schedule of reinforcement the amount of work required to obtain each successive infusion of a drug increases; for example one lever press is required for the first infusion but 268 are required for the twentieth infusion. At some point the response requirement is subjectively too high and the animal ceases to respond for the drug. The amount of responding required to obtain the last infusion is termed the "breakpoint." Treatment drugs can be tested within this procedure to measure any *change* in the motivation to self-

administer a particular drug. For instance, pretreatment with the opioid receptor antagonist naltrexone reduces progressive ratio breakpoints for heroin in a dose-dependent manner (Roberts & Bennett, 1993), suggesting a reduction in the motivation to self-administer heroin.

During Extinction of Drug Seeking Behavior. Under extinction conditions animals are free to lever press, but drugs are no longer available, however, some or all of the associated cues remain present in the self-administration environment. Under these conditions, responding for the drug gradually diminishes but early sessions are characterized by an initial "extinction burst," or high level of responding (Yokel & Wise, 1975; Ettenberg, Pettit, Bloom, & Koob, 1982). The number of lever presses and the vigor with which the animal responds is a measure of the motivation to obtain the previously self-administered drug in addition to the persistence of the lever pressing, or resistance to extinction. Substance abuse treatment drugs can be given before daily extinction sessions to reduce drug-seeking behaviors in this initial period of drug abstinence. A pharmacological treatment that reduces drug seeking in extinction is thought to indicate a reduction in craving for the drug.

In Tests for Reinstatement of Drug-Taking Behavior. Following a period of drug abstinence, generally a period of extinction, animals are returned to the self-administration environment and exposed to a stimuli previously associated with drug taking and allowed to lever press. The drug is not available at this time and, thus, the amount and vigor of responding on the lever previously associated with drugs is thought

to be a measure of drug seeking. This is a test for reinstatement of responding for drugs and it thought to model relapse in human substance abuse patients. Research utilizing the reinstatement procedure has shown that, similar to humans, reinstatement can be precipitated by exposure to drug-associated cues (de Wit & Stewart, 1981; Ciccocioppo, Sanna, & Weiss, 2001), stress (Erb, Shaham, & Stewart, 1996; Shaham, Rajabi, & Stewart, 1996a) and the drug itself (de Wit et al., 1981; de Wit & Stewart, 1983) following extinction conditions.

Prior to tests for reinstatement, treatment drugs can be administered to determine their efficacy in preventing relapse, such that a reduction in lever pressing indicates a reduction in drug seeking. Pretreatment with the opioid treatment drugs methadone and naltrexone, as well as various DA receptor antagonists, attenuate heroin-induced reinstatement in rats (Shaham & Stewart, 1996b; Leri, Tremblay, Sorge, & Stewart, 2004). On the other hand, buprenorphine (Comer, Lac, Curtis, & Carroll, 1993), methadone (Leri et al., 2004), and a DA D_1 receptor agonist (Self, Karanian, & Spencer, 2000) reduce cocaine-induced reinstatement. It is not often the case that a drug is effective in reducing both heroin- and cocaine-induced reinstatement, since the two drugs differ in their mechanisms of action (discussed below); however, a drug with this multiple action would be highly sought after for drug abuse treatment.

The Present Experiments

As mentioned above, the experiments included in this thesis involve two opioid and one stimulant drug. The thesis is designed to test the interactions between the opioid (treatment) drug, buprenorphine, and heroin (opioid) and cocaine (stimulant). Prior to a

discussion of the effects of buprenorphine in treatment for opioid abuse, the following sections will provide a background as to the various actions and locations of action of heroin and cocaine. At numerous times in the following sections there will be mention of the dopamine (DA) system and its terminal regions. The mesolimbic DA system consists of the DAergic neuron projections from the ventral tegmental area (VTA) to numerous terminals fields including the NAc, prefrontal cortex (PFC), amygdala, and hippocampus. Activation of the DA system by various drugs of abuse is thought to underlie the reinforcing aspects of these drugs and is one common feature of the majority of drugs of abuse (Di Chiara & Imperato, 1988).

Cocaine and the Dopamine Transporter (DAT)

Cocaine acts primarily in the DA terminal regions to maintain high extracellular DA levels and facilitate extended binding of DA to its receptors. Cocaine binds to the DAT to block reuptake of DA into the presynaptic neuron, thus increasing the levels of synaptic DA available to bind to DA receptors in the NAc and the PFC (Heikkila, Orlansky, & Cohen, 1975; Volkow et al., 1997; Hall et al., 2004). The increase in extracellular DA brought about by cocaine is generally attributed to DAT blockade, however, it has recently been shown that cocaine has the added ability to release DA directly (Venton et al., 2006). Intravenous infusion or systemic injection of cocaine results in a rise in extracellular DA levels within the NAc and PFC as measured by *in vivo* microdialysis (Di Chiara et al., 1988; Pontieri, Tanda, & Di Chiara, 1995; Ikegami & Duvauchelle, 2004) and rats will self-administer cocaine directly into the NAc (Rodd-

Henricks, McKinzie, Li, Murphy, & McBride, 2002), PFC (Goeders, Dworkin, & Smith, 1986), and the VTA (Rodd et al., 2005).

Heroin and the Opioid System

Heroin activates mu and kappa opioid receptors in a much more potent way than do the endogenous ligands, though the majority of the reinforcing effects of heroin are attributed to its mu-opioid receptor agonist actions (Negus et al., 1993). The opioid receptors are of three types: mu, delta, and kappa (Lord, Waterfield, Hughes, & Kosterlitz, 1977; Knapp et al., 1995) and consist of approximately 400 amino acids resulting in seven transmembrane domains. They are all considered G-protein-coupled receptors (Knapp et al., 1995) and are expressed in different concentrations and locations throughout the brain. The mu and kappa opioid receptors are widely distributed throughout the brain, whereas the delta opioid receptors are sparsely distributed (McLean, Rothman, & Herkenham, 1986; Tempel & Zukin, 1987). In particular, the mu-opioid receptor has been isolated on GABA interneurons within the VTA (Svingos, Garzon, Colago, & Pickel, 2001; Garzon & Pickel, 2001) as well as on medium spiny neurons in the NAc (Mansour, Khachaturian, Lewis, Akil, & Watson, 1988; Mansour, Fox, Akil, & Watson, 1995; Olive, Anton, Micevych, Evans, & Maidment, 1997). In the VTA, stimulation of mu-opioid receptors leads to inhibition of the GABA neuron, thereby releasing the DA neuron from inhibition and indirectly stimulating DA release (Svingos et al., 2001). Thus, administration of heroin results in an increase in DA levels in the NAc of rats (Di Chiara et al., 1988). Interestingly, although heroin causes an increase in DA in the NAc, likely through activation of mu-opioid receptors in the VTA,

heroin remains reinforcing in the absence of DA activity in the NAc (Gerrits & van Ree, 1996; Alderson, Parkinson, Robbins, & Everitt, 2001), suggesting that the rise in NAc DA is secondary to the reinforcing effect of heroin; a DA-independent reinforcing effect. Furthermore mu-opioid receptor agonists are self-administered directly into the VTA (Bozarth & Wise, 1981), NAc (Olds, 1982), and hippocampus (Self & Stein, 1993).

Buprenorphine

The success of buprenorphine as an opioid abuse treatment is the result of two important factors: safety and effectiveness. In humans, buprenorphine has a half-life in blood of up to 35 hours (Kuhlman, Levine, Johnson, Fudala, & Cone, 1998) and can be prescribed at doses between 40 and 70 times the analgesic dose with few side effects (Walsh et al., 1994; McAleer et al., 2003). At opioid abuse treatment doses there is a ceiling effect with respect to blood plasma levels and mu-opioid receptor occupation in the brain (Greenwald et al., 2003) and the long half-life of the drug allows dosing to be done on alternate days in intervals up to 96 hours with very few withdrawal symptoms (Petry et al., 2000). There are no cases of fatal overdose with buprenorphine alone. All reported cases have resulted from concomitant ingestion of at least one additional substance, most often benzodiazepines (Tracqui, Kintz, & Ludes, 1998; Kintz, 2001; Kintz, 2002; Schifano et al., 2005). Furthermore there have been reports of heroin users successfully 'treating' overdosed friends with buprenorphine (Boyd, Randell, Luurila, & Kuisma, 2003) and one case study reports a patient consuming 11 tablets (8 mg/tablet) at one time resulting in severe withdrawal, but no significant respiratory depression – a common fatal effect of opioid drugs (Clark, Lintzeris, & Muhleisen, 2002).

The attributes of buprenorphine for treatment of opioid abuse comes from its unique pharmacology. As opposed to full agonist treatments like methadone, buprenorphine is a partial opioid receptor agonist-antagonist, meaning it has low intrinsic activity. Buprenorphine activates mu-opioid receptors (Lee et al., 1999) and chronic administration down regulates these receptors (Debruyne et al., 2005). Acute buprenorphine elicits a rise in extracellular DA in the NAc (Brown, Finlay, Wong, Damsma, & Fibiger, 1991), supports a place preference (Tzschentke, 2004), and reduces thresholds for brain stimulation reward (Hubner & Kornetsky, 1988). Buprenorphine is self-administered (Mello, Bree, & Mendelson, 1981), but, at high doses, can precipitate morphine withdrawal in rhesus monkeys (Gmerek, 1984). Buprenorphine is a full agonist at the opioid-like receptor (ORL-1) (Wnendt, Kruger, Janocha, Hildebrandt, & Englberger, 1999) and an antagonist at both the kappa (Traynor, Guo, Coop, Lewis, & Woods, 1999) and delta opioid receptors (Negus et al., 2002).

As a treatment for opioid abuse, buprenorphine reduces opioid-positive urine samples in patients undergoing treatment for opioid abuse and increases retention in treatment (Strain et al., 1994a; Strain et al., 1994b; Ling et al., 1998; Montoya et al., 2004). In addition, buprenorphine reduces the subjective effects of heroin (Comer, Walker, & Collins, 2005) and morphine (Teoh et al., 1994) and reduces heroin choice in a dual choice task completed by heroin addicts (Mello et al., 1980). As an alternative to methadone, the comparisons between these drugs have been mixed. Comparative studies in opioid abuse patients have found buprenorphine to be less (Kosten, Schottenfeld, Ziedonis, & Falcioni, 1993; Ling, Wesson, Charuvastra, & Klett, 1996; Schottenfeld, Pakes, Oliveto, Ziedonis, & Kosten, 1997), equally (Strain et al., 1994b), or more

effective than methadone (Johnson et al., 1992; Strain et al., 1996) in reducing opioid use, but these discrepancies can be accounted for by unequal dosing of the two drugs.

There are high levels (up to 75%) of cocaine co-abuse within the opioid abusing patient population (Kosten, Rounsaville, & Kleber, 1987; Kosten, Rounsaville, & Kleber, 1988; Schottenfeld, O'Malley, Abdul-Salaam, & O'Connor, 1993; Schottenfeld et al., 1997; Leri, Bruneau, & Stewart, 2003; Guichard et al., 2003; Tassiopoulos et al., 2004). For this reason, studies have also evaluated the potential of buprenorphine treatment to reduce cocaine use as well. Multiple studies of opioid abuse patients co-abusing cocaine have found that buprenorphine reduced intake of cocaine, as well as opioids (Strain et al., 1994a; Strain et al., 1994b; Montoya et al., 2004). Furthermore, buprenorphine reduces craving for cocaine, although the subjective effects of cocaine were unaffected (Teoh et al., 1994; Foltin & Fischman, 1996). Thus, the safety of buprenorphine and its effectiveness to reduce opioid *and* cocaine use lends support for the utility of this drug in this patient population.

Buprenorphine has been shown to be effective in treating opioid and cocaine use in human patients, but the mechanism of action is somewhat unclear, especially with respect to the interactions with cocaine. To this end, the effects of buprenorphine have been evaluated in animal models of drug taking with both heroin and cocaine. Daily treatment with buprenorphine reduces heroin (Mello, Bree, & Mendelson, 1983; Mello & Negus, 1998), heroin-cocaine "speedball" (Mello et al., 1998), and cocaine (Winger, Skjoldager, & Woods, 1992; Mello et al., 1993; Lukas, Mello, Drieze, & Mendelson, 1995) intake in monkeys and cocaine intake in rats (Carroll & Lac, 1992). An acute

injection of buprenorphine does not reinstate cocaine-seeking in rats, but does prevent reinstatement induced by cocaine (Comer et al., 1993).

It would seem, for the most part, that findings using animal models of drug taking support those in human patients. There is, however, one collection of findings that require mention. A subthreshold dose of buprenorphine (to establish a conditioned place preference) coupled with a subthreshold dose of cocaine produces a stronger preference than that by either drug alone, as well as a potentiated NAc DA response (Brown et al., 1991). This synergism has also been observed in measures of locomotion (Smith et al., 2003) and rotational behavior in substantia nigra lesioned rats (Kimmel, Tallarida, & Holtzman, 1997). Although this has not been reported in the clinical literature, these findings suggest that there is a potential for increased cocaine intake and/or impact while under buprenorphine treatment.

Buprenorphine via Osmotic Minipump

The majority of the studies in the animal literature support the clinical findings in opioid abuse patients, but there are aspects of the effects of buprenorphine treatment that are currently unknown. One such aspect is the effect of chronic, continuous administration of buprenorphine on measures of intake of heroin and/or cocaine during maintenance of self-administration, extinction, or on tests for reinstatement of responding. For this reason we have adopted a strategy to achieve steady state maintenance levels throughout the experiments via subcutaneously implanted buprenorphine-filled osmotic minipumps. This allows constant release of the drug into the circulatory system of the rats and our experiments have shown that these levels do not

significantly differ across four weeks of continuous treatment, suggesting maintained receptor occupation.

The Thesis Chapters

The experiments in Chapter 1 were designed to investigate the potential of the opioid treatment drug, buprenorphine, to reduce heroin and cocaine seeking in tests of extinction and reinstatement in rats trained to self-administer both drugs on alternate days. Reinstatement was induced via acute injections of heroin, cocaine and intermittent footshock stress. In addition, the locomotor and NAc DA response to acute injections of heroin and cocaine was measured while under chronic buprenorphine treatment. The experiments in Chapter 2 were completed to determine whether chronic buprenorphine treatment altered heroin and/or cocaine self-administration when assessed with different doses of heroin and cocaine and on different schedules of reinforcement. The results of the self-administration experiment were further investigated through measurement of NAc DA with the use of *in vivo* microdialysis during the daily self-administration session. Blood samples were also taken from rats under buprenorphine treatment at 1-week intervals over 4 weeks of treatment to determine the amount of buprenorphine levels in plasma during treatment. Finally Chapter 3 sought to investigate the temporal nature of the effects of locomotion and NAc DA over the course of long-term buprenorphine treatment. Locomotor activity and the NAc DA response to acute injections of heroin and cocaine were measured in separate animals after 5, 13-15 or 25-27 days of treatment.

CHAPTER 1

Rats Maintained Chronically on Buprenorphine Show Reduced Heroin and Cocaine Seeking in Tests of Extinction and Drug-Induced Reinstatement

Robert E Sorge, Heshmat Rajabi, and Jane Stewart

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ABSTRACT

Buprenorphine is being introduced as a maintenance therapy in opioid addiction, but it is not clear how buprenorphine will affect co-use of cocaine in opioid users. We examined the effects of chronic buprenorphine (BUP0: 0.0 mg/kg/day; BUP1.5: 1.5 mg/kg/day; BUP3: 3.0 mg/kg/day) on the locomotor activity effects of acute heroin (0.25 mg/kg, sc) and cocaine (20 mg/kg, ip). Buprenorphine had no effect on the stimulatory effect of heroin, but potentiated the locomotor response to cocaine. To investigate further the interactions between buprenorphine (BUP1.5 and BUP3) and heroin (0.125, 0.25 and 0.375 mg/kg, sc) and cocaine (10, 20 and 30 mg/kg, ip), we used *in vivo* microdialysis and HPLC to analyze extracellular levels of dopamine (DA) in the nucleus accumbens (NAc). Buprenorphine attenuated the heroin-induced rise in NAc DA, but greatly potentiated the cocaine-induced rise. Finally, we examined the potential of the highest dose of buprenorphine (BUP3) to reduce heroin and cocaine seeking in the presence of drug-associated cues under extinction conditions and in tests for reinstatement induced by heroin (0.25 mg/kg, sc), cocaine (20 mg/kg, ip) and 15-min footshock stress (0.8 mA, 0.5s/shock, 40s mean OFF time) in rats trained to self-administer both drugs. Buprenorphine reduced heroin and cocaine seeking during extinction and following acute heroin and cocaine priming injections, but had no effect on stress-induced reinstatement. These results indicate that the suppression of responding following priming injections of drugs did not result from reduced motor activity, but possibly from a reduction in the salience of drug-associated cues induced by chronic buprenorphine treatment.

Keywords: buprenorphine, heroin, cocaine, relapse, dopamine, microdialysis

INTRODUCTION

Co-abuse of cocaine by patients receiving treatment for opioid addiction often compromises the success of the pharmacological strategies. Cocaine use during treatment reduces retention rates and increases use of illicit drugs reducing the effectiveness of the maintenance treatment drugs (Kosten et al., 1987; Kosten et al., 1988; Schottenfeld et al., 1993; Schottenfeld et al., 1997; Leri et al., 2003; Guichard et al., 2003; Tassiopoulos et al., 2004) Thus, it is important to know how pharmacological treatments for opioid addiction interact with the response to cocaine and opioids and to the effects of cues associated with the self-administration of these drugs.

To date, the two opioid drugs most often use in maintenance treatments are methadone and buprenorphine (Kosten, 1990; Kleber, 2003; Gonzalez, Oliveto, & Kosten, 2004; Krantz & Mehler, 2004). Both are agonists at the *mu-opioid* receptor, though there are fundamental differences in their actions. Methadone is a full *mu-opioid* receptor agonist, whereas buprenorphine is a partial *mu* agonist with antagonist effects at the *delta* and *kappa* opioid receptors (Tzschentke, 2002; Walsh & Eissenberg, 2003; Gonzalez et al., 2004). Studies of the treatment effectiveness of maintenance doses of buprenorphine are few compared to those of methadone, and the results are mixed. However, on measures of retention rates and illicit opioid use, buprenorphine has been found to be as effective as, or better than, methadone when prescribed at higher doses (8-16 mg/day) (Kosten, Kleber, & Morgan, 1989; Johnson et al., 1992; Strain et al., 1994a; Strain et al., 1994b; Strain et al., 1996; Foltin et al., 1996; Ling et al., 1996; Schottenfeld et al., 1997), whereas lower dose regimens (2-4 mg/day) are less effective (Ling et al., 1998; Greenwald, Schuh, Hopper, Schuster, & Johanson, 2002; Montoya et al., 2004).

Furthermore, there are reports that buprenorphine maintenance reduces craving for cocaine (Foltin et al., 1996) and, to varying degrees, reduces cocaine consumption as measured by urine analysis in human opioid addicts (Kosten et al., 1989; Johnson et al., 1992; Strain et al., 1994a; Strain et al., 1994b; Strain et al., 1996; Ling et al., 1996; Schottenfeld et al., 1997). In monkeys trained to self-administer cocaine, heroin or heroin-cocaine "speedball," *daily injections* of buprenorphine have been found to reduce intake (Mello et al., 1992; Mello et al., 1993; Lukas et al., 1995; Mello et al., 1998) without changing the intake of food or sweetened fluid.

Many drugs of abuse increase extracellular dopamine (DA) in terminal regions including the nucleus accumbens (NAc), though by somewhat different mechanisms (Di Chiara et al., 1988). As mentioned, buprenorphine is an agonist at the *mu-opioid* receptor; it has low intrinsic activity, high affinity and slow dissociation from the receptor (Walsh et al., 1994; Tzschentke, 2002). *Mu-opioid* agonists are thought to activate receptors on GABA interneurons in the ventral tegmental area (Garzon et al., 2001), releasing DA neurons from inhibition and increasing firing rates. On the other hand, cocaine acts primarily to block the DA transporter reducing DA reuptake at terminals, thereby increasing extracellular levels of DA (Gysling & Wang, 1983; Matthews & German, 1984; Ritz, Lamb, Goldberg, & Kuhar, 1988; Ritz, Cone, & Kuhar, 1990; Rothman & Baumann, 2003; Nestler, 2004). Consequently, it has been found in experiments using *acute* injections, that both buprenorphine (0.01 mg/kg) and cocaine (5.0 mg/kg) increase extracellular levels of DA in the nucleus accumbens in rats (each approximately 180% of baseline). As might be expected, an *acute* injection of buprenorphine given with an acute injection of cocaine produced an increase in DA in the

NAc that was higher (approximately 260% of baseline) than that produced by that particular dose of either drug alone. This synergism was also reflected in the reinforcing effects of these drugs when they were administered together during the conditioning of a place preference (Brown et al., 1991). It is not clear how these results from studies using acute injections of buprenorphine can be related to those using chronic treatment with buprenorphine in human opioid addicts and in monkeys trained to self-administer opioids and cocaine. Interestingly, however, there is one report that twice daily injections of buprenorphine, which might be considered to be chronic treatment, attenuated the development of a cocaine conditioned place preference (CPP) (Kosten, Marby, & Nestler, 1991). As pointed out by Tzschentke (Tzschentke, 2004), however, the lack of CPP under such a regime might result from the fact that the dose used by Kosten and colleagues (0.5 mg/kg, twice daily) has been shown to produce a CPP on its own, thus confounding the establishment of the cocaine CPP; rats placed in the saline paired compartment would remain under the influence of a putatively rewarding dose of buprenorphine.

In this paper, therefore, we sought to resolve some of these issues by asking whether and how chronic treatment with buprenorphine alters the behavioral and neurochemical response to heroin and cocaine in naïve rats and drug seeking in rats trained to self-administer both heroin and cocaine. Spontaneous drug seeking was studied in extinction and in reinstatement of drug seeking induced by heroin, cocaine and footshock stress. Buprenorphine was administered chronically through in-dwelling osmotic minipumps allowing for continuous stable exposure to the drug for the duration of testing. In the first set of experiments we studied the effects of acute injections of

heroin and cocaine on locomotor activity and extracellular levels of DA in the NAc in rats with and without buprenorphine minipumps. These experiments helped to determine the dose to be used in the second set of experiments in which rats were trained to self-administer both heroin and cocaine. Following training we studied heroin and cocaine seeking under chronic treatment with buprenorphine via minipump in two separate conditions: during extinction and in tests for reinstatement

MATERIALS AND METHODS

Subjects

The subjects were 107 male Long-Evans rats (350-375g, Charles River, St. Constant, Quebec), housed singly in hanging wire cages in a reverse light-dark cycle room (light onset 2000h, offset 0800h). The rats had food (Rat Chow, Purina Foods) and water *ad libitum* for the duration of the experiment. Testing was conducted during the dark cycle between 0800 and 1700. All experimental procedures followed the guidelines of the Canadian Council on Animal Care and were approved by the Animal Care Committee at Concordia University.

Surgery – Osmotic Minipumps

Chronic exposure to buprenorphine was achieved through the use of osmotic, buprenorphine-filled minipumps implanted subcutaneously (sc). Rats were anesthetized using Isoflurane (Vetoquinol N.A. Inc, Lavaltrie, QC) and a small incision was made between the scapulae. Using a hemostat, a small pocket was created by separating the connective tissues under the skin. Once the pocket was formed, osmotic buprenorphine-

filled minipumps (Alzet model 2ML2, Durect Corp., Cupertino, CA) were implanted with the flow modulator pointed away from the incision to avoid leakage of the drug. The incision was closed using wound clips. Pumps were removed under anesthesia using the same surgical methods. In the case of the 0 mg/kg/day buprenorphine (BUP0) rats, the same surgical procedures were employed with the exception of the insertion of a pump (i.e. an incision and pocket were made and the wound was clipped following surgery).

Surgery-Intracranial Cannulation

The rats were anesthetized with sodium pentobarbital (Somnotol™, MTC Pharmaceuticals, Cambridge, ON; intraperitoneally [ip]) and then unilateral stainless steel 20 gauge cannula (Plastics One, Roanoke, VA) were implanted aimed at the NAc (NAc: AP +1.6mm, ML +2.8mm, DV -5.5mm from bregma) at an angle of 10 degrees in order to avoid extensive damage to the ventricle above the NAc, while maximizing the surface area of the probe within the NAc. Cannula were cemented in place with dental acrylic and the rats were placed in recovery following an injection of penicillin (Pen G, Vetoquinol, Lavaltrie, QC; intramuscularly [im]) and Ketoprofen.

Surgery-Intravenous Catheterization

For the self-administration and reinstatement experiment the rats had intravenous catheters implanted in the right jugular vein. The rats were anesthetized with sodium pentobarbital (Somnotol™, ip) and given a subcutaneous (sc) injection of atropine sulfate (MTC Pharmaceuticals, Cambridge, ON) prior to surgery. The silastic (Dow Corning,

Midland, MI) catheters were implanted and secured to the right jugular vein with silk sutures and passed subcutaneously to the top of the skull where it was attached to a modified plastic cannula (Plastics One, Roanoke, VA) and fixed with jeweler's screws and dental cement to the skull surface. A plastic blocker was placed over the opening of the cannula (Tygon™ Tubing, Fisher Scientific, Montreal, QC) and protected from the rat with a metal cap. Following surgery, rats were injected with penicillin to prevent infection and Ketoprofen as post-surgery pain management. The blocker and cap were left in place at all times except when the rats were participating in self-administration, extinction or reinstatement sessions. On every second day, following the self-administration sessions, the rats were flushed with 0.2 mg/ml heparin-saline solution (ICN Biomedicals, Cleveland, OH).

Apparatus

Locomotor Activity Boxes

Locomotor activity was assessed in a bank of twelve activity boxes. Boxes were constructed of white pressed wood on three walls and clear Plexiglas for the front wall (20 x 41 x 25 cm, custom made, Concordia University). The top of the box was constructed of wire mesh, while the floor was evenly spaced stainless steel tubing. Two photocell pairs, positioned 3.5 cm from the floor, were located along the front and back wall of the chamber and provided a measure of horizontal locomotion. The bank of activity boxes was isolated in a room that was left in complete darkness for the duration of the testing. The photocells were connected, through a wall port, to a computer located in an adjacent room that ran the custom-made software.

Microdialysis and High Performance Liquid Chromatography

Four hexagonal, chambers were used for microdialysis. Each chamber (42 x 39 x 33.5 cm, custom made, Concordia University) consisted of Plexiglas walls with wooden ceilings and stainless steel grid floors. They were individually housed in wooden cubicles and lighting was provided on a reverse cycle by overhead lights.

Microdialysis Probe. The dialysis probe (HRS Scientific, Montreal, QC) consisted of a 2.5mm length of semi-permeable dialysis membrane (Fisher Scientific, 240 um OD, 13000 MW cutoff), closed at one end and attached to a 21 mm long, 26 gauge piece of stainless steel tubing. The flared end of a 40-50 cm long piece of PE tubing connected one end of the stainless steel shaft to a single channel liquid swivel (custom made) stationed above the testing chamber that was, in turn, connected to a variable speed electric syringe infusion pump (Harvard Apparatus, South Natick, MA). Small diameter fused silica tubing extended internally through the probe with one end resting 0.5 mm from the tip of the probe with the other end exiting the PE tubing 35 cm below the infusion swivel. The opposite end of the silica tubing was attached to the PE tubing near the liquid swivel with masking tape in order to attach the sample collection vials. The probe was secured in place by stainless steel collars that were screwed onto the guide cannula. The external length of the PE tubing was protected from chewing by steel spring casing. The probes were inserted the day before the beginning of microdialysis testing. To prevent occlusion, artificial CSF (145 mM Na^+, 2.7 mM K^+, 1.2 mM Ca^{2+},

1.0 mM Mg^{2+}, 150 mM Cl^-, 0.2 mM ascorbate, 2 mM Na_2HPO_4, pH, 7.4 + 0.1) was perfused overnight at a rate of 0.03 µl/min.

High Performance Liquid Chromatography. A 10 µl volume of dialysate was extracted from each sample and analyzed immediately using one of two similar high-performance liquid chromatography systems with electrochemical detection (HPLC-EC). The samples were loaded onto C-18 reverse-phase columns (5 µm, 15 cm) through manual injection ports (Reodyn 7125; 20 µl loop); reduction and oxidation currents for DA and its metabolites [dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) and 5-hydroxyindole acetic acid (HIAA)] were measured with dual-channel ESA coulometric detectors (Coulochem III, with a model 5011 analytical cell). The currents for DA were measured independently of those for DOPAC, HVA and HIAA using separate channels of the Coulochem detectors. The mobile phases (20% acetonitrile, 0.076 M SDS, 0.1 M EDTA, 0.058 M $NaPO_4$, 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 EZChrom Chromatography Data System (Scientific Software Inc, San Ramon, CA). The mobile phase was adjusted to allow for the separation and quantification of DA, DOPAC, HVA and HIAA in a single run.

Self-Administration

Eighteen Plexiglas custom-made operant boxes (Concordia University, Montreal, QC) were used for this experiment and each was enclosed in a sound-attenuating plywood chamber. Each operant box had four levers (Med Associates, Lafayette, IN)

located 10 cm above the floor of the box: two retractable ("active") and two stationary ("inactive"). One of each type was located on opposite walls of the chamber, 10 cm apart from each other. The retractable levers were connected to an infusion pump for the delivery of drugs (Razel Scientific Instruments, Stamford, CT) positioned outside the sound-insulating chamber. The stationary levers served to control for baseline, non-reinforced, operant behavior; depression of this lever had no consequences, but all presses were recorded. There were two white light stimuli (one constant light and one flashing) 3 cm above each active lever that came on for 30 s at the beginning of the session, and for the duration of each drug infusion, thus serving as a discrete conditioned stimuli (CS) for drug delivery. Throughout the experiment, each self-administration session was started by the illumination of a red house light that remained on for the duration of the session. Each self-administration chamber was fitted to deliver constant-current, intermittent, inescapable, electric footshock through a scrambler to the grid floor (Med Associates, Lafayette, IN).

Drugs

Heroin (diacetylmorphine HCl) was purchased from Almat Pharmachem Inc (Concord, ON), cocaine (cocaine HCl) from Medisca Pharmaceutique (Montreal, QC) and buprenorphine HCl from Reckitt Benckiser Healthcare Limited (Hull, UK).

The doses used for the self-administration experiment (heroin, 0.05 mg/kg/inf; cocaine, 0.5 mg/kg/inf) were chosen on the basis of previous research in this laboratory showing that these doses will produce similar levels of responding under extinction conditions when animals are trained to self-administer both drugs (Leri & Stewart, 2001).

Furthermore, Leri et al (Leri et al., 2004) used these doses in a similar study to test the efficacy of methadone on spontaneous and drug- and stress-induced reinstatement of heroin- and cocaine-seeking allowing for comparisons between the studies of the two opioid addiction treatment drugs. The high dose of buprenorphine used in this experiment (3.0 mg/kg/day; BUP3) was chosen because it is the highest dose able to be put into solution without the addition of alcohols and because high doses of buprenorphine are well tolerated (Walsh et al., 1994).

PROCEDURES

Locomotor Activity

One week after arrival in the colony, 31 rats were placed in the activity chambers in order to habituate to the chambers and provide a measure of baseline locomotion that was utilized to match the experimental groups. On each day of testing, the rats were placed in the chambers in a darkened room and the photocells were activated. The computer started the 120 min-session as soon as the rats were in the chambers. For each chamber the total number of horizontal infrared beam breaks provided the measure of horizontal locomotion. Once the groups were matched on the basis of the locomotion scores, osmotic minipumps were implanted creating three buprenorphine treatment groups (0 mg/kg/day [BUP0], 1.5 mg/kg/day [BUP1.5] and 3.0 mg/kg/day [BUP3]). Three days after surgery (Day 3), rats were given saline injections and returned to the locomotor chambers (Saline Test) for the 120 min test. On Days 7 and 10 the rats received heroin (0.25 mg/kg, sc) and cocaine (20 mg/kg, ip) injections in a counterbalanced fashion such that every rat received both drugs, but half the rats in each

group received heroin first (Heroin Test), and the other half received cocaine (Cocaine Test).

Microdialysis

Different groups of rats (n=58) were used for the microdialysis experiments. Three days before the studies were initiated, osmotic minipumps were implanted creating three treatment groups (0 mg/kg/day [BUP0], 1.5 mg/kg/day [BUP1.5] and 3.0 mg/kg/day [BUP3]). In the late afternoon of Day 3 after minipump implantation, microdialysis probes were inserted into the guide cannula of 4 rats (at least one from each group) and dialysate was infused at a rate of 0.3 µl/min overnight. Dialysate sampling and activity monitoring began the next morning. The dialysate flow rate was increased to 0.7 µl/min, and baseline dialysate samples (approximately 14 µl) were collected every 20 min and analyzed immediately. Dialysate samples from individual rats were analyzed consistently using one of the two HPLC-EC systems and the assignment of the animals to each system was counterbalanced across all treatment conditions. Once stable baseline levels of DA and its metabolites (DOPAC and HVA) were attained (less than 10% variation in three consecutive samples), the rats were injected with one of three doses of heroin (0.125, 0.25 or 0.375 mg/kg, sc) or cocaine (10, 20 or 30 mg/kg, ip) and samples were collected at 20-min intervals for 140 min. On the following day, the identical protocol was used and each rat received an injection of one of the doses of the other drug, such that each rat was given one dose of heroin and one of cocaine. The order of the drug administration was counterbalanced within the groups. Food was removed from the chambers before sampling, but a water drinking tube was available throughout.

Postmortem Tissue Analysis. Following the two test days, the rats were perfused intracardially with saline and formaldehyde (Formalin 10%V/V, Anachemia, Montreal, QC) before having their brains removed. In order to identify the placements of the cannula tract and probes, horizontal frozen sections were taken using a cryostat, mounted and stained with cresyl violet.

Self-Administration

An additional eighteen rats were trained to self-administer both heroin (0.05 mg/kg/inf) and cocaine (0.5 mg/kg/inf) in the same chambers on alternate days in a counterbalanced fashion, such that some rats had access to cocaine first and others heroin first. Each drug was paired with a distinct lever and light pattern as outlined above such that one "active" and one "inactive" lever were in use each day. Half of the rats had heroin paired with the right lever (constant light) and cocaine paired with the left lever (flashing light).

Training. Rats were given access to drugs for a total of 16 3-h self-administration sessions, 8 sessions with each drug on alternate days. The sessions were conducted once per day with two groups of rats commencing their sessions at 0800h and 1100h respectively. Each rat had access to a specific chamber and had access at the same time each day. For each self-administration session the rats were transported from the colony to the experimental room and placed in the operant chambers. Each rat had its protective cap and blocker removed and was connected to the drug infusion tubing with a protective spring sleeve screwed to the cannula mounted on the skull. This functioned to protect the tubing from the rat and to secure the tubing to the cannula. Once the rats were secured in

their respective chambers there was a 5-min time-out period that allowed the rats to acclimate to the chambers. Following the 5-min period, the red house light was turned on and remained on for the duration of the session. Ten sec later the retractable lever was extended and the cue light was activated for 30 sec. This light remained on for 30 sec unless a response was made; if there was a response, it stayed on for the duration of the 10-sec drug infusion. A fixed ratio 1 schedule of reinforcement (FR1) was used such that the first active lever press resulted in a 10-sec drug infusion (approximately 64 μl). During the infusion, presses on the active lever had no additional consequence, but were recorded, as were presses on the inactive lever. Following the 180-min session, the active lever was retracted and the house light was extinguished.

Surgery. Following the last self-administration day (Day 16), all rats were prepared for surgery as outlined above. The rats in the buprenorphine (BUP3) experimental group had pumps implanted subcutaneously, and those in the control group (BUP0) had sham surgery performed but no pump implanted. After surgery, rats were returned to their home cages and allowed 24 h to recover. This was thought to be enough time to recover and allowed an evaluation of the immediate effects of the chronic buprenorphine treatment on spontaneous heroin- and cocaine-seeking during extinction.

Extinction. The next day, rats were brought back to the self-administration boxes and connected as before. On this first day of extinction the procedure was identical to that used in the self-administration sessions with the exception both the heroin and cocaine levers were present and no drugs were available during the 3-h session. During the six days of extinction, a depression either of the two retractable levers resulted in the appropriate cue light and the activation of the pump for 10 sec. In each case, an empty

syringe was connected to the tubing to seal the system, but was not placed in the syringe pump.

Drug-Induced Reinstatement. On the seventh day, rats were placed into the self-administration boxes for another extinction session lasting only 1 h. If the extinction criterion was met in the first hour (less than 15 active lever presses), the session was terminated and the first reinstatement session was initiated. When more than 15 responses were made on either active lever, another 1-h extinction session was started 15 min following the termination of the previous session until extinction criterion was achieved for each rat. For the drug-induced reinstatement, half of the rats received an injection of heroin (0.25 mg/kg, sc), and the other half received an injection of cocaine (20 mg/kg, ip) 15 min before returning to the self-administration boxes. These doses were chosen on the basis of previous work in this laboratory demonstrating that these doses are effective at reinstating heroin and cocaine-seeking to similar levels in rats trained to self-administer both drugs (Leri et al., 2001; Leri et al., 2004). Once the rats were returned to the chambers, following a 15-minute timeout period, the session began as each previous extinction session with the house light illuminated for 10 sec before the extension of the two retractable levers and the activation of both cue lights for 30 sec. As in previous sessions, the number of active responses on both levers was recorded as well as the number of inactive lever responses. Following the reinstatement session rats were returned to their home cages.

The next day the rats were given a 3-h extinction session to extinguish any residual responding before the next reinstatement session. On the next day rats were given another 1-h extinction session to determine whether the extinction criterion had

been met. If so, the next reinstatement session was started and, if not, another 1-h extinction session was given until the criterion was met. On the second reinstatement session the rats received the drug that they had not received on the first reinstatement session.

Footshock Stress-Induced Reinstatement. On the day after the last drug test, rats were returned to the self-administration boxes for a 3-h extinction session. The final day of testing began with a 1-h extinction session to determine whether the extinction criterion had been met. Once this was attained, the rats were exposed to 15 min of intermittent, inescapable, footshock stress in the self-administration boxes (0.8 mA, 0.5s/shock, 40s mean OFF time). A pilot study showed these to be the optimal intensity and duration of exposure to footshock stress for reinstatement in rats trained to self-administer cocaine; in addition, these parameters had been used in previous studies in this laboratory (Leri et al., 2001; Leri et al., 2004). Following the shock exposure, the 3-h session began with the illumination of the red house light for 10 sec prior to extension of the two retractable levers and the activation of the cue lights for 30 sec.

Statistical analyses. Data from each of the experiments were analyzed using ANOVAs for Treatment groups by time, as appropriate. Post-hoc comparisons between means were made using Fisher's LSD test ($p<0.05$)

RESULTS

Locomotor Activity

Figure 1 shows the mean total activity levels of the three buprenorphine treatment groups on each of the test days. An ANOVA conducted on the 2-h activity scores

revealed a significant main effect of Group ($F(2,28)=3.41$, $p<0.05$) and Test ($F(4,112)=80.78$, $p<0.0001$) and a Group by Test interaction ($F(8,112)=4.22$, $p<0.0001$). This Group by Test interaction reflects the fact that rats treated with buprenorphine, BUP1.5 and BUP3 had higher scores than the BUP0 only on the saline and cocaine tests; the response to heroin was unaffected by buprenorphine. We next examined the locomotor activity in 20-min intervals across the 2-h session and noted that the differences between groups remained stable across time (data not shown). Separate ANOVAs were carried out for each of the three tests and in each case the Group by Time interactions were not significant (Saline: $F(10,140)=1.542$, $p=0.131$; Heroin: $F(10,140)=0.374$, $p=0.956$; Cocaine: $F(10,140)=0.814$, $p=0.615$).

It can also be noted that on the initial test given before pump implantation, there was no significant main effect of Group ($F(2,28)=0.15$, $p=0.86$), showing that the groups were matched for their basal activity scores. The effect of chronic buprenorphine on activity can be seen on the Saline test given after implantation of the pumps. Though not significant, there was a tendency for the buprenorphine groups to be more active ($F(2,28)=2.77$, $p=0.08$) (Figure 1).

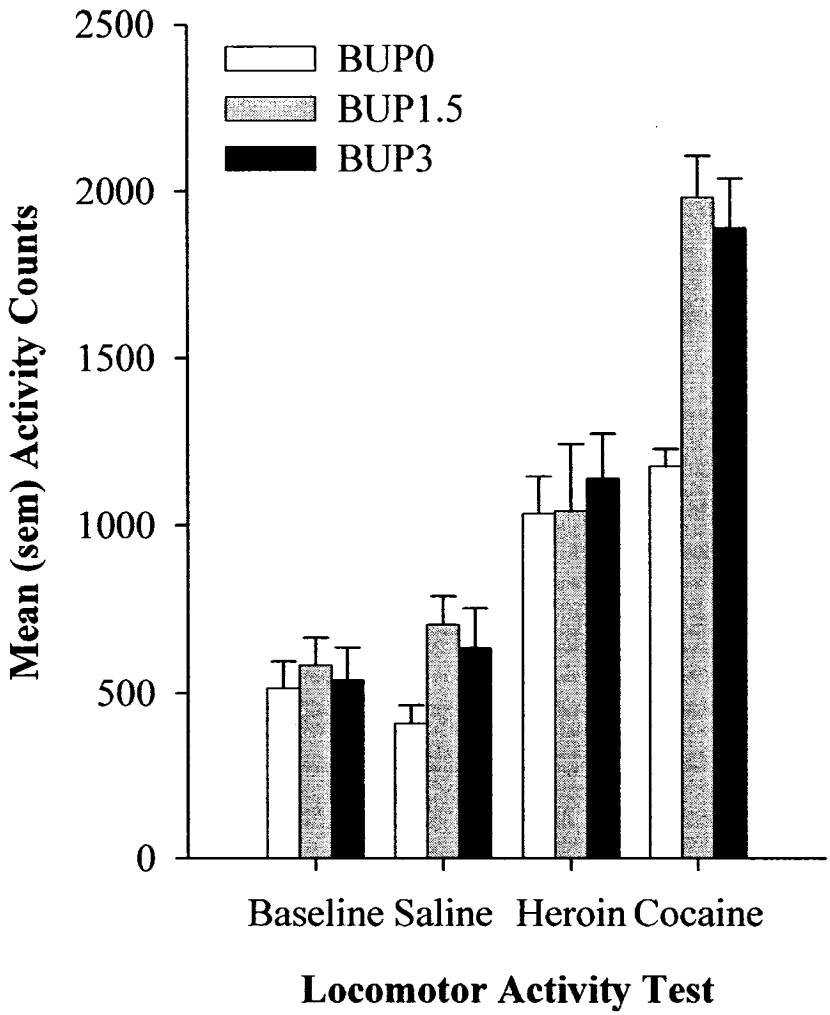


Figure 1
Mean (SEM) locomotor activity counts in buprenorphine treatment groups (BUP0, n=10: BUP1.5, n=10: BUP3, n=11) before implantation of minipumps (baseline), and in response to saline (day 4), heroin (0.25 mg/kg, sc, day 7-10) and cocaine (20 mg/kg, ip, day 7-10) injections during buprenorphine maintenance.

Microdialysis

The effect of acute administration of heroin on extracellular levels of DA in the NAc in buprenorphine-maintained rats is shown in Figure 2. It can be seen that the response to all doses of heroin was suppressed in buprenorphine-maintained rats. An ANOVA comparing the groups at each dose of heroin revealed significant main effects of Group ($F(2,41)=6.607$, $p<0.01$), Heroin Dose ($F(2,41)=5.940$, $p<0.01$) and Time ($F(5,205)=7.191$, $p<0.0001$), and a Heroin Dose by Time interaction ($F(10,205)=2.186$, $p<0.05$). Separate ANOVAs were carried out on the post-injection samples (20-120 min) for each dose of heroin. At the lowest dose of heroin (0.125 mg/kg, sc) there was no significant effect of buprenorphine treatment (Figure 2a). At the intermediate dose of heroin (0.250 mg/kg, sc), however, buprenorphine treatment suppressed the levels of DA (see Figure 2b). The ANOVA carried out on the post-injection samples revealed significant effects of Group ($F(2,13)=6.125$, $p<0.05$) and Time ($F(5,65)=3.840$, $p<0.005$). Post-hoc comparisons indicated that the BUP0 group differed significantly from both the BUP1.5 and BUP3 groups. Similar effects were seen at the highest dose of heroin (0.375 mg/kg, sc); the ANOVA revealed significant Group ($F(2,15)=3.583$, $p=0.05$) and Time ($F(5,75)=4.952$, $p<0.001$) effects. Once again the post-hoc comparisons showed that the BUP0 group had significantly higher levels of DA than either the BUP1.5 or the BUP3 buprenorphine treatment groups (see Figure 2c).

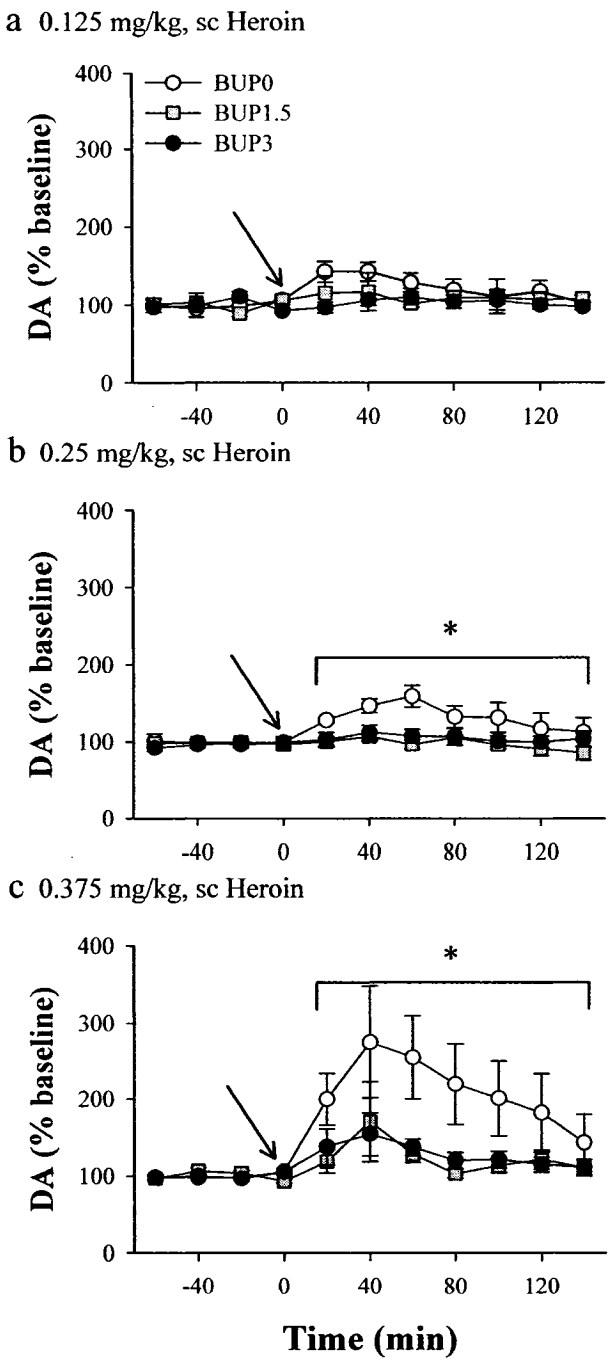


Figure 2

Mean percent increase in extracellular dopamine in the nucleus accumbens in response to acute injections of heroin (day 4 or 5) in the buprenorphine treatment groups (BUP0, BUP1.5 and BUP3). (a) 0.125 mg/kg, sc, heroin, n=6 per group. (b) 0.25 mg/kg, sc, heroin (BUP0, n=4: BUP1.5, n=6: BUP3, n=6. (c) 0.375 mg/kg, sc, heroin (BUP0, n=5: BUP1.5, n=7: BUP3, n=6). * Significant Group effects following heroin injection, $p<0.05$. Arrow indicates when the injection was given. All groups received only one dose of heroin and one of cocaine in a counterbalanced order.

The effect of buprenorphine treatment on the effects of acute cocaine injections are illustrated in Figure 3. It can be seen that the extracellular DA response in the NAc to cocaine was potentiated in buprenorphine-maintained rats. An overall ANOVA was carried out to examine the effects of each dose of cocaine in each of the buprenorphine-treated groups. This ANOVA revealed significant Group ($F(2,44)=5.532$, $p<0.01$) and Cocaine Dose ($F(2,44)=11.296$, $p<0.001$) effects, and Group by Cocaine Dose ($F(4,44)=2.874$, $p<0.05$) and Group by Cocaine Dose by Time ($F(20,220)=1.606$, $p<0.05$) interactions. Separate ANOVAs were then carried out on the data from each dose of cocaine. No Group differences were found at the lowest dose of cocaine (10 mg/kg, ip), though there was a significant effect of Time ($F(5,65)=8.652$, $p<0.001$) (Figure 3a). At the intermediate dose of cocaine (20 mg/kg, ip), buprenorphine enhanced DA overflow; there were significant effects of Group ($F(2,14)=4.739$, $p<0.05$) and Time ($5,70)=20.175$, $p<0.001$) and a Time by Group interaction ($F(10,70)=2.964$, $p<0.01$). Post-hoc analyses showed that the BUP0 group had significantly lower scores than both the BUP1.5 and BUP3 buprenorphine treatment groups (see Figure 3b). Similar effects were seen at the highest dose of cocaine (30 mg/kg, ip); as shown in Figure 3c buprenorphine augmented the effects of cocaine on NAc DA. The ANOVA revealed a significant effect of Time ($F(5,85)=2.731$, $p<0.001$); Group effect ($p=0.07$); the BUP0 group differed significantly from both the BUP3 and BUP1.5 groups.

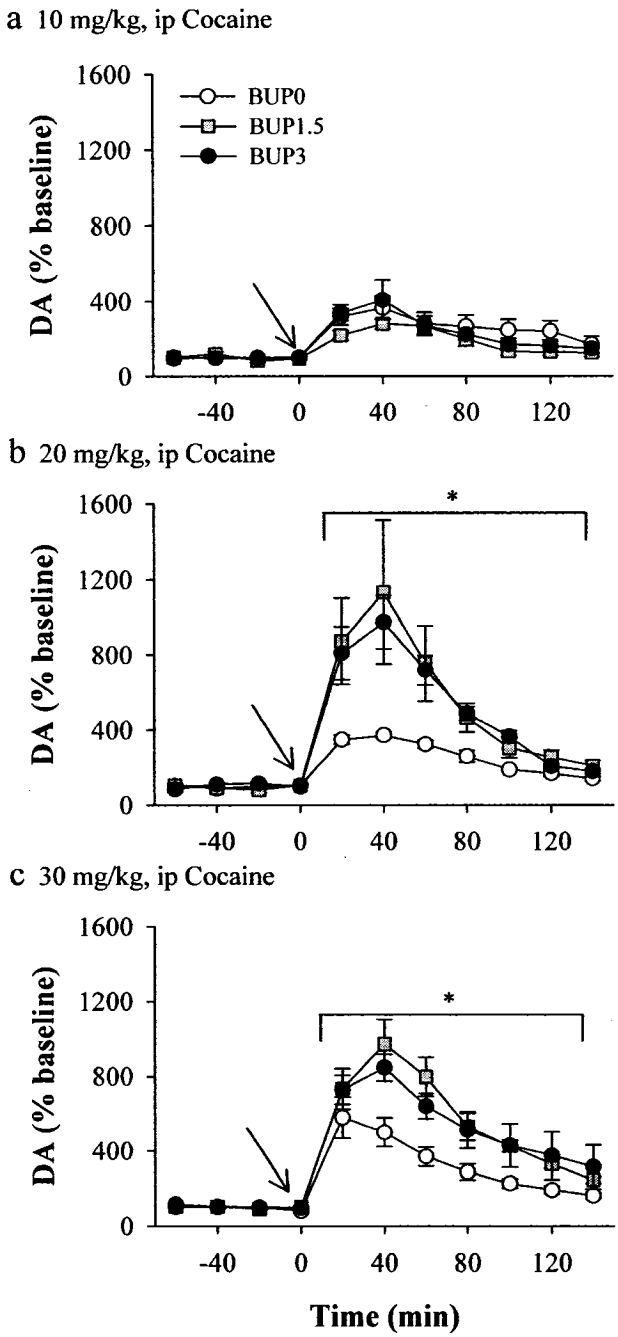


Figure 3
Mean percent increase in extracellular dopamine in the nucleus accumbens in response to acute injections of cocaine (day 4 or 5) in the buprenorphine treatment groups (BUP0, BUP1.5 and BUP3). (a) 10 mg/kg, ip, cocaine, (BUP0, n=4: BUP1.5, n=6: BUP3, n=7). (b) 20 mg/kg, ip, cocaine (BUP0, n=6: BUP1.5, n=7: BUP3, n=7. (c) 30 mg/kg, ip, cocaine (BUP0, n=5: BUP1.5, n=7: BUP3, n=6). * Significant Group effects following cocaine injection, $p<0.05$. Arrow indicates when the injection was given. All groups received only one dose of heroin and one of cocaine in a counterbalanced order.

Inspection of microdialysis probe placements in animals in all of the groups revealed considerable variability resulting in variations in basal NAc DA levels. In order to determine whether buprenorphine affected basal levels of DA we examined the actual levels in each of the treatment groups comparing only those animals with nearly identical placements within the NAc core and shell at three points anterior to Bregma (+1.2, 1.6, and 1.7 mm). Some animals were eliminated from the analysis at each distance from Bregma due to the fact that the placement of the probe was either lateral, medial or dorsal from the majority. It can be seen from Figure 4 that at each distance from Bregma there was a clear tendency for BUP1.5 and BUP3 groups to have higher levels of DA than BUP0 over the four samples. The ANOVA carried out on the data from each time point at each distance from Bregma revealed only a trend towards a Group effect ($F(2,36)=17.744$, $p=0.11$).

Taken together, irrespective of location from Bregma, there were no significant main effects, though there was a trend towards a Group effect. Though unwarranted, post-hoc comparisons revealed that, across each location, basal NAc DA levels in BUP3 rats were significantly higher than BUP0 rats ($p<0.05$) (see Figure 4). Microdialysis probe placements for all rats are shown in Figure 5.

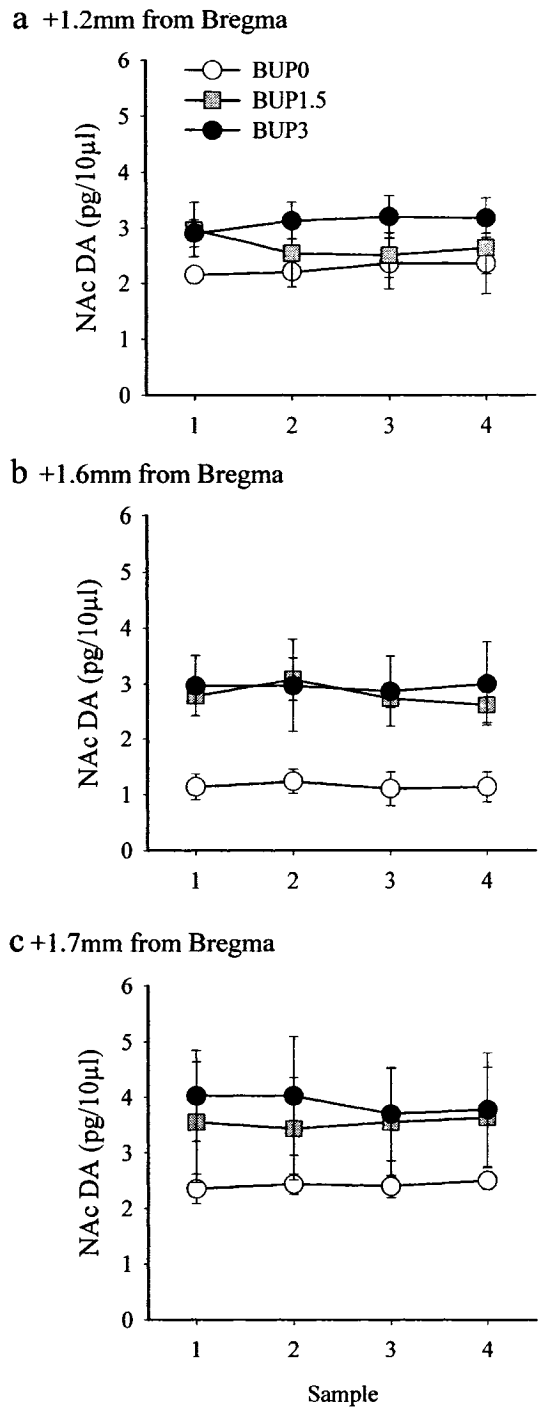


Figure 4
Mean (SEM) basal DA level (pg/ 10 µl) in four consecutive 20-min samples during in vivo microdialysis at (a) +1.2mm from Bregma (BUP0, n=2 : BUP1.5, n=3 : BUP3, n=4), (b) +1.6 mm from Bregma (BUP0, n=3: BUP1.5, n=6: BUP3, n=3) and (c) +1.7mm from Bregma (BUP0, n=8: BUP1.5, n=6: BUP3, n=4) for each buprenorphine treatment group.

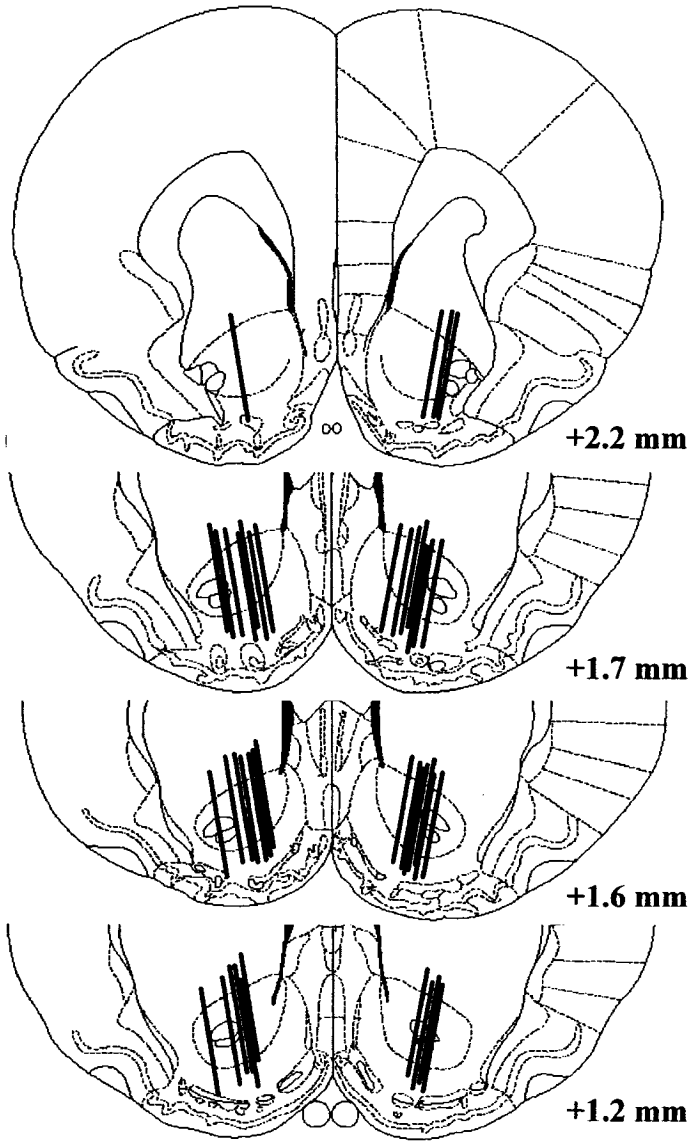


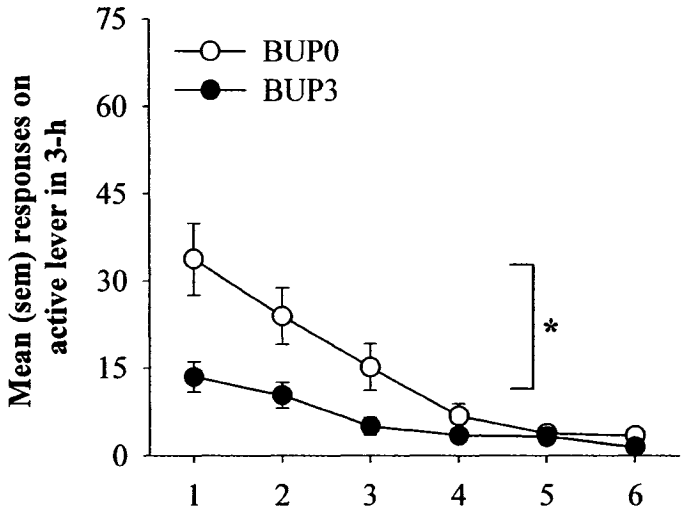
Figure 5
Microdialysis probe placements for all rats. Black lines indicate probable probe sampling area verified following histological preparation.

Self-Administration

Training. Rats developed reliable heroin and cocaine self-administration during the 8 training sessions with each drug. On the final three days of heroin self-administration, the mean number of responses on the two levers (active ± sem vs inactive ± sem) were: 32.00 ± 20.11vs 0.72 ± 0.29, 17.39 ± 5.66 vs 0.78 ± 0.26, and 13.94 ± 2.26 vs 1.06 ± 0.34. The number of responses on the cocaine levers (active ± sem vs inactive ± sem) on the last three days of training were: 47.28 ± 15.28 vs 3.50 ± 1.67, 57.78 ± 20.05 vs 4.17 ± 1.67, and 31.50 ± 4.746 vs 3.17 ± 1.40. These results show clearly that rats responded preferentially on the drug-associated levers. Rats were subsequently assigned to treatment groups matched on the basis of scores during training, at which time pumps were implanted into the BUP3 group only (n=10), while the remaining 8 rats received sham surgery (BUP0 group). The animals were returned to their home cages following surgery and extinction training began the next day.

Extinction. Buprenorphine treatment reduced drug seeking during extinction in these rats previously trained to self-administer both heroin and cocaine. It can be seen in Figure 6 that when both the heroin- and cocaine-associated levers were present responding on both levers was reduced over the first three extinction sessions in the BUP3 group compared to that in the BUP0 group. An ANOVA on data from the heroin-associated lever (Figure 6a) revealed significant effects of Group ($F(1,16)=11.11$, $p<0.005$) and Time ($F(5,80)=28.30$, $p<0.001$) and a significant Group by Time interaction ($F(5,80)=5.90$, $p<0.001$). Similarly, in the case of the cocaine-associated lever (Figure 6b), buprenorphine reduced responding; the ANOVA revealed significant effects

a Heroin Associated Lever



b Cocaine Associated Lever

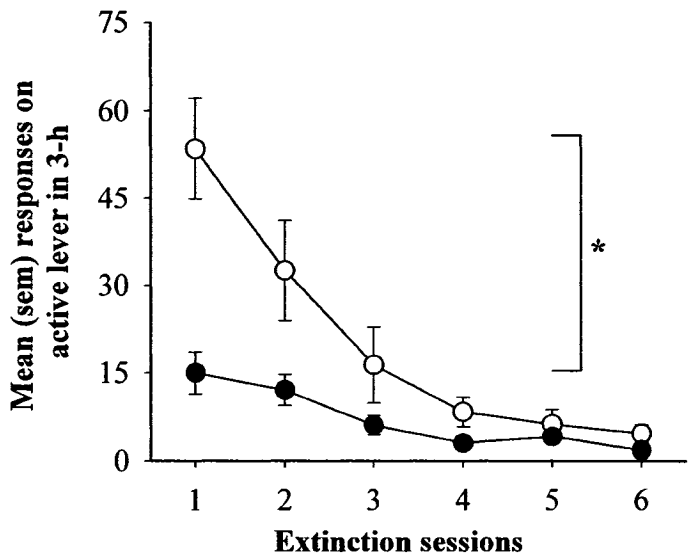
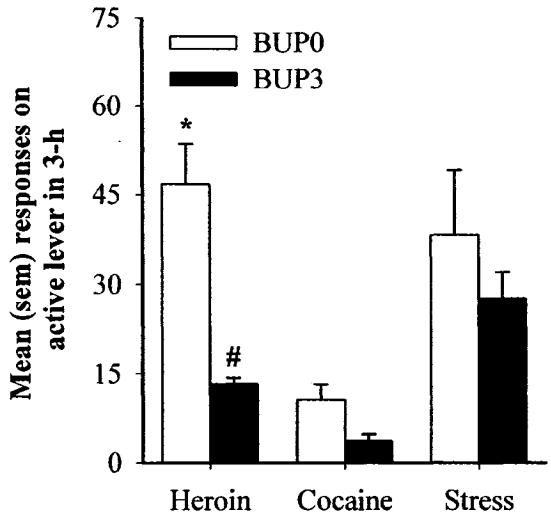


Figure 6
Total responses during extinction in each 3-h daily session. (a) Mean (SEM) responses made on the active lever previously associated with heroin by the BUP0 and BUP3 groups (b) Mean (SEM) responses made on the active lever previously associated with cocaine by the BUP0 and BUP3 groups. During extinction sessions all cues previously associated with drug availability and infusions were present. *Significant Group effect, $p<0.005$.

of Group ($F(1,16)=11.18$, $p<0.005$) and Time ($F(5,80)=29.60$, $p<0.001$) and a significant Group by Time interaction ($F(5,80)=9.79$, $p<0.001$).

Reinstatement. Three tests for reinstatement were conducted following the extinction sessions, a heroin, a cocaine and a footshock test. During these tests, both the heroin and cocaine levers were present, but pressing led only to the onset of the drug-associated cues. An overall ANOVA for Group x Test was carried out on the data from both drug-associated levers. In both the heroin and cocaine tests for reinstatement (in the BUP0 group), responding was lever selective as previously reported in such test (Leri et al., 2001, 2004); rats responded more on the heroin-associated lever after a heroin injection and more on the cocaine-associated lever after an injection of cocaine; in the test for footshock-induced reinstatement, responding on the two levers did not differ. The ANOVA revealed significant Group by Test ($F(2,32)=4.083$, $p<0.05$), Lever by Test ($F(2,32)=36.744$, $p<0.001$) and Group by Lever by Test ($F(2,32)=24.328$, $p<0.001$) interactions. As shown in Figures 7a and 7b, and supported by post-hoc analysis, buprenorphine suppressed responding after both heroin and cocaine priming injections on the lever associated with the priming drug. There was no effect of buprenorphine on the test with footshock stress.

a Heroin Associated Lever



b Cocaine Associated Lever

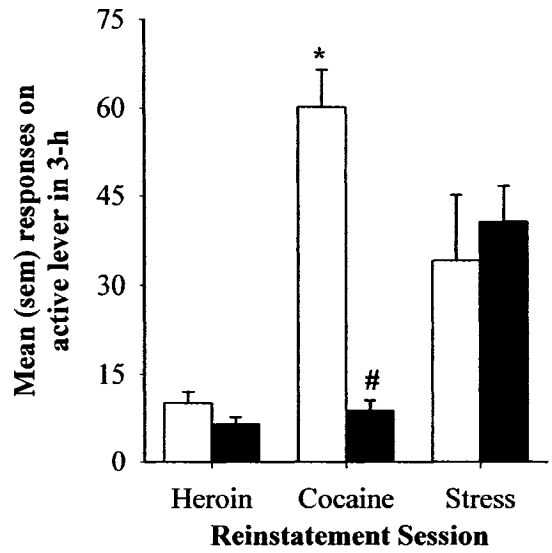


Figure 7
Total responses on the active levers during 3-h tests for reinstatement . (a) Mean (SEM) responses made on the active lever previously associated with heroin by the BUP0 and BUP3 groups following heroin (0.25 mg/kg sc), cocaine (20 mg/kg, ip, and 15 min of intermittent footshock stress (0.8 mA, 0.5s/shock, 40s mean OFF time). (b) Mean (SEM) responses made on the active lever previously associated with cocaine by the BUP0 and BUP3 groups in each of the 180-min reinstatement sessions following heroin, cocaine and footshock stress. Responding was lever selective following heroin and cocaine priming: # significant difference between the number of responses on the heroin- and cocaine-associated levers, $p<0.001$. Buprenorphine suppressed drug induced responding selectively: * significant Group effect, $p<0.05$.

DISCUSSION

The primary findings of this study are that chronic maintenance on buprenorphine significantly reduces drug seeking during extinction in the presence of drug associated cues and reduces reinstatement of drug seeking in response to both heroin and cocaine, but has no effect on reinstatement induced by footshock stress. Interestingly, these findings parallel those recently reported from this laboratory on the effects of methadone maintenance via minipump (Leri et al., 2004) and suggest that maintenance on buprenorphine has the potential to reduce both heroin- and cocaine-seeking. These data cannot be explained by a suppression of general activity by buprenorphine; in fact, rats maintained on buprenorphine had slightly higher basal levels of activity than rats without buprenorphine. In this regard it is interesting that the buprenorphine-maintained rats also had higher basal levels of DA in the NAc, suggesting that chronic low level activation of DAergic function might play a role in suppression of drug seeking, but in our view this, in itself, seems an unlikely explanation. However, because of these findings on the effects of buprenorphine on basal levels of DA in the NAc and because the effects of buprenorphine paralleled so closely the effects seen previously with methadone in our studies (Leri et al., 2004), we carried out a subsequent study on the effects of methadone delivered by osmotic minipump on basal levels of DA. Interestingly, the effect of methadone was similar to that of buprenorphine; 30.0 and 40.0 mg/kg/day methadone yielded mean basal levels of 7.5 pg and 7.6 pg/10 µl respectively, compared to 4.5 pg/10 µl for the control group. (Note that the overall levels of basal DA in this experiment were higher than in the buprenorphine study probably as a result of more dorsal placements of the dialysis probes.)

Even if the higher basal levels of DA in the NAc were contributing to the reduced effectiveness of drug-related cues in extinction conditions, it is not easy to relate the findings on suppression of drug-induced reinstatement by buprenorphine to the effects of buprenorphine on the acute DAergic response to heroin and cocaine. Buprenorphine attenuated the extracellular levels of DA in the NAc in response to heroin, but potentiated the DA response to cocaine. Furthermore, chronic buprenorphine had no effect on heroin-induced locomotor activity, but significantly elevated cocaine-induced activity, as previously reported with acute buprenorphine (Smith et al., 2003). It can be noted that significant differences were never found between the doses of buprenorphine used. This may be due to the finding of Greenwald et al. (Greenwald et al., 2003) that a dose of 16 mg results in 80 $\pm$ 2% mu-opioid receptor occupancy, whereas twice this dose (32 mg) results in only 84 $\pm$ 2% occupation.

Buprenorphine is a partial *mu-opioid* agonist with antagonistic properties at the *kappa* and *delta* opioid receptors. It is characterized primarily by its actions at the *mu* receptor and its therapeutic effects are attributed to its high affinity and slow dissociation (Tzschentke, 2002). Buprenorphine, like morphine, is thought to exert its effects in the brain by activation of *mu-opioid* receptors located on GABA interneurons in the ventral tegmental area (VTA) (Devine, Leone, Pocock, & Wise, 1993; Garzon et al., 2001). Chronic administration of buprenorphine would maintain continuous activation of opioid receptors, thus causing chronic elevation of DA levels within the mesolimbic system (see Figure 4), unless tolerance were developing. The finding that chronic buprenorphine maintenance (3.0 mg/kg/day) reduced heroin-induced reinstatement of drug responding is consistent with the findings from microdialysis and what could be predicted from the

pharmacokinetics of the two drugs. The high affinity for, and the slow dissociation from, the *mu-opioid* receptor would make buprenorphine somewhat of a "sticky' drug that would not be readily displaced by heroin. However, buprenorphine (BUP1.5 and BUP3) did not block the stimulatory effect of the acute injection of heroin, and only attenuated the accompanying rise in extracellular DA in the NAc, suggesting that tolerance had not developed.

Despite the fact that buprenorphine reduced cocaine-induced reinstatement, there was a clear potentiation of the locomotor effect of an acute injection of cocaine. Additionally, a significant increase in extracellular DA levels following cocaine was observed in the buprenorphine-treated rats. This effect was not surprising; synergistic effects between opiates and stimulants have been found in many situations including: place conditioning (Brown et al., 1991), locomotion (Smith et al., 2003), conditioned reward (Cunningham & Kelley, 1992), rotational behavior in rats with lesions of the substantia nigra (Kimmel et al., 1997) and drug toxicity (Plunkett, Seifen, & Kennedy, 1989). Therefore, it remains somewhat puzzling that buprenorphine (3.0 mg/kg/day) reduced cocaine-induced reinstatement of drug-seeking over the full 3-h session, while potentiating the rise in extracellular DA levels and the accompanying rise in locomotion. One explanation might have been that the combination of buprenorphine and cocaine and the accompanying high levels of DA were experienced as a very high dose of cocaine making it likely that responding (cocaine seeking) would be low initially, but persist as the effects of the drug diminished. The fact that this is not what was observed, suggests that the effect of buprenorphine was to reduce drug seeking directly and, as in the case of extinction, the salience or effectiveness of the drug-related cues.

Although buprenorphine treatment (3.0 mg/kg/day) was effective at reducing spontaneous and drug-induced reinstatement of responding for both heroin and cocaine, it was ineffective at reducing responding following footshock stress. This finding is consistent with previous research from this lab demonstrating that neither heroin (Shaham et al., 1996a) nor methadone (Leri et al., 2004) maintenance, nor other opiate antagonists (Shaham et al., 1996b) are effective at reducing footshock stress-induced reinstatement. Once again, this is evidence for a dissociation between the systems underlying drug- and stress-induced responding (Stewart, 2000; Stewart, 2003). As mentioned previously, the constellation of findings seen here in rats given buprenorphine chronically via osmotic minipumps resembles closely that found in similar experiments from this laboratory in rats treated chronically with methadone (Leri et al., 2004). Methadone, significantly reduced extinction responding in rats trained to self-administer both heroin and cocaine and reduced reinstatement of lever-pressing after priming injections of heroin and cocaine selectively on the heroin- and cocaine-associated levers without affecting reinstatement induced by footshock stress. Furthermore, these effects were found in spite of the fact that methadone slightly enhanced spontaneous locomotor activity and did not suppress the effects of cocaine on locomotion. Taken together these finding suggest that chronic treatment with relatively high doses of drugs such as buprenorphine and methadone, both of which have been found to have beneficial effects in the treatment of opioid addiction, have the capacity to suppress the effectiveness of drug-related cues (as in extinction conditions) and to reduce drug seeking induced by both opioid and stimulant drugs.

Finally, although it has been shown that both buprenorphine and methadone are effective in reducing spontaneous and drug-induced reinstatement of heroin- and cocaine-seeking, we have yet to determine the mechanisms whereby they act. To date, we have searched for a relation between their effects on drug seeking and their effects on DAergic activity. Both drugs enhance basal levels of DA within the NAc, do not suppress cocaine induced locomotor activity, and in the case of buprenorphine at least, enhance the DAergic response to cocaine. Thus is seems clear that in the presence of buprenorphine enhanced dopaminergic activity in the NAc is not sufficient to induce drug seeking. Recent studies suggest that the actions of glutamate in the NAc are critical for the induction of cocaine seeking by priming injections of cocaine in otherwise drug-free rats. Furthermore, there is evidence that it is a projection from the medial prefrontal cortex that is involved (Capriles, Rodaros, Sorge, & Stewart, 2003; McFarland, Lapish, & Kalivas, 2003; Kalivas, 2004). Interestingly, in this context, it has been shown in slices taken from rats chronically treated with morphine that NMDA activity and possible glutamate release in the NAc core is significantly decreased (Martin, Przewlocki, & Siggins, 1999; Martin et al., 1999) Thus, it may prove interesting to study the effects of buprenorphine on the activity of these prefrontal glutamate projections and on levels of glutamate in the NAc.

ACKNOWLEDGEMENTS

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The previous chapter entitled "Rats maintained chronically on buprenorphine show reduced heroin and cocaine seeking in tests of extinction and drug-induced reinstatement" was published in *Neuropsychopharmacology* in 2005 and was based on an earlier article from our lab with chronic methadone treatment. Chapter 1 includes data indicating that buprenorphine, when administered via osmotic minipump, reduces heroin and cocaine seeking in extinction and following drug prime injections of either drug. This effect is in spite of a blockade of the NAc DA response to acute injections of heroin and a potentiated response to acute injections of cocaine. The timing of the buprenorphine treatment immediately following self-administration training was done to model the period of drug abstinence at the outset of opioid abuse treatment when drug seeking behavior is most likely to occur and treatments must be initially very effective. The results are discussed in terms of a reduced responsiveness to drug-associated cues as a result of buprenorphine treatment. There is evidence in humans and monkeys that buprenorphine reduces heroin and cocaine intake and so Chapter 2 sought to investigate the effect of chronic buprenorphine of heroin and cocaine intake. The previous chapter found that there was less drug-seeking behavior, but the drug was not available to the rats in that case, whereas, in this chapter, we allowed the rats to self-administer heroin or cocaine while under treatment.

The following manuscript, entitled "The effects of chronic buprenorphine on intake of heroin and cocaine in rats and its effects on nucleus accumbens dopamine levels during self-administration," is currently in the journal *Psychopharmacology*. The following series of experiments endeavors to determine the unique interactions between buprenorphine and heroin and cocaine through the use of various schedules of

reinforcement, various doses of heroin and cocaine and through the use of *in vivo* microdialysis during self-administration. In addition, we analyzed the self-administration session in 10-minute intervals to determine if there was a change in the pattern of intake of heroin or cocaine while under chronic buprenorphine treatment.

CHAPTER 2

The effects of chronic buprenorphine on intake of heroin and cocaine in rats and its effects on nucleus accumbens dopamine levels during self-administration

Robert E. Sorge and Jane Stewart

Sorge, R. E., & Stewart, J. (2006). The effects of chronic buprenorphine on intake of heroin and cocaine in rats and its effects on nucleus accumbens dopamine levels during self-administration. *Psychopharmacology, 188:* 28-41. With kind permission of Springer Science and Business Media.

Abstract

Rationale: Buprenorphine reduces both heroin and cocaine intake in opioid addicts, but the mechanisms remain unclear. *Objectives*: To determine the effects of chronic buprenorphine treatment on intake of heroin and/or cocaine and measure nucleus accumbens (NAc) dopamine (DA) levels during self-administration. *Methods*: In Experiment 1, plasma levels of buprenorphine were determined in rats with buprenorphine osmotic minipumps (3.0 mg/kg/day) using an ELISA. In Experiment 2, rats self-administered (FR1) one dose of heroin (0.025, 0.05 or 0.1 mg/kg/inf) and one dose of cocaine (0.25, 0.5 or 1.0 mg/kg/inf), before and under sham or chronic buprenorphine treatment (1.5 or 3.0 mg/kg/day). In Experiment 3, the effect of sham or chronic buprenorphine treatment (3.0) on heroin (0.05) or cocaine (0.5) self-administration under FR5 and progressive ratio (PR) schedules was evaluated. In Experiment 4, *in vivo* microdialysis sampling from the nucleus accumbens (NAc) was carried out during heroin (0.05) or cocaine (0.5) self-administration (FR1) under sham or buprenorphine treatment (3.0). *Results:* Buprenorphine levels in plasma were stable over time. Buprenorphine treatment had no effect on total heroin intake at any dose or under any schedule, whereas it suppressed cocaine intake at all doses and under all schedules. Buprenorphine enhanced basal levels of DA, attenuated the NAc DA response to heroin and enhanced the DA response to cocaine. Interestingly, buprenorphine increased the latency to respond to drug-associated cues at the start of self-administration sessions. *Conclusions:* Chronic buprenorphine reduces cocaine, but not heroin, intake and possibly reduces drug seeking by reducing the salience of the drug-associated cues.

Keywords: buprenorphine, heroin, cocaine, self-administration, microdialysis, addiction, dopamine, nucleus accumbens, cues, reinforcement

Introduction

The partial mu-opioid receptor agonist, buprenorphine, is currently one of two opioid agonist treatments for opioid addiction. Interestingly, among opioid addicts, a high proportion of whom co-use cocaine (Kosten, Gawin, Rounsaville, & Kleber, 1986; Schutz, Vlahov, Anthony, & Graham, 1994), there is evidence that buprenorphine, in addition to reducing opioid use (Mello et al., 1980), can reduce cocaine use (Strain et al., 1996; Montoya et al., 2004). Although this is not found consistently (Compton, Ling, Charuvastra, & Wesson, 1995; Schottenfeld et al., 1997), there is sufficient evidence to stimulate an interest in determining the mechanisms that underlie the effectiveness of buprenorphine on drug-seeking and taking of both heroin and cocaine.

Mello and colleagues found that daily or alternate day *bolus* injections of buprenorphine were effective in reducing heroin (Mello et al., 1998), cocaine (Mello et al., 1992), or heroin-cocaine "speedball" self-administration in monkeys working on a complex second order, cue-controlled, schedule of reinforcement (FR4[VR16:S]) while leaving food self-administration unaffected (Mello et al., 1998). Similarly, Winger et al (1992) found, using an FR30 schedule with 45-sec periods of time out in monkeys, that buprenorphine reduced responding for the opioid alfentanil and cocaine. Interestingly, however, although responding was reduced in this experiment, the dose-effect curves for these drugs were not shifted laterally. In previous studies, we showed that chronic buprenorphine delivered by osmotic minipumps reduced both heroin and cocaine seeking under extinction conditions with drug-related cues present and following drug priming injections in rats trained to self-administer both drugs (Sorge, Rajabi, & Stewart, 2005). These findings suggested to us that buprenorphine might have its common effects on

heroin and cocaine seeking by reducing the ability of drug-related cues to initiate and maintain behavior. Furthermore, we speculated that the common effects of buprenorphine reported for heroin and cocaine self-administration might be accounted by similar mechanisms. Thus, the present experiments were carried in rats to study the effect of buprenorphine on self-administration of heroin and cocaine in an attempt to determine the basis of its effect on drug taking.

Because we used osmotic minipumps to provide continuous infusion of buprenorphine in all experiments, blood levels were analyzed in a group of rats after 3, 14, 21 and 28 days of continuous treatment and 7 days after minipump removal (Experiment 1). In Experiment 2, three groups of rats were trained to self-administer one dose of heroin (0.025, 0.05 or 0.1 mg/kg/inf) and one dose of cocaine (0.25, 0.5 or 1.0 mg/kg/inf) on alternate days and then each group was divided into three and exposed to one of three treatments (sham, or 1.5 [BUP1.5] or 3.0 [BUP3.0] mg/kg/day buprenorphine via minipump) for a total of nine groups. In Experiment 3, another four groups of rats were trained to self-administer either heroin (0.05 mg/kg/inf) or cocaine (0.5 mg/kg/inf) on FR1, FR5 and progressive ratio (PR) schedules before and during exposure to either sham or 3.0 mg/kg/day of buprenorphine via minipump. Finally, in Experiment 4, four additional groups of rats were trained to self-administer either heroin or cocaine and were then exposed to sham or 3.0 mg/kg/day of buprenorphine. On the third day of treatment, during 3-hour self-administration sessions, levels of dopamine (DA) were measured in the nucleus accumbens (NAc) in microdialysis dialysate samples collected every 10 min.

Materials and Methods

Subjects. The subjects were 115 male Long-Evans rats (Charles River, St. Constant, QC) weighing approximately 300-350g at the start of the experiments. The rats were singly housed in stainless steel hanging wire cages or in plastic housing cages within the Concordia University Animal Care Facility in a reverse light-dark cycle room (light onset 2000h, offset 0800h). The rats had food (Rat Chow, Purina Foods) and water *ad libitum* in their home cages. All training and testing was carried out during the dark cycle starting at 0900h or 1200h. All procedures followed the guidelines of the Canadian Council on Animal Care and were approved by the Animal Care Committee at Concordia University.

Intravenous Catheterization. All rats had intravenous catheters implanted in the right jugular vein as previously described (Sorge et al., 2005). Immediately following surgery, rats were administered penicillin (Pen G, 40 000 IU/kg) and ketoprofen (Ketofen™, 0.5 mg/kg). The blocker and cap were removed during self-administration sessions. Rats were flushed with 0.2 mg/ml heparin-saline solution (ICN Biomedicals, Cleveland, OH) to prevent blood clotting in the catheter following the self-administration sessions every second day. A subset of the rats, namely those in Experiment 4, had unilateral stainless steel 20 gauge cannula (Plastics One, Roanoke, VA) implanted aimed at the NAc (NAc: AP +1.6 mm, ML +2.8 mm, DV -5.5 mm from bregma) at an angle of 10° to avoid extensive damage to the ventricle, while maximizing the surface area of the probe within the NAc (core and shell) (Paxinos & Watson, 1986).

Osmotic Minipump Implantation. Chronic exposure to buprenorphine was achieved through the use of osmotic, buprenorphine -filled, minipumps implanted subcutaneously

(sc), in most cases, following the daily self-administration session to allow at least 21 hours of exposure to buprenorphine and recovery from surgery before the next session. Rats were lightly anesthetized using Isoflurane (Vetoquinol N.A. Inc, Lavaltrie, QC) and a small incision was made in the skin between the scapulae. Using a hemostat, a small pocket was created by separating the connective tissues under the skin into which the osmotic buprenorphine-filled (BUP1.5 or BUP3.0) minipumps (Alzet model 2ML2, 0.5 µl/hr flow rate, Durect Corp., Cupertino, CA) were implanted. The flow modulator was directed away from the incision to avoid leakage of the drug prior to healing of the wound. The incision was closed using wound clips. Pumps were removed under anesthesia in a similar manner. In the case of the sham groups, rats underwent similar surgical procedures with the exception of the insertion of a pump. It is to be noted that, in pilot experiments conducted in this laboratory using methadone minipumps in studies of heroin and cocaine reinstatement of drug seeking, saline-filled pumps were implanted in control group rats. Rats readily tolerated these pumps and no effects on ongoing self-administration behavior were observed in rats with saline-filled pumps. Similar findings have been reported elsewhere (Kunko, French, & Izenwasser, 1998; Rada, Jensen, & Hoebel, 2001; Vann, Balster, & Beardsley, 2006).

In Vivo Microdialysis and High Performance Liquid Chromatography. All *in vivo* microdialysis sampling was done via dual channel swivels (Instech Corporation, Plymouth Meeting, USA) in the self-administration chambers described below.

Microdialysis Probe. The dialysis probes (custom made) were made from a 2.5 mm length of semi-permeable dialysis membrane (Fisher Scientific, 240 um OD, 13000 MW cutoff), closed at one end and attached to a 21 mm long, 26 gauge piece of stainless steel

tubing. The complete microdialysis experimental setup has been previously described (Sorge et al., 2005). The probes were inserted the evening before microdialysis testing and, to prevent occlusion, aCSF (145 mM Na^+, 2.7 mM K^+, 1.2 mM Ca^{2+}, 1.0 mM Mg^{2+}, 150 mM Cl^-, 0.2 mM ascorbate, 2 mM Na_2HPO_4, pH, 7.4 ± 0.1) was perfused steadily at a rate of 0.15 µl/min from the time of implantation.

High Performance Liquid Chromatography. Dialysate was collected and frozen immediately upon collection. During HPLC dialysate analysis, a 10 µl volume of dialysate was extracted from each thawed sample and analyzed using one of two similar HPLC systems with electrochemical detection (HPLC-EC) (Sorge et al., 2005). The mobile phase was adjusted to allow for the separation and quantification of DA, DOPAC, HVA and HIAA within a single run (though only DA is shown).

Drugs. Heroin (diacetylmorphine HCl) was purchased from Almat Pharmachem Inc (Concord, ON), cocaine (cocaine HCl) from Medisca Pharmaceutique (Montreal, QC) and buprenorphine HCl from Reckitt Benckiser Healthcare Limited (Hull, UK). The doses of buprenorphine used in this experiment ([BUP1.5] 1.5 mg/kg/day or [BUP3.0] 3.0 mg/kg/day via 14-day minipumps) were chosen on the basis of previous research in our laboratory (Sorge et al., 2005). In cases where only one dose of buprenorphine was used, we used the highest dose (3.0 mg/kg/day).

Self-Administration Apparatus. Sixteen custom-made Plexiglas operant conditioning chambers enclosed in sound-attenuating plywood box (Concordia University, Montreal, QC) were used in these experiments. Each operant conditioning chamber had four levers (Med Associates, Lafayette, IN) located 10 cm above the floor of the chamber: two retractable ("active") and two stationary ("inactive"), such that the left and right walls

were equipped with one active and one inactive lever. The retractable levers signaled to an infusion pump (Razel Scientific Instruments, Stamford, CT) positioned outside the chamber for the delivery of drugs. The stationary levers served to control for baseline, non-reinforced, operant behavior. Two white light stimuli located 3 cm above the active levers were illuminated for 30 sec (left: constant light, right: flashing light) at the beginning of the session, and for the duration of each drug infusion (10 sec), thus serving as a discrete conditioned stimuli (CS) for drug delivery. Throughout the experiment, each self-administration session commenced by the illumination of a red house light that remained on for the duration of the session. Rats in Experiment 3 and 4 self-administered either heroin or cocaine paired with the constant light CS.

Self-Administration Procedures. Rats were given daily drug self-administration sessions lasting 3 hours/day, 7 days a week. Each rat had access to a specific chamber and had access at the same time (0900 or 1200h) each day. For each self-administration session, the rats were transported from the colony to the experimental room and placed in the operant conditioning chambers (with the exception of rats in Experiment 4, see below). Once the protective cap and blocker were removed, the rat was connected to the drug infusion tubing with a protective spring sleeve screwed to the skull-mounted cannula. This functioned to protect the tubing from the rat and to prevent the tubing from detaching from the cannula. Once the rats were secured in their respective chambers they were subjected to a 5-min time-out period that allowed the rats to acclimate to the chambers. Following the 5-min period, the red house light was illuminated and remained on for the duration of the session. Ten sec later the retractable lever was extended and the cue light was activated in its predetermined pattern for 30 sec. This

light remained activated for 30 sec unless a response was made; if there was a response, it stayed on for the duration of the 10-sec drug infusion. A fixed ratio 1 schedule of reinforcement (FR1) was used initially in all experiments such that the first active lever press resulted in a 10-sec drug infusion (approximately 64 µl). During the infusion, presses on the active lever had no additional consequence, but were recorded, as were presses on the inactive lever. Following the 3-hour session, the active lever was retracted and the house light was extinguished.

Inactive Lever Presses. At no time were there effects of buprenorphine on the levels of inactive lever presses and, thus, these data are not shown.

HPLC Analysis. Dialysate samples from individual rats were thawed and analyzed consecutively using one of two HPLC-EC systems and the assignment of the animals to each system was counterbalanced across all treatment conditions.

Postmortem Tissue Analysis. Following the microdialysis session, the rats were anesthetized and perfused intracardially with saline and formaldehyde (Formalin 10%V/V, Anachemia, Montreal, QC) before brain removal. Frozen sections were taken, mounted and stained with cresyl violet.

Statistical Analysis. All data were analyzed using analysis of variance (ANOVA) with post-hoc comparisons made using Fisher's LSD test ($p<0.05$).

Experiment 1: Determination of buprenorphine plasma levels in rats

Procedures. Four rats had buprenorphine-filled osmotic minipumps (BUP3.0) implanted following catheter surgery and one rat had sham surgery. Blood was withdrawn via the intravenous catheters after 3, 14, 21 and 28 days of buprenorphine treatment and 7 days following removal of the minipumps. It is to be noted that the minipumps were replaced

after 14 days. At each time point the blood was centrifuged and plasma was extracted and frozen at -80°C until analysis. A specific enzyme-linked immunosorbant assay (ELISA) for buprenorphine (Neogen Corp., Lexington, KY) was performed following product directions for quantification based on comparison to a calibrated standard curve. Blood plasma samples were diluted (1:5) in the supplied buffer and kit sensitivity was reported to be 1 ng/ml for buprenorphine. A one-way ANOVA (Days) was performed on values obtained from the ELISA.

Results. The mean plasma level of buprenorphine over the period of treatment (28 days) was 10.85 ± 0.98 ng/ml. The ANOVA showed no effect of Days ($F(3, 7)=0.781$, ns), indicating that the levels remained constant as long as pumps were present. Interestingly the levels were 5.23 ± 0.68 ng/ml one week following removal of the buprenorphine-filled minipumps.

Experiment 2: Effects of chronic buprenorphine on heroin and cocaine self-administration.

Procedures. Three groups of rats were trained to self-administer one dose of heroin (0.025, 0.05 or 0.1 mg/kg/inf) and one dose of cocaine (0.25, 0.5 or 1.0 mg/kg/inf) in the same operant chambers on alternate days for 8 days. The choice of the cocaine and heroin doses for each group was based on an earlier study in which we found similar self-administration dose response curves for these drugs whether rats were trained to take both or only one of the drugs (Leri et al., 2001). The order of the first drug exposure was counterbalanced, such that some rats had access to heroin first and others cocaine first. Each drug was paired with a distinct lever and light pattern as outlined above, such that an "active" and an "inactive" lever were in use each day. Half of the rats had heroin

paired with the right lever (flashing cue light) and cocaine with the left lever (constant cue light), whereas the remaining rats had the opposite association.

Following the 8th self-administration session (i.e. 4 days with access to each drug), each group was divided into three and exposed to one of three buprenorphine treatments (sham, 1.5 [BUP1.5] or 3.0 [BUP3.0] mg/kg/day buprenorphine via minipump) for a total of nine groups. With the exception of those groups that self-administered the intermediate doses of heroin and cocaine, group size ranged from 4-6 rats. Because all subsequent experiments were done using these intermediate doses of heroin (0.05) and cocaine (0.5), two replications were done at these doses resulting in 11 rats in each of the buprenorphine conditions. Following surgery for implantation of minipumps rats were returned to their home cages and allowed to recover overnight. The next day self-administration sessions continued as before for an additional 8 days.

Results. Heroin Self-Administration. All rats acquired reliable self-administration of heroin before buprenorphine treatment (less than 20% variability over 2 consecutive sessions). Separate ANOVAs (for before and during buprenorphine treatment) carried out on the number of infusions per day for all groups at all doses of heroin revealed no effect of Day, although there was a tendency for responding to increase across days of training for both groups (Before: $F(3, 159) = 2.49$, $p = 0.06$) but not during treatment (During: $F(3, 159) = 0.39$, $p = 0.76$); thus the data were averaged across days and subsequent analyses were carried out on the mean number of infusions per day for each rat in each condition.

Figure 8a shows the mean daily number of infusions of heroin in the nine treatment groups before and during buprenorphine treatment. An ANOVA carried out on

infusions per day during training revealed a significant effect of Heroin Dose ($F(2, 53) = 12.98$, $p<0.001$). As expected, rats took more infusions at the lower doses (Figure 8a-Before). The ANOVA for the number of infusions of heroin per day during buprenorphine treatment again revealed a significant effect of Heroin Dose ($F(2, 53) = 8.68$, $p<0.001$). Interestingly, however, there was no significant effect of buprenorphine on the number of infusions of heroin taken, regardless of dose of heroin, though there was a trend ($F(2, 53) = 2.81$, $p=0.07$). We noted, however, that buprenorphine-treated rats tended to make fewer non-reinforced lever presses during the presentation of light cues that accompanied each infusion than did untreated rats (sham, 21.96 ± 10.17; BUP1.5, 7.56 ± 1.19; BUP3.0, 7.69 ± 1.20). We have recently found that chronic buprenorphine treatment reduced non-reinforced responses in rats trained to self-administer sucrose pellets while also reducing intake of sucrose pellets (unpublished observations).

Cocaine Self-Administration. All rats acquired reliable self-administration of cocaine before buprenorphine treatment began (less than 20% variability over 2 consecutive days). Figure 8b shows the mean daily number of infusions of cocaine in the same nine treatment groups before and during buprenorphine treatment. Analysis of the number of infusions of cocaine before and during buprenorphine treatment revealed no significant Day effect in either period (Before: $F(3, 159) = 0.87$, ns; During: $F(3, 159) = 0.36$, ns); therefore, as in the case of heroin, the data were analyzed and displayed as the mean number of infusions per day in each of the two conditions. An ANOVA of the number of infusions per day during training revealed a significant main effect of Cocaine Dose ($F(2, 53) = 33.36$, $p<0.001$); more infusions were taken at the lowest dose. More importantly,

rats treated with buprenorphine significantly reduced their cocaine intake. There were significant main effects of both Buprenorphine Dose ($F(2, 53) = 42.89$, $p<0.001$) and Cocaine Dose ($F(2, 53) = 7.27$, $p<0.01$) and a significant Buprenorphine Dose by Cocaine Dose Interaction ($F(4, 53) = 7.10$, $p<0.001$). Across all doses of cocaine, sham rats were significantly different from both the BUP1.5 and the BUP3.0 groups ($p's<0.001$) during buprenorphine treatment.

Experiment 3: Effect of chronic buprenorphine on heroin and cocaine self-administration under different schedules of reinforcement.

Procedures. Because of the nature of the effects of buprenorphine on self-administration on the FR1 schedule in Experiment 2 (no effect on heroin intake and reduction of cocaine intake), it was important to determine whether these effects would be altered under schedules with greater response requirements. In the case of heroin, it might have been that, although buprenorphine had no effect when drug was readily available on an FR1 schedule, responding would be reduced under an FR5 or a PR schedule when the work requirements were greater. In the case of cocaine, it seemed possible that the reduced intake under the FR1 schedule in buprenorphine-treated rats could have resulted from an enhanced DA response to cocaine (Sorge et al. 2005) and/or an enhanced reinforcing impact of cocaine (Brown et al. 1991). Thus, four additional groups of rats were trained to self-administer heroin (0.05 mg/kg/inf) or cocaine (0.5 mg/kg/inf) on an FR1 schedule of reinforcement in 3-hour sessions for 3 days, followed by 2 days on an FR5 schedule and one day on a progressive ratio (PR) schedule. The formula for the progressive ratio was as follows: $5e^{(0.2 \times \text{Infusion \#})-5}$ and the session length was maintained at 3 hours. After this PR session, minipumps were implanted as described (sham or 3.0 mg/kg/day

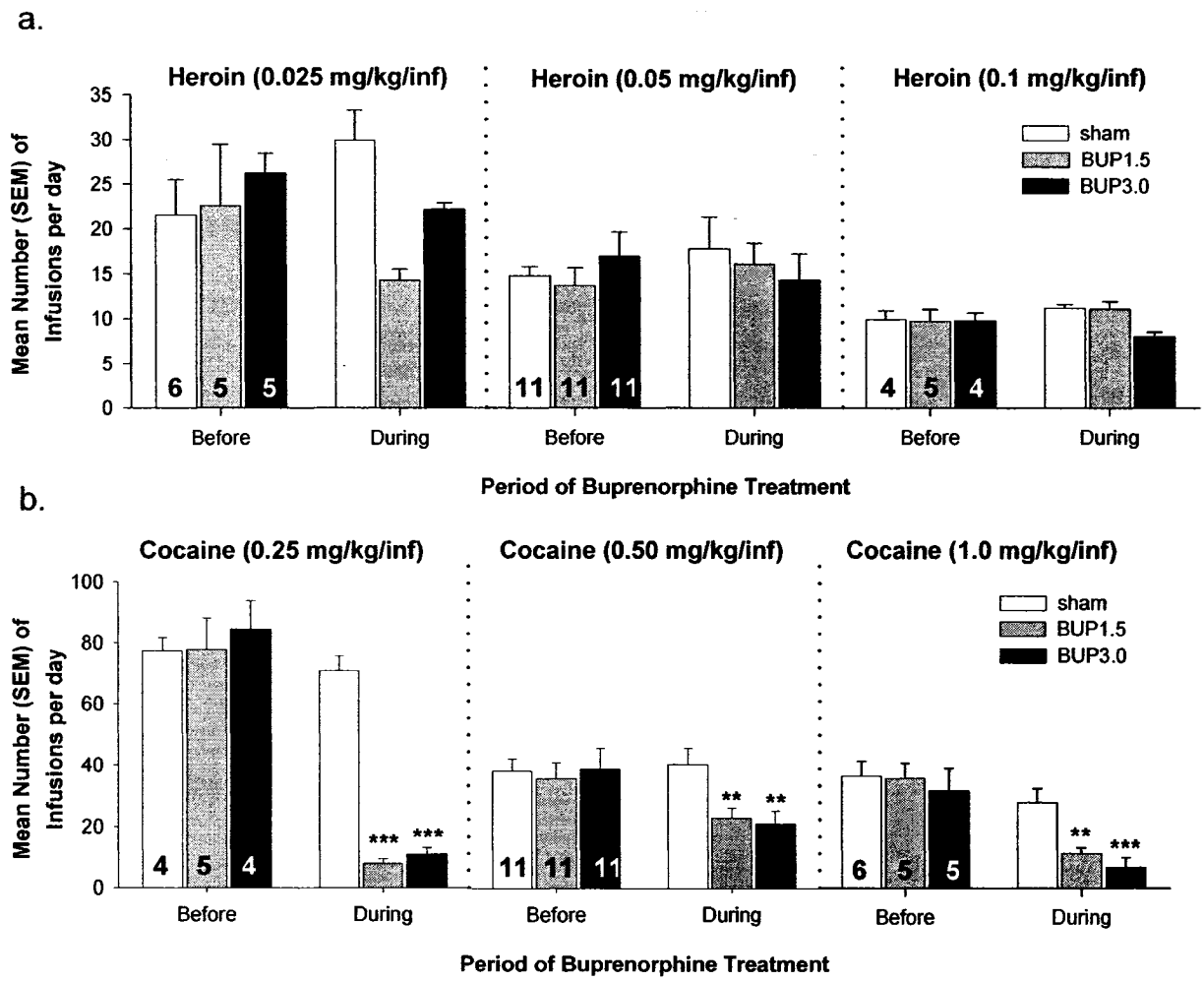

Figure 8.
Heroin and cocaine self-administration before and during buprenorphine treatment for rats in Experiment 2. (a) Mean number (± SEM) of infusions of heroin per day over 4 days of alternate day training and 4 days of buprenorphine treatment for each of the three buprenorphine treatment groups (BUP0, n=21;BUP1.5, n=21; BUP3.0, n=20) at each dose of heroin (3 hours/day, FR1 schedule). Buprenorphine treatment had no effect on heroin intake at any dose of heroin. Rats did, however, take more infusions of lower than of higher doses of heroin. (b) Mean number (± SEM) of infusions of cocaine per day over 4 days of alternate day training and 4 days of buprenorphine treatment for each of the buprenorphine treatment groups (BUP0, n=21; BUP1.5, n=21; BUP3.0, n=20) for each dose of cocaine (3 hours/day, FR1 schedule). Rats took more infusions of the lowest dose of cocaine. Chronic buprenorphine treatment significantly reduced cocaine intake across all doses of cocaine. Note: Separate subgroup ns are shown on the figure bars. ** $p<0.01$, *** $p<0.001$.

buprenorphine) and the rats were then returned to their home cages. This was followed by 2 days of self-administration under the FR1 schedule followed by a day on the FR5 and a final day on the PR schedule.

Results. Heroin Self-Administration under a Progressive Ratio Schedule. Figure 9a shows the mean number of infusions of heroin taken on the PR schedule before and during buprenorphine treatment; following surgery, rats in both the sham and buprenorphine treatment groups took about 7 infusions and made about 20 responses for the last infusion. Thus, buprenorphine had no effect on the rate of responding for heroin under this schedule, nor did it affect responding on the FR5 schedule (data not shown).

Cocaine Self-Administration under a Progressive Ratio Schedule. It can be seen in Figure 9b that chronic buprenorphine reduced the number of responses and number of infusions of cocaine taken on the PR schedule. Buprenorphine also significantly reduced cocaine self-administration on the FR5 schedule (data not shown). Analysis of the number of cocaine infusions taken under the PR schedule during buprenorphine treatment revealed a significant main effect of buprenorphine treatment ($F(1, 16) = 34.96$, $p<0.001$).

Experiment 4: Effects of chronic buprenorphine on NAc DA response to self-administered heroin or cocaine.

Procedures. The somewhat puzzling set of results from Experiments 2 and 3 led us to explore the effects of buprenorphine on the initiation and the time-course of drug taking and the temporal relation between this and NAc DA levels in response to infusions of heroin or cocaine during sessions of self-administration on an FR1 schedule. In addition,

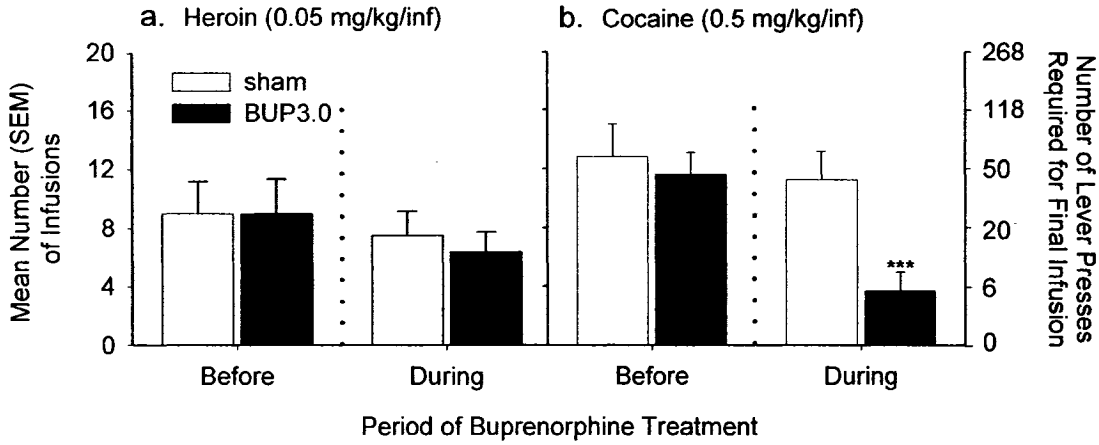

Figure 9.
Mean number (± SEM) of infusions taken under the progressive ratio schedule of reinforcement and required lever active lever presses for the indicated infusions during training and during buprenorphine treatment (3 hour session). (a) Self-administration of heroin under PR schedule of reinforcement in both buprenorphine treatment groups (BUP0, n=6; BUP3.0, n=6). Chronic buprenorphine treatment had no effect on heroin intake under this schedule. (b) Self-administration of cocaine under PR schedule of reinforcement in both buprenorphine treatment groups (BUP0, n=8; BUP3.0, n=10). Chronic buprenorphine treatment significantly reduced cocaine intake at this schedule. *** $p<0.001$.

the effects of buprenorphine on basal levels of DA were determined. We chose to measure DA in the NAc because of our previous finding that buprenorphine attenuated the DA response to experimenter delivered heroin, and enhanced the DA response to cocaine (Sorge et al. 2005) while enhancing basal levels of DA. Thus four additional groups of rats (ns=6) were trained to self-administer either heroin (0.05 mg/kg/inf) or cocaine (0.5 mg/kg/inf) for 5 consecutive days in 3-hour sessions while living in the operant conditioning chambers. Food and water were available at all times except that food was removed one hour before the self-administration sessions. After the fifth day of self-administration, rats were removed from the operant conditioning chambers, matched for their heroin or cocaine intake and assigned to a buprenorphine treatment group (sham or BUP3.0). After surgery for minipumps, rats were taken back to the operant chambers for recovery. On the following 2 days, rats were given access to heroin or cocaine under the same conditions as before surgery (3 hours, FR1). After the self-administration session on the second day of buprenorphine treatment, rats were anesthetized under light Isoflurane anesthesia, had microdialysis probes inserted into surgically implanted cannula, and were then placed back into their operant conditioning chambers overnight. The following day, dialysate was collected every 10 min for 1 hour before, for 3 hours during, and for 1 hour following the self-administration session for a total of 30 consecutive samples. Samples were labeled and frozen with dry ice immediately after collection to prevent degradation before analysis. Due to malfunction of dialysis probes the data from some of the rats in the sham groups could not be analyzed. Thus, the final

group sizes for both the behavioral data and the microdialysis data in this experiment were 3 for each of the sham groups and 6 for each of the buprenorphine treated groups.

Results. Heroin Self-administration and Microdialysis. Figure 10a shows the mean number of infusions of heroin taken over days by the two groups of rats (sham or 3.0 mg/kg/day) before (5 days) and during buprenorphine treatment (2 days before plus 1 day during microdialysis sampling). It can be seen that the mean number of infusions of heroin did not differ between the two buprenorphine conditions and remained stable during each phase of the experiment (Before: Group, $F(1,7) < 1$, ns, Day, $F(4,28) < 1$, ns; During: Group, $F(1,7) < 1$, ns, Day, $F(2,14) < 1$, ns).

Figure 11 shows the records from individual rats taking heroin on the day of microdialysis sampling. The number of infusions taken by the rats in the two groups (sham, # 12, 16 and 17: left panel; BUP3.0, # 11, 13, 14, 15, 18, 19: right panel) during 10-min intervals throughout the 3-hour session of self-administration are indicated by the bar graph (right axis). The levels of DA in the NAc recorded at 10-min intervals before (basal), during and after the self-administration sessions are shown by the line graph (left axis). Sham-treated rats showed patterns of self-administration characterized by responding immediately upon presentation of the lever and cues, and a relatively constant intake of heroin throughout the session. These infusions were accompanied by increases in NAc DA levels that, on the whole, remained elevated throughout the period of self-administration and differed significantly from baseline ($t(2) = 3.86$, $p<0.05$). Interestingly, although there was no difference in total intake between sham and buprenorphine groups, rats given buprenorphine (# 11 was the exception) showed very little responding at the beginning of the session in response to cues and lever entry. Two

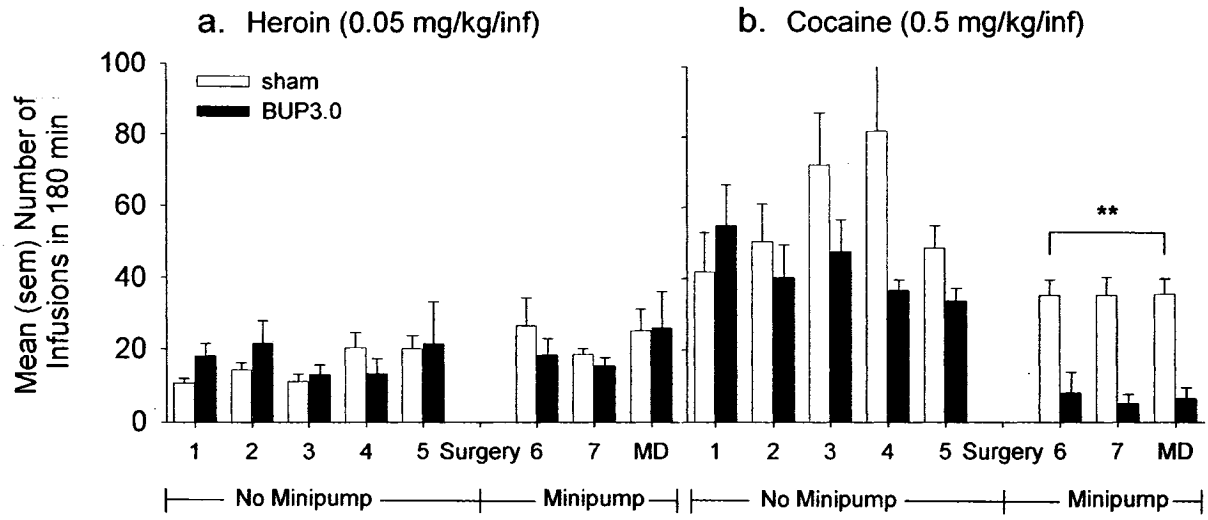

Figure 10.
Heroin and cocaine self-administration for rats in Experiment 4 before and during buprenorphine treatment. (a) Mean number (± SEM) of infusions of heroin per day over 5 days of training and 3 days of buprenorphine treatment for each of the buprenorphine treatment groups (BUP0, n=3; BUP3.0, n=6). Buprenorphine had no effect on heroin intake. (b) Mean number (± SEM) of infusions of cocaine per day over 5 days of training and 3 days of buprenorphine treatment for each of the buprenorphine treatment groups (BUP0, n=3; BUP3.0, n=6). Buprenorphine significantly reduced cocaine intake. Surgery = buprenorphine minipump implantation. MD = Microdialysis sampling day.
** $p<0.01$

rats did not begin responding at all until after the 30-min time bin, but nonetheless, continued to respond throughout the session once they began. In contrast to what was seen in the sham group, NAc DA levels remained unchanged in the BUP3.0 group despite heroin self-administration ($t(5) = 1.17$, ns). Analysis of number of infusions within the first 10 minutes of the session revealed a significant effect of Group ($F(1, 7) = 12.44$, $p<0.01$). Thus, buprenorphine reduced responding to the initial presentation of heroin-associated cues, attenuated the NAc DA response to self-administered heroin, but as in Experiments 2 and 3, did not reduce total heroin intake.

Cocaine Self-Administration and in vivo Microdialysis. Figure 10b shows the mean number of infusions of cocaine taken over days by the two groups of rats (sham or BUP3.0) before (5 days) and during (2 days before plus 1 day during microdialysis sampling). It can be seen that buprenorphine reduced cocaine intake similarly during the three self-administration sessions (Before: Group, $F(1,7) = 3.32$, ns, Day, $F(4,28) = 1.64$, ns; During: Group, $F(1,7) = 21.73$, $p <.01$, Day, $F(2,14) <1$, ns).

Figure 12 shows the records from individual rats self-administering cocaine on the day of microdialysis sampling. Rats in the sham group (left panel, rats, # 2, 5, and 8) showed a typical pattern of short latency responding to the presentation of the lever and cues and steady intake throughout the session. DA levels rose at the start of the session in these rats and remained elevated throughout ($t(2) = 3.52$, $p<0.05$). In contrast, rats treated with BUP (# 1, 3, 4, 6, 7, and 9, right panel) failed to respond at the beginning of the session (only one rat took an infusion in the first 30 min), and when they did eventually start to take cocaine, responded sporadically. Interestingly, the DA responses to individual infusions appeared to be greater than those seen in the sham rats (see

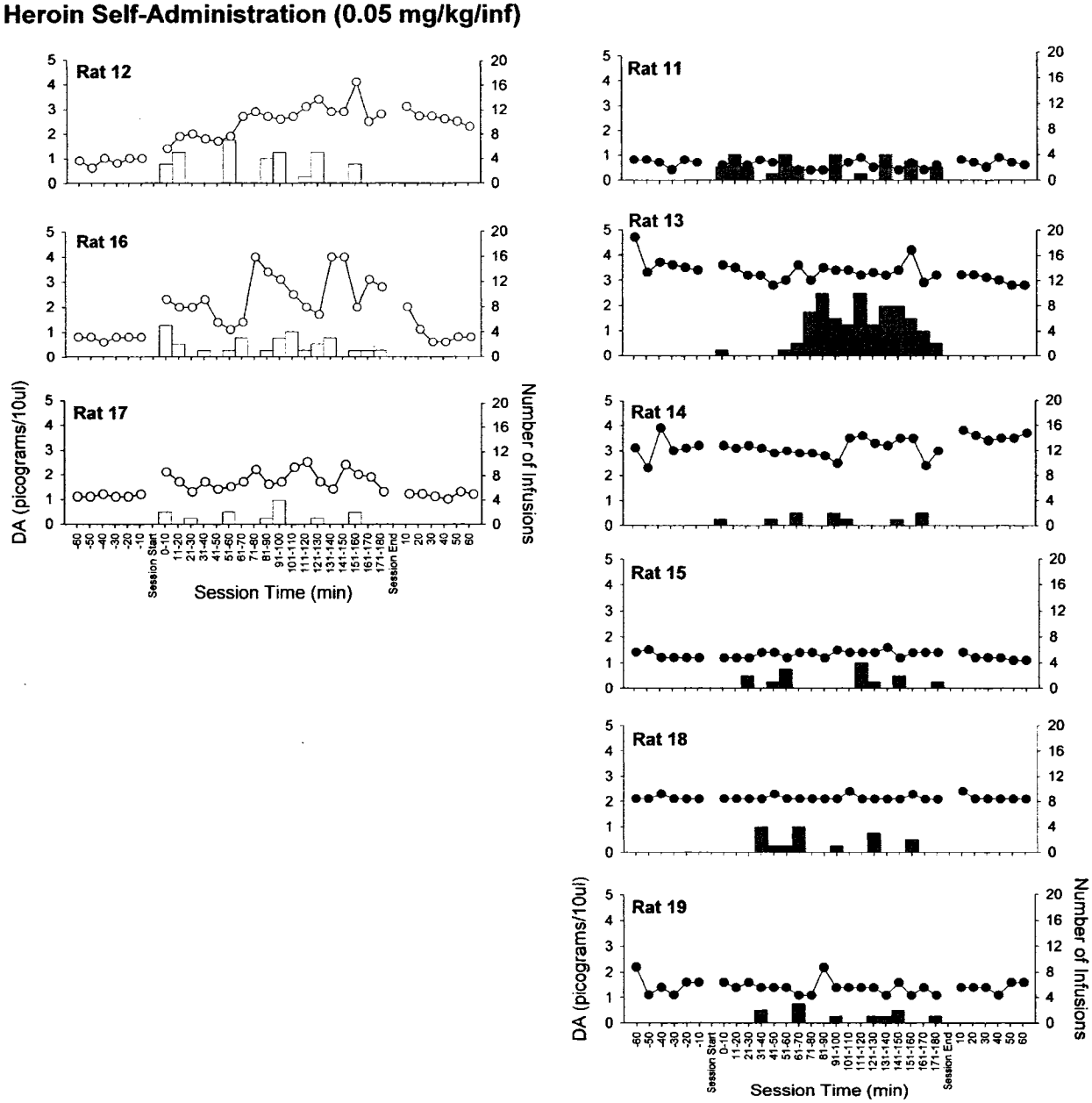

Figure 11.
Number of heroin infusions (right axis, bar graph) taken in each of the 10-min intervals throughout the 60-min pre-session, 180-min self-administration session, and 60-min post-session periods for rats in the BUP0 (left panel, rats #12, 16, and 17; open bars) and BUP3.0 groups (right panel, rats #11, 13, 14, 15, 18, 19; gray bars). Concurrent *in vivo* microdialysis sampling of extracellular DA in the NAc before, during and following the heroin self-administration session. Data are expressed as raw DA levels in picograms per 10µl of dialysate (left axis, line graph) for the BUP0 (open circles) and BUP3.0 (filled circles) groups. Buprenorphine suppressed responding early in the session, but did not significantly reduce heroin intake, though the NAc DA response to heroin infusions was virtually non-existent.

records for rats # 3 and 4) and were similarly elevated over baseline throughout the session ($t(5) = 2.59$, $p<0.025$). Rat 6, like the others, had an increased latency to initiate responding, showed large increases in DA levels in response to infusions, but did respond throughout the last two hours of the session. Analysis of number of infusions within the first 10 minutes of the session revealed a significant effect of Group ($F(1, 7) = 35.18$, $p<0.001$).

Probe Placements and Basal Levels of DA in the NAc. Figure 13 (a) shows the probable sampling area of the microdialysis probes of all rats (dotted line indicate sham rats) and a photomicrograph of the probe location for one rat (b). The mean basal levels of DA are shown below (c) for rats with placements at the three distances from Bregma. Only one rat (buprenorphine treated) had the most anterior placement. It can be seen, however, that when probes were at +1.6-1.7 from Bregma, the basal levels of DA were significantly higher in buprenorphine-treated animals (Group, $F(1, 16) = 7.19$, $p<0.05$).

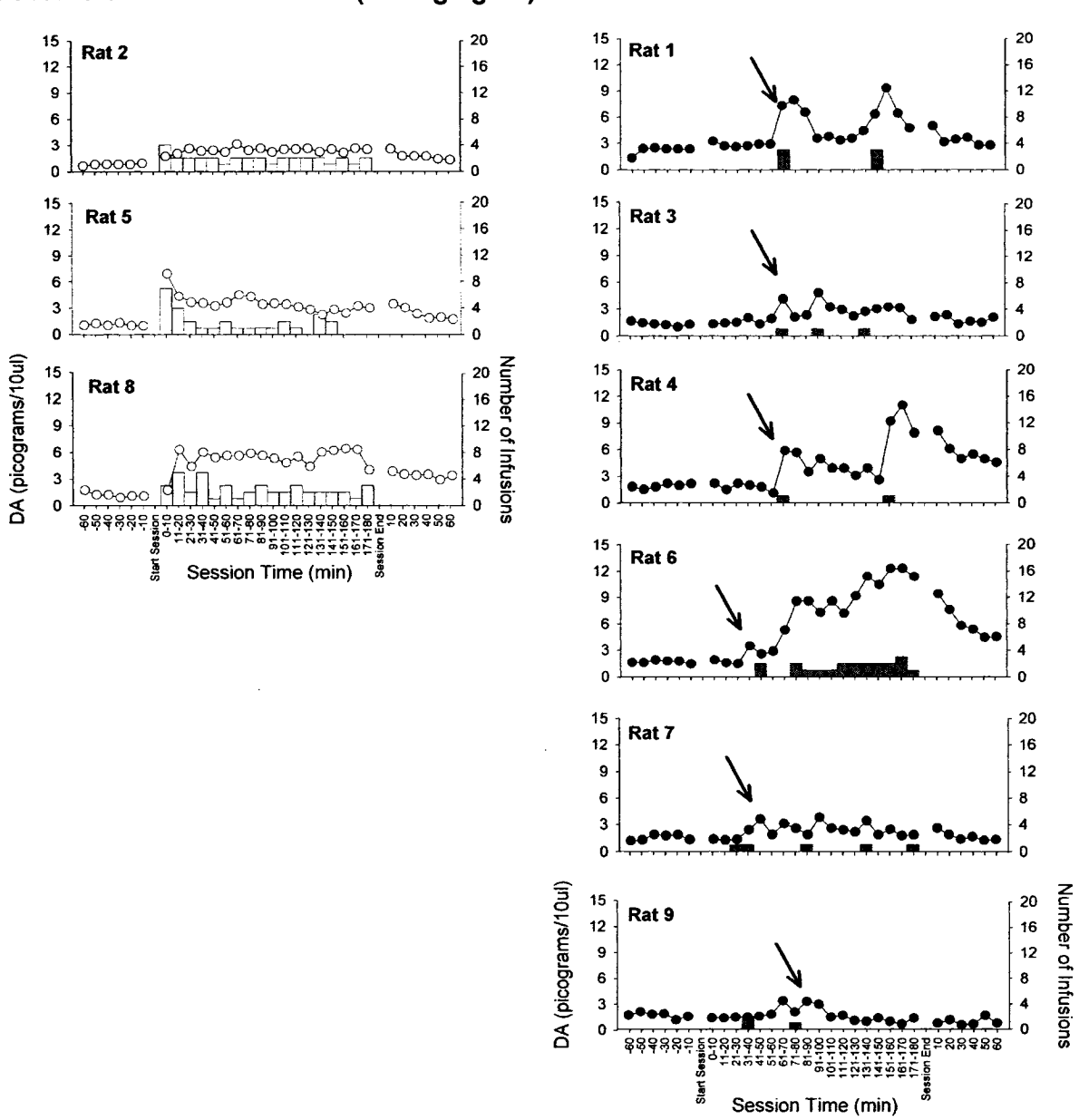

Figure 12.
Number of cocaine infusions (right axis, bar graph) taken in each of the 10-min intervals throughout the 60-min pre-session, 180-min self-administration session, and 60-min post-session periods for rats in the BUP0 (left panel, rats #2, 5, and 8; open bars) and BUP3.0 groups (right panel, rats #1, 3, 4, 6, 7, 9; gray bars). Concurrent *in vivo* microdialysis sampling of extracellular DA in the NAc before, during and following the cocaine self-administration session. Data are expressed as raw DA levels in picograms per 10µl of dialysate (left axis, line graph) for the BUP0 (open circles) and BUP3.0 (filled circles) groups. Buprenorphine suppressed responding early in the session and suppressed overall cocaine intake for all rats in the BUP3.0 group. Arrows denote times at which cocaine infusions resulted in enhanced dopamine levels.

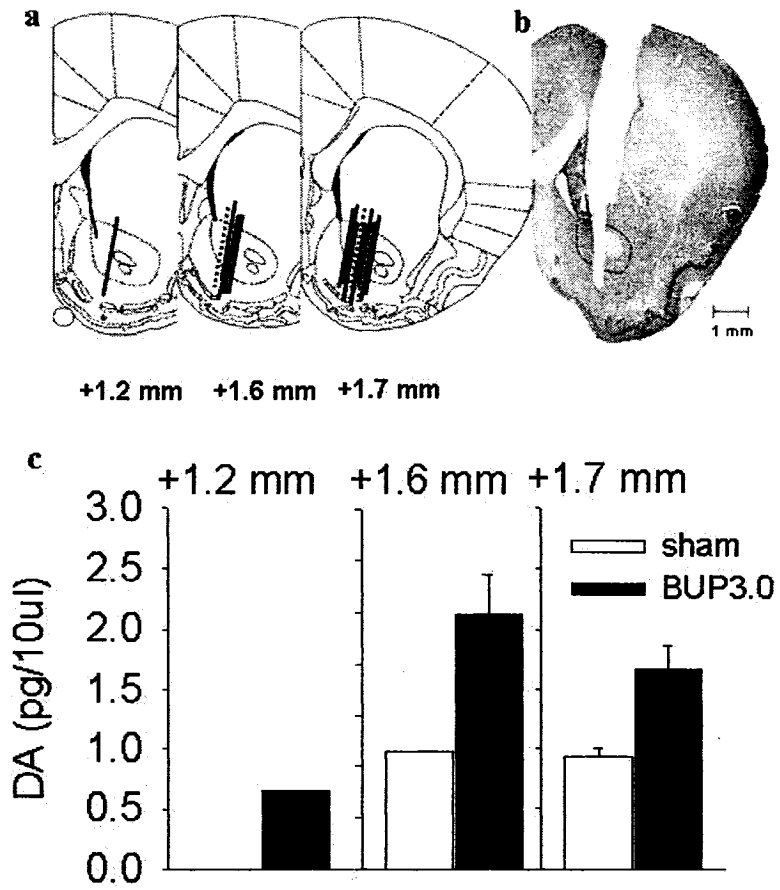

Figure 13.
Microdialysis probe placements and basal dopamine levels. (a) Histologically verified probable sampling area of the microdialysis probes of all rats in Experiment 4 (dotted line indicate sham rats). (b) Photomicrograph of guide cannula and microdialysis probe placement for one animal. Dialysate sampling was likely to have occurred from both the nucleus accumbens core and shell as each probe traversed these areas. Hatched area represents nucleus accumbens core region and scale bar represents 1 mm. (c) Mean (± SEM) basal levels of DA for rats with placements at the three distances from Bregma. The basal levels of DA were significantly higher in buprenorphine-treated animals at +1.6 and +1.7 mm from Bregma. * $p<0.05$

Discussion

Chronic high-dose buprenorphine had differential effects on intake of heroin and cocaine as well as on the NAc DA response to these drugs when measured concurrently using microdialysis. A common effect of buprenorphine on self-administration behavior was the reduction in responding to cues associated with both heroin and cocaine. There was an increased latency to respond upon presentations of the cue light and the active lever and, in the case of heroin, a slight reduction in non-reinforced responding typically seen during heroin self-administration. The major differential effect was that, although cocaine intake was greatly reduced in buprenorphine-treated rats, heroin intake was maintained after self-administration began, regardless of dose and performance requirement. This latter finding was most surprising in view of the absence of an increase in NAc DA to self-administered heroin and an enhanced DA response to self-administered cocaine in buprenorphine-treated rats.

The dose of buprenorphine used in our studies resulted in a steady state level close to twice that achieved with high dose buprenorphine treatment (i.e. 32 mg/day) in human patients, but does correspond to the peak levels reached 1 hour following administration of this dose (Greenwald et al., 2003). Regardless of this high dose of buprenorphine, total heroin intake was not significantly suppressed under any of the conditions, whereas the DA response in the NAc to heroin infusions was totally absent; thus there is a dissociation between the effectiveness of heroin as a reinforcer and the magnitude of the DA response to the infusion in the NAc. Not surprisingly, buprenorphine blocks the subjective and behavioral effects of other mu receptor agonists in a variety of species in various tests (Filibeck, Castellano, & Oliverio, 1981; Leander,

1983; Bickel et al., 1988) likely due to its high affinity for and slow dissociation from the mu-opioid receptor. Furthermore, it is known that antagonism of opioid receptors in the NAc (Vaccarino, Bloom, & Koob, 1985) or VTA (Britt & Wise, 1983), which would effectively reduce the "dose" of heroin, results in an increase in heroin self-administration in rats, suggesting that chronic treatment with buprenorphine might have led to an initial increase in heroin intake due to competition at the mu-opioid receptor, but this did not occur (see Figures 8a, 9a, 10a and/or 11). Interestingly, chronic treatment with the mu agonists, morphine or methadone, has been shown to shift the analgesic response of heroin from predominantly mu- to delta-1-receptor mediated (Rady, Holmes, Portoghese, & Fujimoto, 2000). If buprenorphine, a weak delta antagonist (Negus et al., 2002), did shift the agonist action of heroin to the delta opioid receptor, this agonist action might be sufficient to maintain self-administration (Shippenberg, Bals-Kubik, & Herz, 1987; Devine & Wise, 1994). Although this delta receptor agonist action might explain in part the lack of effect of buprenorphine on heroin intake under the conditions of our experiments, in other circumstances and under differing schedules of reinforcement, buprenorphine has been found to attenuate opioid-seeking and intake of opioids. For example, in patients, buprenorphine reduced overall intake in an experimental hospital setting (Mello et al., 1980), reduced craving for heroin and choice of hydromorphone over money (Greenwald et al., 2002) and, when given as buprenorphine/naloxone mixture, reduced subjective responses to heroin and choice of heroin over money (Comer et al., 2005). In monkeys working on a complex second order, cue-controlled, schedule of reinforcement, self-administration of heroin was reduced by buprenorphine (Mello et al., 1998). Negus (Negus, 2006), however, has

reported no reduction in heroin intake in self-administering monkeys given buprenorphine, but a reduction in choices of heroin over food at higher doses. Thus, though all of these studies point to the usefulness of buprenorphine in the treatment of opioid addiction, they do not speak directly to the mechanism through which it acts to change behavior.

In contrast to its effects on heroin intake, chronic buprenorphine treatment significantly reduced the intake of cocaine at all doses under all schedules of reinforcement tested. The fact that cocaine intake was reduced regardless of the self-administered dose and the schedule of reinforcement suggests that the reduction cannot be accounted for by a reduction in the reinforcing properties. A reduction in reinforcing efficacy of cocaine, similar to substitution with a lower dose, is generally accompanied by an increase in intake, whereas an increase in reinforcing efficacy often leads to short latencies to initiate responding and results in higher breakpoints under a PR schedule (Lorrain, Arnold, & Vezina, 2000; Morgan, Brebner, Lynch, & Roberts, 2002). Neither of these effects was seen under chronic buprenorphine. Our finding is, however, consistent with those of one study in rhesus monkeys where it was found that buprenorphine resulted in a downward shift in the dose-effect curve for self-administered cocaine (Winger et al., 1992). An additional study in rats demonstrated that twice daily, intravenous, buprenorphine reduced cocaine intake across a variety of doses of both buprenorphine and cocaine, however there was tolerance to this effect as early as the second day of buprenorphine administration (Carroll et al., 1992).

Although intake was reduced at all doses in the present experiments, the rise in DA in response to self-administered infusions of cocaine was enhanced under

buprenorphine. A similar enhancement of DA is seen after infusions of a combination of heroin and cocaine (Hemby, Co, Dworkin, & Smith, 1999) and is sometimes accompanied by enhanced reinforcing efficacy as measured by drug choice procedure (Ward, Morgan, & Roberts, 2005). Thus we might have predicted that, under buprenorphine, self-administered infusions of cocaine would increase breakpoints on a PR schedule and serve to prime subsequent responding. This did not occur during the PR schedule, and under the FR1 schedule initial rises in DA did not appear to prime subsequent responding. Furthermore cocaine did not induce reinstatement in rats under chronic buprenorphine treatment in our previous study (Sorge et al., 2005). Thus the interactions between the pharmacological effects of buprenorphine and cocaine on the DA response in the NAc do not provide an explanation for the reduction in intake.

As mentioned previously, there was one effect of buprenorphine on self-administration that was common to both heroin and cocaine; in both cases buprenorphine appeared to reduce the effectiveness of drug-related cues. In untreated rats, cues signaling drug availability (i.e. cue light presentation and lever entry) were highly effective stimuli for the initiation of lever-pressing. As shown in Fig. 11 and Fig. 12, chronic buprenorphine increased the latency to respond to these cues. Whereas rats in the sham condition responded within the first 10 min of the session, most rats in the BUP3.0 condition did not begin to lever press for at least 30 min into the session. This suggests a reduction in the salience of drug-related cues, an idea supported by our previous work showing that chronic treatment with buprenorphine, as well as methadone, reduces heroin and cocaine seeking in the presence of drug-related cues during extinction and during tests for drug-induced reinstatement (Leri et al., 2004; Sorge et al., 2005). These effects

cannot be attributed directly to sedation in that chronic treatment with either buprenorphine or methadone does not reduce locomotor activity, but in fact slightly enhances it.

Studies have shown that the non-contingent presentation of a CS that predicts a cocaine infusion elicits a modest rise in extracellular DA within the NAc core (Ito, Dalley, Howes, Robbins, & Everitt, 2000), which has been illustrated as well using *in vivo* voltammetry (Gratton & Wise, 1994; Richardson & Gratton, 1996). Furthermore, this DA signal in the NAc in response to a drug-associated cue is relatively small, of short-duration, and is followed by a steeper and longer rise that coincides with the lever press (Roitman, Stuber, Phillips, Wightman, & Carelli, 2004). Fig. 13 (bottom panel) reveals that BUP3.0 rats had higher basal DA levels than sham rats, consistent with previous work from our laboratory that has shown that chronic treatment with buprenorphine via osmotic minipump results in higher basal levels of DA in the NAc than those seen in control animals (Sorge et al., 2005). It is possible that the higher basal DA levels in buprenorphine-treated rats attenuate the signal to noise ratio of the modest rise in DA levels in the NAc in response to presentation of drug-related cues, thus masking the effectiveness of the cue.

Another possible line of speculation is that chronic exposure to BUP might have its effects by reducing glutamate function. Chronic morphine treatment results in decreased glutamate and increased DOPAC in the NAc and the striatum (Huang, Tseng, Wong, & Tung, 1997) as well as a decrease in NMDA-receptor mediated synaptic transmission and evidence for an enhanced inhibition of glutamate release in the NAc (Martin et al., 1999). Consequently, one might suppose that chronic treatment with

buprenorphine would also result in decreased glutamatergic activity in the NAc and striatum. There is some evidence that global reduction of glutamate release, brought about by the metabotropic glutamate receptor 2/3 agonist (mGluR2/3), LY379268, affects responsivity to drug-related cues. For example, systemic injections of LY379268 attenuate cue-induced reinstatement of heroin seeking in rats, leaving heroin intake unaffected (Bossert, Busch, & Gray, 2005), similar to our findings with buprenorphine. LY379268 has also been shown to reduce cocaine self-administration and to attenuate cue-induced reinstatement of cocaine seeking in rats (Baptista, Martin-Fardon, & Weiss, 2004). Interestingly, it has been shown recently that the NMDA receptor antagonist, LY235959, reduces cocaine self-administration early in the session (Allen, Carelli, Dykstra, Suchey, & Everett, 2005), suggesting that it reduces the effectiveness of drug-related cues in a manner similar to that of buprenorphine seen in our study.

In summary, we found in the present experiments that, although heroin and cocaine intake were differentially affected by buprenorphine, the initiation of responding to drug-related cues was delayed in the case of both heroin and cocaine self-administration. These findings support our previous suggestion that buprenorphine has its effects on heroin and cocaine seeking by reducing responsiveness to cues associated with these drugs (Sorge et al., 2005). This idea is supported further by our finding that buprenorphine reduces responding to cues associated with a sucrose reward in non-deprived rats (unpublished observations). We do not have an explanation for the reduced responsiveness to cues; it can be pointed out, however, that a reduction in responses to drug-related cues, whether they be the initiation of drug-seeking, drug-related thinking or drug craving, would be a desirable property of a pharmacological treatment for drug

abuse.

Acknowledgements

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In the preceding chapter, entitled "The effects of chronic buprenorphine on intake of heroin and cocaine in rats and its effects on nucleus accumbens dopamine levels during self-administration" it was demonstrated that chronic treatment with buprenorphine reduced cocaine, but not heroin, intake across doses and schedules of reinforcement. Furthermore, there was a common effect of a reduced responsiveness to cues associated with either drug during microdialysis testing. These results suggest a common effect of buprenorphine on drug-associated cues, but cannot be explained by the unique interactions of buprenorphine with either heroin or cocaine. Specifically, buprenorphine treatment blocked the NAc DA response to infusions of heroin and potentiated the response to cocaine infusions.

The above results show promise for the potential of buprenorphine to reduce heroin and cocaine seeking in extinction and following contingent (Chapter 2) and noncontigent (Chapter 1) exposure to heroin and cocaine, but the effects are shown early in treatment. In particular, we have revealed a potentiated locomotor (Chapter 1 only) and NAc DA response to experimenter-delivered (Chapter 1) and self-administered (Chapter 2) cocaine. The synergism of these drugs is not new, but is potentially troubling for opioid abuse treatment programs including cocaine co-abusing patients. For this reason, the Chapter 3 addresses the possibility that the potentiated locomotor and NAc DA response to acute cocaine and the attenuated NAc DA response to acute heroin, seen in Chapter 1 and 2, may change over the course of long-term treatment. Therefore, the manuscript on which Chapter 3 is based, entitled "The effects of long-term chronic buprenorphine treatment on the locomotor and nucleus accumbens dopamine response to acute heroin and cocaine in rats," seeks to determine the interactions between

buprenorphine and heroin and cocaine over protracted treatment. This manuscript is in *Pharmacology, Biochemistry and Behavior.*

CHAPTER 3

The effects of long-term chronic buprenorphine treatment on the locomotor and nucleus accumbens dopamine response to acute heroin and cocaine in rats

Robert E. Sorge and Jane Stewart

Sorge, R. E., & Stewart, J. (2006). The effects of long-term chronic buprenorphine treatment on the locomotor and nucleus accumbens dopamine response to acute heroin and cocaine in rats. *Pharmacology. Biochemistry & Behavior, 84*: 300-305. Reprinted with permission from Elsevier.

Abstract

We have previously shown that chronic treatment with the partial mu-opioid receptor agonist, buprenorphine, blocks the nucleus accumbens dopamine response to an acute injection of heroin, whereas it potentiates the response to an acute injection of cocaine after 4-5 days of treatment. Here we studied the effects chronic exposure to buprenorphine via osmotic minipumps for up to 28 days (1.5 or 3.0 mg/kg/day) on responses to acute injections of heroin and cocaine. Increases in locomotion induced by heroin (0.25 mg/kg, sc), given on the 5th, 15th or 25th day of treatment were unaffected by buprenorphine, whereas increases induced by cocaine (20 mg/kg, ip) were enhanced early in treatment but not on the 15th or 25th days. Using *in vivo* microdialysis we found that both the suppression of the dopaminergic response in the nucleus accumbens to heroin and the potentiation to cocaine seen early in treatment diminished over the 26-27 days, whereas basal dopamine levels remained elevated throughout. Therefore, although these studies do not explain the mechanism whereby buprenorphine reduces heroin and cocaine intake, they do indicate that there is little tolerance to the presence of chronic buprenorphine.

Keywords: buprenorphine, heroin, cocaine, dopamine, locomotion, microdialysis, tolerance, addiction

Introduction

The primary drugs used in treatment of opioid addiction are the full mu-opioid receptor agonist, methadone, and the partial mu-opioid receptor agonist buprenorphine, both of which are slow to dissociate from receptors and have long half-lives (Tzschentke, 2002). In studies with opioid addicts there are reports that cocaine use is not significantly affected (Strain et al., 1996; Schottenfeld et al., 1997). However, in one study, specifically examining concomitant cocaine use in opioid addicts, significant decreases in cocaine use were reported during buprenorphine treatment in a dose-dependent manner (Montoya et al., 2004). Consistent with the latter finding, studies in monkeys have shown that intermittent or daily injections of buprenorphine can reduce cocaine self-administration under complex schedules of reinforcement (Mello et al., 1992), as well as cocaine-heroin "speedball" and heroin self-administration (Mello et al., 1998). In fact, in one study it was found that buprenorphine significantly reduced cocaine seeking for more than 120 days (Mello et al., 1992), indicating little tolerance to the effects of repeated acute buprenorphine treatment over time.

We have recently shown that chronic treatment with buprenorphine reduces cocaine, but not heroin, intake in rats trained to self-administer both drugs on an FR1 schedule of reinforcement (Sorge & Stewart, 2006), although it reduces responding in extinction and reinstatement induced by either drug (Sorge et al., 2005). In addition, we found that, in tests performed after less than 10 days of treatment, chronic buprenorphine attenuated the nucleus accumbens (NAc) dopaminergic (DA) response to heroin without affecting heroin-induced locomotor activity. In contrast, buprenorphine potentiated the locomotor and NAc DA responses to cocaine (Sorge et al., 2005).

To determine whether these behavioral and neurochemical responses to acute injections of heroin and cocaine would be modified by longer exposure to buprenorphine, we examined the locomotor and NAc DA responses to acute injections of heroin (0.25 mg/kg, sc) or cocaine (20 mg/kg, ip) after either 13-15 or 25-27 days of chronic buprenorphine treatment (sham, BUP1.5: 1.5 mg/kg/day and/or BUP3.0: 3.0 mg/kg/day). In the experiment on locomotion, separate groups of rats were tested on either the 5th, 15th or 25th day of buprenorphine treatment, with injections of either heroin or cocaine and locomotor activity was assessed. In the microdialysis experiment, heroin and cocaine injections were administered on the 13-14th or 26-27th day of buprenorphine exposure (sham, BUP1.5 or BUP3.0) to separate groups of rats.

General Methods

Subjects

A total of 107 male Long-Evans rats (5-6 rats per group, Charles River, St. Constant, QC) weighing 325-350 g at the start of the experiments were used. Animals were singly housed in reverse cycle rooms (lights OFF at 0800h; ON at 2000h) with food (Rat Chow, Purina Foods) and water *ad libitum*. All experimental procedures followed the guidelines of the Canadian Council on Animal Care and were approved by the Animal Care Committee at Concordia University.

Drugs

Chronic buprenorphine (3.0 mg/kg/day, buprenorphine HCl purchased from Reckitt Benckiser Healthcare Limited, Hull, UK) treatment was achieved via subcutaneous (sc) implantation of osmotic, buprenorphine-filled, minipumps (Alzet model 2ML2, Durect Corp., Cupertino, CA). Surgical procedures, using Isoflurane

anesthesia (Vetoquinol N.A. Inc, Lavaltrie, QC) have been previously described (Sorge et al., 2005). No pump was implanted in the sham rats, though the same surgical procedures were employed. (Preliminary results in our lab found no significant behavioral effects of saline-filled osmotic minipumps on self-administration behavior so, to reduce experimental costs, the practice was discontinued.) Heroin (0.25 mg/kg, sc, diacetylmorphine HCl) was purchased from Almat Pharmachem Inc (Concord, ON) and cocaine (20 mg/kg, ip, cocaine HCl) from Medisca Pharmaceutique (Montreal, QC). The doses of heroin and cocaine chosen were those previously used in our laboratory to elicit both locomotor activity (without sedation or stereotypy) and a significant NAc DA response (Sorge et al., 2005).

Apparatus

Locomotor activity and microdialysis chambers have been described elsewhere (Sorge et al., 2005). Briefly locomotor activity was monitored in a bank of 12 activity chambers by dual infrared beams located on each of the long sides of the rectangular chamber, positioned 3.5 cm from the stainless steel bar floor and 10 cm from each other. Microdialysis chambers were custom made hexagonal chambers with Plexiglas walls, wooden ceilings and stainless steel bar floor. The microdialysis probe, also described previously (Sorge et al., 2005), consisted of a 2.5 mm length of semi-permeable dialysis membrane (Fisher Scientific, 240 um OD, 13000 MW cutoff) connected to a 21 mm long, 26 gauge piece of stainless steel tubing. This tubing was connected to a variable speed electric syringe infusion pump (Harvard Apparatus, South Natick, MA) that delivered artificial cerebrospinal fluid (aCSF: 145 mM Na^+, 2.7 mM K^+, 1.2 mM Ca^{2+}, 1.0 mM Mg^{2+}, 150 mM Cl^-, 0.2 mM ascorbate, 2 mM Na_2HPO_4, pH, 7.4 ± 0.1) to the

system. Small diameter fused silica tubing extended internally through the probe. The probes were inserted the day before the start of microdialysis testing and, to prevent occlusion, aCSF was perfused overnight at a rate of 1.0 µl/min.

Procedures

Microdialysis guide cannulae (Plastics One, Roanoke, VA) were implanted under sodium pentobarbital (Somnotol™, MTC Pharmaceuticals, Cambridge, ON; 65 mg/kg intraperitoneally [ip]) anesthesia for rats in the microdialysis experiment. Cannulae were targeted above the NAc (NAc: AP +1.6mm, ML +2.8mm, DV -5.5mm from bregma) at an angle of 10° (Paxinos et al., 1986) and were fixed in place with dental acrylic. Rats were placed in recovery following an injection of penicillin (Pen G, Vetoquinol, Lavaltrie, QC; intramuscularly [im]).

For the experiment on locomotion, rats were given a baseline test at day 0 for 120 min at either 0900h or 1200h. Groups were matched on the basis of locomotor activity on this test and rats received osmotic minipumps or sham surgery. The rats were left in their home cages until testing on either day 5, 15 or 25. Different groups of rats were tested at each time point such that each rat had its exposure to heroin or cocaine at the time of test.

Microdialysis testing was begun 13 or 26 days following intracranial cannulation and buprenorphine minipump or sham surgery. Probes were inserted on days 12 or 25 and rats were taken to the microdialysis chambers where they were connected to the infusion pumps overnight. The next day (starting at 0900h) samples were collected at 20-min intervals and 10 µl of dialysate was injected and analyzed using one of two similar HPLC systems with electrochemical detection (HPLC-EC). Once baseline levels were

stable (less than 10% variability in three consecutive samples) rats were injected (between 1100h and 1300h) with either heroin (0.25 mg/kg, sc) or cocaine (20 mg/kg, ip) and samples were taken for another 120 min. Rats remained connected overnight and were tested the following day with the opposite drug in a counterbalanced fashion such that each rat was tested with both heroin and cocaine, but at only one time point.

The HPLC-EC apparatus has been previously described (Sorge et al., 2005). The currents for DA were measured independently of those for DOPAC and HVA using separate channels and were quantified by EZChrom Chromatography Data System (Scientific Software Inc, San Ramon, CA) such that two rats were analyzed simultaneously on two identical systems.

Following microdialysis sampling, rats were anesthetized and perfused intracardially with saline and formaldehyde (Formalin 10%V/V, Anachemia, Montreal, QC) before brain removal. To identify placements of the cannula tract and probes, coronal frozen sections were taken using a cryostat, mounted and stained with cresyl violet.

Data Analysis

All data were analyzed using the analysis of variance (ANOVA) with Fisher's LSD comparisons conducted as post-hoc analysis. The alpha level was set to 0.05.

Results

Figure 14a shows the effect of chronic buprenorphine treatment on the locomotor response to an acute injection of heroin for the different groups of rats (n=5-6) tested for the first time at one of the three time points after buprenorphine treatment. It can be seen that heroin increased locomotion over baseline (dotted line) equally in buprenorphine-

treated and untreated rats to a similar magnitude. An ANOVA conducted on the activity counts during the Baseline and Heroin Tests revealed a significant effect of Test (i.e. Baseline vs. Heroin Test) ($F(1, 29) = 216.67$, $p<0.001$), but no effect of Buprenorphine treatment ($F(2, 29) = 0.001$, ns) and no Buprenorphine treatment by Time of Test interaction ($F(2, 29) = 0.27$, ns).

Figure 14b shows the locomotor response to an acute injection of cocaine in different groups of rats (n=5-6). It can be seen that buprenorphine enhanced the response to cocaine only on day 5; although cocaine increased activity above baseline on days 15 and 25, the groups did not differ. The ANOVA revealed significant effects of Test (Baseline vs. Cocaine) ($F(1, 29) = 279.21$, $p<0.001$), Buprenorphine treatment ($F(1, 29) = 7.75$, $p<0.01$), and Time of Test ($F(2, 29) = 15.24$, $p<0.001$) as well as a significant Test by Buprenorphine treatment interaction ($F(1, 29) = 9.00$, $p<0.01$). Post-hoc analysis revealed that BUP1.5 rats differed from sham rats only at Day 5 ($p<0.01$). These findings show that cocaine significantly increased locomotion in both buprenorphine-treated and untreated rats and that buprenorphine treatment significantly enhanced the locomotor response to cocaine but only early in treatment. In addition, the ANOVA revealed a significant Test by Time of Test interaction ($F(2, 29) = 11.23$, $p<0.001$). Groups tested for the first time at the late time point (day 25) were less responsive to cocaine regardless of buprenorphine treatment. We cannot explain the reduced responsiveness to the locomotor stimulatory effect of cocaine, however, this reduction was consistent in rats in both treatment conditions.

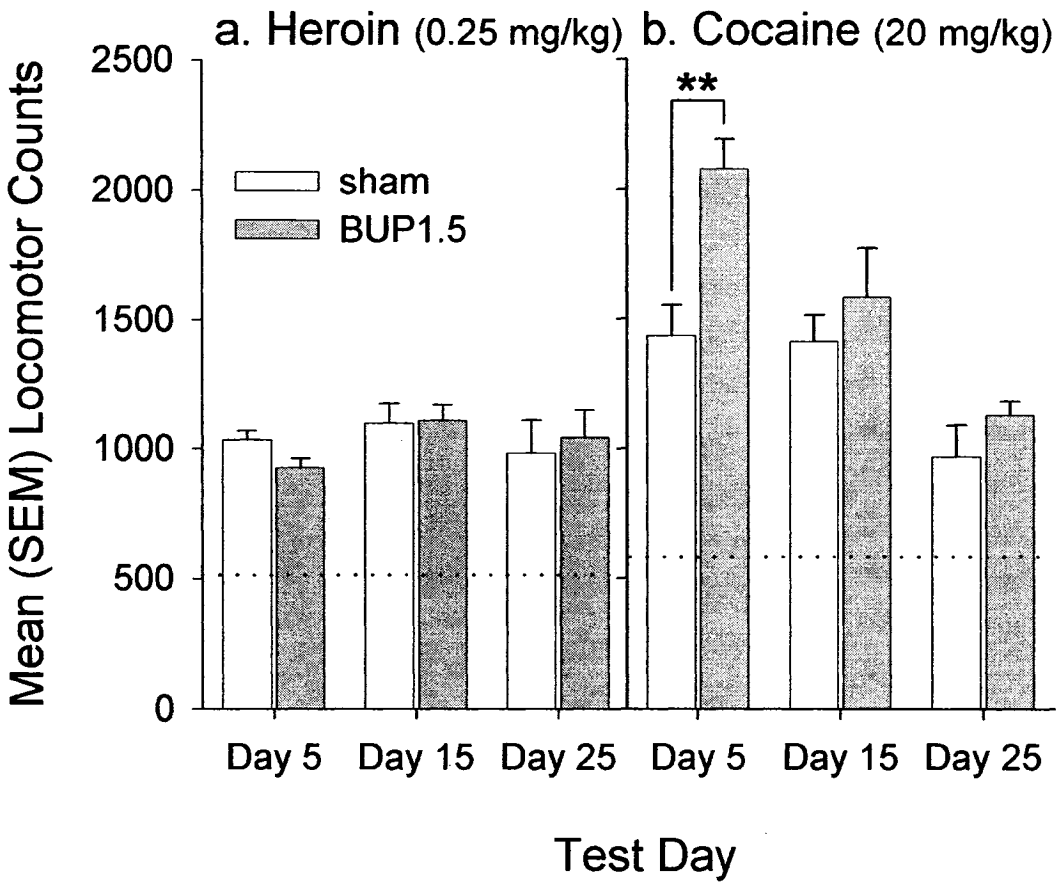

Figure 14.
The effect of chronic buprenorphine on the locomotor response to an acute injection of (a) heroin (0.25 mg/kg, sc) or (b) cocaine (20 mg/kg, ip) after 5, 15 or 25 days of treatment. The graphs display the mean (±sem) locomotor counts with the baseline locomotion indicated by the dotted line (Heroin: sham; Day 5, n=6, Day 15, n=6, Day 25, n=6; BUP1.5, Day 5, n=6, Day 15, n=6. Day 25, n=5; Cocaine; sham; Day 5, n=5, Day 15, n=6, Day 25, n=6; BUP1.5, Day 5, n=6, Day 15, n=6. Day 25, n=6).
** $p<0.01$

Figure 15 (a and b) shows the change in extracellular DA levels in the NAc following an acute injection of heroin after 13-14 (Figure 15a) or 26-27 (Figure 15b) days of chronic BUP treatment (n = 5-7/group). The mean of four baseline samples was used to calculate the percent change in DA around the mean before and following the injection of heroin. It can be seen that although heroin caused an increase in DA levels over baseline in all groups, this increase was less in buprenorphine-treated groups at both time points. This effect of buprenorphine is reflected in the significant Post-Injection Time by Buprenorphine treatment interaction ($F(12, 174) = 1.92$, $p<0.05$) in the ANOVA carried out on the post-injection scores. There was also an unexpected Post-Injection Time by Time of Test interaction ($F(6, 174) = 2.25$, $p<0.05$) reflecting the greater DA response in all groups at the later time point. The main effect of Buprenorphine treatment was marginally significant ($p=0.06$). Thus after 13-14 and 26-27 days of chronic buprenorphine, the blockade seen previously (Sorge et al., 2005) was attenuated though buprenorphine continued to elevate basal levels of DA at day 26 (see Figure 17b). Figure 16 shows the effects of chronic buprenorphine on the NAc DA response to acute injections of cocaine after 13-14 (Figure 16a) or 26-27 days of treatment (Figure 16b). It can be seen that cocaine enhanced DA levels in all groups. The ANOVA revealed significant main effects of Post-Injection Time ($F(6, 156) = 30.69$, $p<0.001$) and Time of Test ($F(1, 26) = 4.37$, $p<0.05$), but no effect of Buprenorphine treatment ($F(2, 26) = 0.41$, ns). Subsequent analyses of buprenorphine-treated groups alone, revealed a significant effect of Time of Test ($F(1, 17) = 5.03$, $p<0.05$), whereas there was no difference in the response of the untreated groups at the two time points ($F(1, 9) = 0.09$, ns). These data reveal that the ability of buprenorphine treatment to potentiate the NAc DA response to

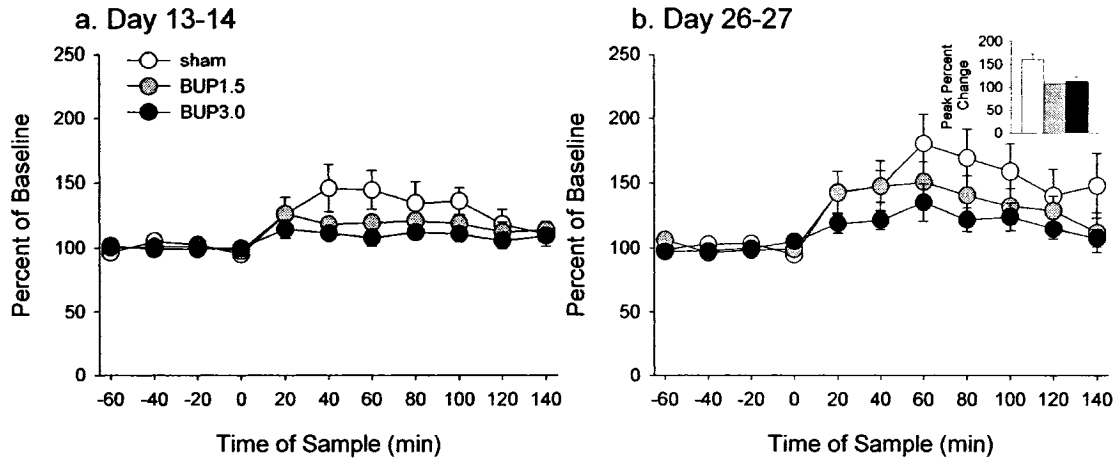

Figure 15
The mean (±sem) percent change from baseline in extracellular DA in the NAc following an acute injection of heroin (0.25 mg/kg, sc) after (a) 13-14 or (b) 26-27 days of chronic buprenorphine treatment. Insert in 2b shows the peak values for rats tested at day 4-5 of treatment from an earlier report (Sorge et al., 2005) (Day 13-14: sham, n=4, BUP1.5, n=5, BUP3.0, n=5; Day 26-27: sham, n=6, BUP1.5, n=4, BUP3.0, n=6).

acute cocaine reported previously (Sorge et al., 2005) is absent and slightly attenuated after long-term chronic treatment.

Figure 17 shows the mean basal levels of DA as determined from the last four samples before heroin or cocaine injections in buprenorphine-treated and untreated groups on the first day of microdialysis sampling (Figure 17a, day 13; Figure 17b, day 26). It can be seen that basal DA levels, at each of the locations anterior to bregma, were higher in buprenorphine-treated groups on both days 13 and 26. The ANOVA revealed a significant main effect of BUP treatment ($F(2, 19) = 10.08$, $p<0.01$) and post-hoc analysis confirmed that the sham group was significantly different from the BUP1.5 ($p<0.01$) and BUP3.0 groups ($p<0.001$) when the groups were collapsed across probe location. At each location there were significant differences seen between the BUP3.0 and sham rats at +1.6 mm from Bregma on Day 13 ($p<0.05$) and at +1.6 mm and +1.7 mm on Day 26 ($ps<0.05$). There was no effect of Length of Treatment ($F(1, 19) = 2.95$, ns), although there appeared to be a trend towards higher levels later in treatment.

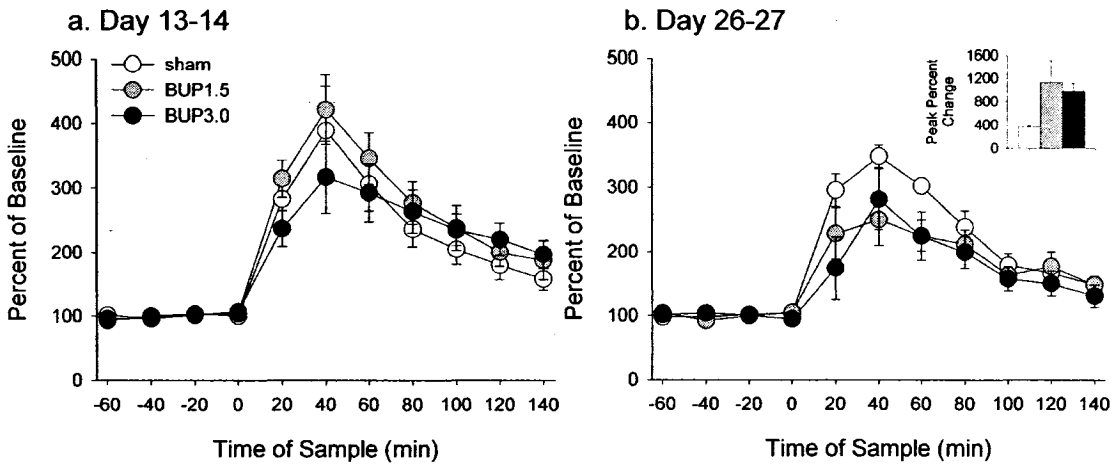

Figure 16
The mean (±sem) percent change from baseline in extracellular DA in the NAc following an acute injection of cocaine (20 mg/kg, ip) after (a) 13-14 or (b) 26-27 days of chronic buprenorphine treatment. Insert in 3b shows the peak values for rats tested at day 4-5 of treatment from an earlier report (Sorge et al., 2005) (Day 13-14: sham, n=5, BUP1.5, n=5, BUP3.0, n=5; Day 26-27: sham, n=4, BUP1.5, n=2, BUP3.0, n=4).

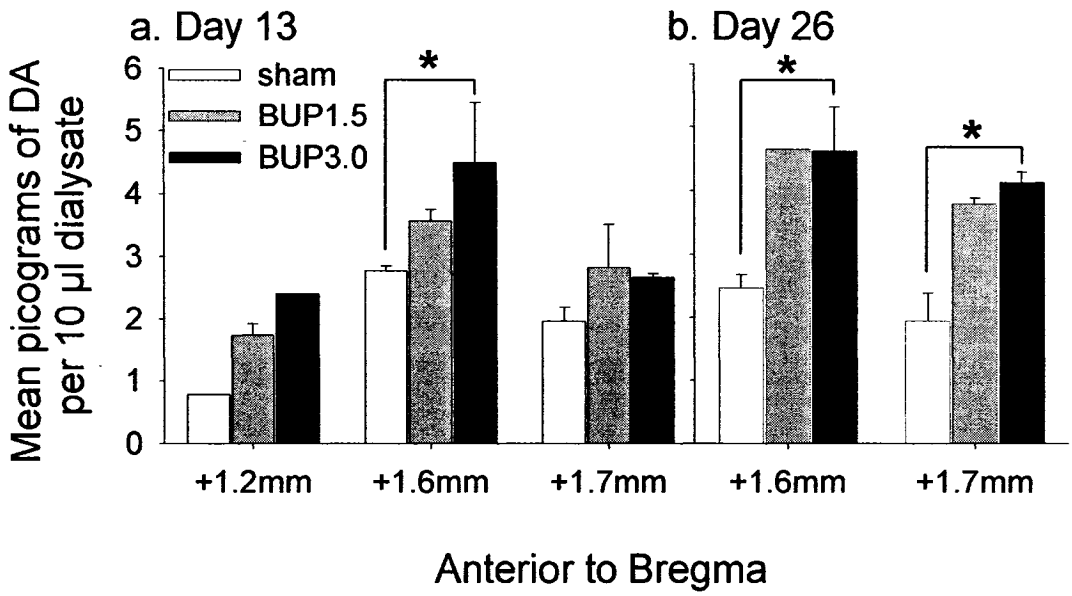

Figure 17
Mean (±sem) basal levels of DA in the NAc at various levels anterior to bregma for rats with similar probe placements after (a) 13 or (b) 26 days of chronic buprenorphine treatment (Day 13: +1.2 mm; sham, n=1, BUP1.5, n=2, BUP3.0, n=1; +1.6 mm; sham, n=2, BUP1.5, n=2, BUP3.0, n=2; +1.7 mm; sham, n=3, BUP1.5, n=3, BUP3.0, n=3; Day 26; +1.6 mm; sham, n=2, BUP1.5, n=1, BUP3.0, n=4; +1.7 mm; sham, n=4, BUP1.5, n=2, BUP3.0, n=2). * $p<0.05$

Discussion

These experiments were carried out to determine whether responses to acute injections of heroin and cocaine would change as a function of duration of exposure to buprenorphine delivered chronically via osmotic minipump. In the case of heroin, it was found that the locomotor response was elevated at all time points in treatment and that buprenorphine neither enhanced nor reduced this response. There was, however, a change in the effect of heroin on the NAc DA response such that the complete blockade of the response seen previously (Sorge et al., 2005) was diminished in tests made after 13-14 or 26-27 days of treatment. In the case of cocaine, it was found that the locomotor response was consistently elevated and that buprenorphine enhanced this response only at the early time point (Day 5). Similarly, the enhanced NAc DA response seen previously (Sorge et al., 2005) was no longer evident at 13-14 days and was below the control levels after 26-27 days. Despite these changes in response to heroin and cocaine, buprenorphine continued to elevate basal levels of DA in the NAc throughout treatment. Furthermore, we have recently found that the level of buprenorphine in plasma, in rats with osmotic minipumps (3.0 mg/kg/day), is stable (approximately 10 ng/ml) over the course of 28 days of chronic treatment (Sorge et al., 2006). Thus, although aspects of these data suggest modest tolerance to some of the effects of buprenorphine, others were unaffected by long-term exposure.

Acute administration of heroin or cocaine results in an increase in extracellular DA within the NAc (Di Chiara et al., 1988). Heroin is thought to increase DA cell firing by acting primarily at mu-opioid receptors located on GABA interneurons in the ventral tegmental area (VTA) (Johnson & North, 1992), whereas cocaine blocks the DA

transporter (DAT) preventing reuptake of DA (Heikkila et al., 1975). Buprenorphine would be expected to enhance extracellular levels of DA in the NAc (Brown et al., 1991) by acting in a manner similar to heroin at mu-opioid receptors in the VTA and, indeed, we found that chronic treatment with buprenorphine raised basal DA levels in NAc. Such chronic elevation of DA might in turn increase or up-regulate the functioning of the DAT in a manner similar to that seen after repeated infusions of cocaine (Letchworth, Nader, Smith, Friedman, & Porrino, 2001), thereby attenuating the effect of cocaine on extracellular DA. Furthermore, the chronic presence of buprenorphine would be expected to compete with heroin for the mu-opioid receptor as it has been shown to induce withdrawal from morphine (Gmerek, 1984). Combined, these two effects could help account for the reduction of heroin-induced DA release and the reduction of the enhanced DA response to cocaine under prolonged buprenorphine treatment.

Another possibility is a down-regulation of mu-opioid receptor over time in rats treated chronically with buprenorphine. Acute or chronic administration of buprenorphine has been shown to reduce mu-opioid receptor number in the frontal cortex, thalamus, hippocampus striatum and brainstem (Belcheva et al., 1996; Debruyne et al., 2005). A reduction in mu-opioid receptor number and/or affinity might explain the attenuated NAc DA response to the acute injection of heroin during buprenorphine treatment. This reduction in binding would not, however, explain why the locomotor response to heroin was unaffected during buprenorphine treatment.

Chronic buprenorphine would be expected to lead to adaptations within other opioid receptor systems. It has been shown that chronic treatment with morphine (Rady et al., 2000) or methadone (Rady, Portoghese, & Fujimo, 2002) pellets shifts the

mediation of the antinociceptive effect of heroin from mu to delta-opioid receptors, where heroin is a potent agonist. Interestingly, delta opioid agonists are self-infused into the VTA (Devine et al., 1994), lead to increases in extracellular DA in the NAc (Devine et al., 1993) and, importantly, increase locomotion (Michael-Titus, Dourmap, & Costentin, 1989). Thus, heroin may have retained its stimulatory and DA-elevating effects through delta-opioid receptor activation during chronic buprenorphine treatment. If this were the case, it would be relevant that intra-VTA infusions of the mu-opioid receptor agonist, DAMGO, have been found to induce greater DA release in the NAc than similar infusions of the delta-opioid receptor agonist, DPDPE (Devine et al., 1993). This might account for the change in the NAc DA response to heroin over the duration of BUP treatment. Initially buprenorphine completely blocked the DA response (Sorge et al., 2005), whereas after long-term chronic treatment acute injections of heroin induced a modest, but lower, rise in DA in the buprenorphine-treated groups possibly due to delta-opioid receptor activation.

Finally, concerning the interaction between cocaine and buprenorphine, additive effects of opioids and psychostimulants have been demonstrated using different experimental procedures. For example, synergistic effects have been seen on acquisition of a conditioned place preference (Brown et al., 1991) and in enhanced locomotor activity (Smith et al., 2003). Therefore, it was expected that treatment with buprenorphine would potentiate the locomotor activity to an acute injection of cocaine. As discussed above, however, there was an attenuation of this response with chronic buprenorphine treatment that might be accounted for by up-regulation of the DAT.

In summary, for up to 25-27 days of chronic administration of buprenorphine there was no change in the locomotor activity induced by acute injections of heroin, whereas the blockade of the heroin-induced increase in extracellular DA in the NAc seen in a previous experiment (Sorge et al., 2005) was reduced. These data were discussed in terms of increased activity at the delta-opioid receptor and reduced activity at the mu-opioid receptor. In the case of cocaine, the potentiation of the locomotor and NAc DA responses seen early in treatment was no longer evident during prolonged chronic treatment. In addition, although these data suggest that after acute injections or early in chronic exposure to buprenorphine responses to cocaine may be enhanced (Brown et al., 1991), with chronic treatment this enhancement is no longer evident. Paradoxically, however, rats treated chronically with buprenorphine maintained significantly elevated basal extracellular DA levels in the NAc throughout long-term treatment. Although these studies do not shed direct light on how buprenorphine acts to reduce self-administration of heroin and cocaine, they are consistent with findings in monkeys that there is little tolerance to the suppressant effect of buprenorphine on self-administration (Mello et al., 1992). Thus, from the perspective treatment drug abuse, it is encouraging that there seems little reason to be concerned that the useful effects of buprenorphine will show tolerance.

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General Discussion

In the experiments reported in this thesis, the opioid abuse treatment drug, buprenorphine, had both common and differential effects on the behavioral and neurochemical response to heroin and cocaine in rats. Chronic treatment with buprenorphine via osmotic minipump reduced responding for heroin and cocaine-associated cues under extinction conditions and during tests for drug-induced reinstatement. Furthermore, this reduction in responsiveness to drug-associated cues was seen at the beginning of *daily* self-administration sessions in the experiment in which sampling of DA levels in the NAc was carried out using microdialysis. Specifically, buprenorphine-treated rats showed an increased latency to respond to activation of the cue light and entry of the lever signaling drug availability at the start of the session. This reduction in responding could not be accounted for by sedation or 'anhedonia' inasmuch as chronic treatment increased activity slightly and significantly increased basal levels of DA in the NAc for up to 28 days of treatment. Although reduced responsiveness to cues was seen in both the heroin and cocaine self-administration experiments, buprenorphine had no effect on heroin intake at any dose or under any schedule of reinforcement in spite of attenuating the NAc DA response to self-administered infusions *and* to experimenter-delivered acute injections. On the contrary, chronic buprenorphine reduced cocaine intake across all doses and schedules tested in one series of experiments (Chapter 2), even though the NAc DA and the locomotor response to self-administered infusions or acute injections of cocaine was potentiated in other experiments (Chapters 1 and 3). It was found, however, that the potentiated NAc DA and locomotor response to acute

injections of cocaine was diminished over the course of 28 days of chronic buprenorphine treatment in spite of elevated plasma levels of buprenorphine and basal NAc DA levels.

As discussed in the introduction, buprenorphine is as effective as methadone in measures of reduction in opioid use and retention in treatment (Johnson et al., 1992; Strain et al., 1996). Administration of buprenorphine reduces intake of heroin (Mello et al., 1980) and cocaine (Foltin et al., 1996) and reduces craving for heroin (Greenwald et al., 2002) and cocaine (Foltin et al., 1996) in human patients. In animal studies buprenorphine reduces intake of heroin (Mello et al., 1983), cocaine (Mello et al., 1990) and heroin-cocaine 'speedball' (Mello et al., 1998) in monkeys trained on complex, cue-controlled schedules of reinforcement. Although the evidence seems to indicate that buprenorphine is highly effective in reducing heroin intake under these conditions and schedules, chronic administration via osmotic minipump did not reduce heroin intake across the doses and schedules of reinforcement measured in the above series of experiments. Heroin infusions did not result in an increase in NAc DA as measured by concurrent *in vivo* microdialysis and so, given the extant literature on the effectiveness of buprenorphine to reduce heroin intake, it is surprising that we found no change in heroin intake in our rats. For example, patients given buprenorphine have shown reduced heroin self-administration (Mello et al., 1980), reduced craving for heroin and choice of hydromorphine over money (Greenwald et al., 2002) and, when given a buprenorphine/naloxone combination, patients have reported reduced subjective effects of heroin (Comer et al., 2005). Chronic daily administration of buprenorphine to monkeys working on complex schedules of reinforcement reduced responding for heroin

(Mello et al., 1998), though this reduction in intake is not always found in spite of reduced choice of heroin over food (Negus, 2006).

The common effect of buprenorphine found in Chapters 1 and 2 is a reduction in the effectiveness of drug-associated cues to elicit responding. This effect was discussed as possibly resulting from elevated basal DA levels as a result of chronic buprenorphine masking the cue-induced DA signal as well as a reduction in glutamate activity preventing/blunting this neurochemical response to drug-associated cues. In both drug- and food-trained animals there is a modest increase in DA cell firing in anticipation of reward (Gratton et al., 1994; Chang, Sawyer, Lee, & Woodward, 1994) and following presentation of a stimulus that reliably predicts reward (Schultz, Apicella, & Ljungberg, 1993; Schultz, 1998). The increase DA cell firing likely contributes to the elevated firing of NAc (shell) neurons seen in response to cocaine-associated cues in withdrawal from cocaine self-administration (Ghitza, Fabbricatore, Prokopenko, Pawlak, & West, 2003). As a result of prolonged activation of DA receptors there is an increase in post-synaptic D_2 receptor sensitivity (Edwards, Whisler, Fuller, Orsulak, & Self, 2006) and a decrease in D_2 autoreceptor sensitivity in the NAc (Henry, Hu, & White, 1998). This suggests that even a modest rise in DA, elicited by a drug-associated cue, may have a greater impact on behavior following conditioning (i.e. cue-induced reinstatement). In the case of the rats undergoing chronic buprenorphine treatment, however, the elevated basal levels of DA within the NAc may have made post-synaptic DA receptors more sensitive, but the chronic DA neuron activation may have also reduced the signal-to-noise ratio of DA in the NAc. In this case, the rats would be less likely to respond to drug-associated cues.

The increased DA levels as a result of chronic buprenorphine may have reduced responding for drug-associated cues directly through DAergic alterations or indirectly through alterations in other systems. At several points in the discussion of the reduced responsiveness to cues associated with drugs, it has been suggested that altered glutamate function and/or activity may have been responsible and there is reason to believe that chronic administration of buprenorphine resulted in reduced glutamate function. Chronic administration of morphine reduces NMDA-receptor mediated synaptic transmission in NAc cells (Martin et al., 1999) and reduces the affinity of glycine at the NMDA receptor (Siggins et al., 2003). In addition, *in vivo* experiments have shown that chronic treatment with morphine results in a number of alterations to glutamate function, including reduced basal and evoked levels (Huang et al., 1997) and increased GluR1 receptor subunits in the VTA (Fitzgerald, Ortiz, Hamedani, & Nestler, 1996) and the BLA (Glass, Kruzich, Colago, Kreek, & Pickel, 2005). Although no systematic study has yet evaluated the possibility that buprenorphine reduces glutamate activity, it is reasonable to assume that chronic treatment may have had this effect. Chronic treatment with opioids increases basal DA levels (Huang et al., 1997), as seen in the above experiments, and DA has clear effects on glutamate activity. Glutamate release can be reduced via local administration of DA itself, or DA agonists, to striatal tissue *in vitro* (Nieoullon, Kerkerian, & Dusticier, 1982) and via perfusion into the microdialysis probe *in vivo* in both the NAc (Kalivas & Duffy, 1997) and the PFC (Harte & O'Connor, 2004). This modulation of glutamate release is thought to arise through DA activation of D_2 receptors located on glutamatergic terminals (Maura, Giardi, & Raiteri, 1988; Yamamoto & Davy, 1992) and, thus, the high

levels of DA seen in our experiments may have led to inhibition of glutamate release through continuous D_2 receptor occupation.

If chronic administration of buprenorphine were to reduce glutamatergic activity, this, in turn, might explain the reduction in responsiveness to drug-associated cues. In particular the metabotropic glutamate receptor 2/3 agonist LY379268 has been shown to reduce cue-induced reinstatement of responding for heroin (Bossert, Liu, Lu, & Shaham, 2004; Bossert et al., 2005) and cocaine (Baptista et al., 2004). These drugs function to reduce evoked glutamate release and are effective in reducing cue-induced reinstatement when administered centrally, or directly into the NAc (Bossert, Gray, Lu, & Shaham, 2005). This drug, when given systemically or into the NAc, is effective in reducing food-seeking and cocaine-induced cocaine seeking, though a lower priming dose was used than that in the current thesis experiments (Peters & Kalivas, 2006). Furthermore, it has been seen that these drugs reduce cocaine (Baptista et al., 2004), but not heroin intake in rats (Bossert et al., 2005) – an effect paralleled by chronic buprenorphine treatment. Thus, it appears that glutamate has a role in responsiveness to drug-associated cues and that drugs which negatively modulate glutamate disrupt this behavior.

Chronic administration of buprenorphine might have its effects on responsiveness to cues through modulation of glutamate in specific areas critically involved in cue associations. Rats show elevated neural activity (i.e. Fos protein expression) in the PFC, BLA and the NAc following a test for a cocaine-induced CPP (Miller & Marshall, 2004) indicating that both areas are activated by drug-associated cues. The PFC and BLA are reciprocally connected, but both project to the NAc, which is considered a common output pathway for motivated action. Although both the PFC and BLA send excitatory

projections to the NAc, it has been shown through fluorogold iontophoresis (Miller & Marshall, 2005) and lesion experiments (Meil & See, 1997), that the BLA output is the critical one for cue-induced drug seeking behavior. Chronic treatment with morphine has been shown to increase expression of NMDAR1 mRNA (Turchan, Maj, & Przewlocka, 2003) and GluR1 subunits (Glass et al., 2005) in the BLA signifying that opioids modulate glutamate in this area. There is little colocalization of mu opioid receptors and GluR1 receptor subunits in the BLA (Glass et al., 2005) and so it can be assumed that the regulation of glutamate receptors is indirect and possibly the result of increased DA activity.

The integrity of the BLA is critical for learning drug-cue associations and responding as the result of exposure to these cues. Lesions of the BLA have no effect on acquisition of heroin (Alderson, Robbins, & Everitt, 2000) or cocaine self-administration (Whitelaw, Markou, Robbins, & Everitt, 1996; Meil et al., 1997), but do disrupt second-order conditioning (Whitelaw et al., 1996) and the acquisition of the associative learning of a cocaine-paired cue (Kruzich & See, 2001). BLA lesions block cue-induced reinstatement of heroin (Fuchs & See, 2002) and cocaine self-administration (Meil et al., 1997; Grimm & See, 2000; Kruzich et al., 2001) as well as the expression of a cocaine CPP (Fuchs, Weber, Rice, & Neisewander, 2002). Lesions of the BLA reduce heroin-primed reinstatement (Fuchs et al., 2002), but have no effect on cocaine-induced reinstatement (Grimm et al., 2000). These data suggest, when taken with the lack of effects on acquisition of self-administration, that the integrity of the BLA is critical for the associations between heroin and cocaine and predictive stimuli.

The mechanism responsible for the differential effects of buprenorphine on the intake of heroin and cocaine is currently unknown, but we have speculated that they could have arisen as a result of a shift in receptor action of heroin or decreased glutamate activity affecting cocaine reinforcement. The common effect whereby chronic administration of buprenorphine reduced the responsiveness to drug-associated cues may have been the result of a combination of increased DA and the resulting decreased glutamate activity. Increased basal DA in the BLA may have resulted in decreased glutamate release, which in turn reduced BLA output to the NAc. This may have effectively mirrored a BLA lesion, which was manifested as a reduced ability of drug-associated cues to motivate responding. Therefore, future experiments with chronic administration of buprenorphine via osmotic minipump should investigate the alterations in glutamate activity globally and in specific regions in the brain. In addition, experiments specifically examining responses to cues associated with drugs while under buprenorphine treatment are critical to evaluate the above hypotheses.

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