INVESTIGATIONS OF THE NEUROBIOLOGICAL AND BEHAVIOURAL ACTIONS OF COCAINE

By

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We accept this thesis as conforming
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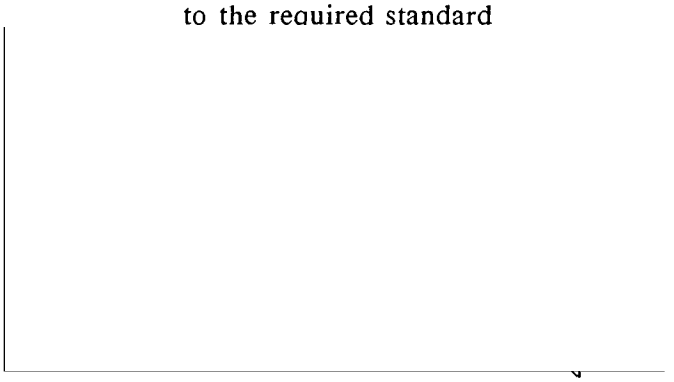

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Abstract

Although once considered a benign recreational stimulant, cocaine is now recognized to possess substantial abuse potential with considerable medical and social consequences. Accordingly, these experiments examined the behavioural and neurobiological effects of cocaine in the rat.

The behavioural and neurochemical interactions between cocaine and buprenorphine were examined using a conditioned place preference (CPP) procedure and *in vivo* microdialysis. Cocaine and buprenorphine both elicited CPP; moreover, these drugs interacted to produce significantly larger CPPs when given in combination. Both cocaine and buprenorphine increased interstitial concentrations of dopamine in the nucleus accumbens; the effect of cocaine was potentiated by the coadministration of buprenorphine. Taken as a whole, these results indicate that buprenorphine can interact with cocaine in a synergistic manner.

The ability of stimuli previously paired with cocaine to elicit similar neurochemical changes as cocaine was assessed by *in vivo* microdialysis. Although acutely administered cocaine produced a significant increase in interstitial dopamine concentrations in the nucleus accumbens, the presentation of a cocaine-paired environment did not. Despite the absence of a conditional neurochemical effect, significant conditioned locomotion was observed. These data do not support the hypothesis that stimuli paired with cocaine produce their behavioural effects by eliciting similar neurochemical effects as cocaine.

To understand better the neurobiology of cocaine-induced environment-specific conditioning, expression of *c*-Fos, a putative marker of neuronal activity, was examined in the forebrain of rats exposed to an environment in which they had previously received cocaine. Compared to saline-treated controls, cocaine produced an increase in locomotor behaviour that was accompanied by an increase in *c*-Fos expression within specific limbic regions, as well as the basal ganglia. Exposure of rats to the cocaine-paired environment

also produced an increase in locomotion that was associated with an increase in *c*-Fos expression within specific limbic regions, but not within the basal ganglia. These findings suggest that specific limbic regions exhibit increased neuronal activation during the presentation of cocaine-paired cues and may be involved in the formation of associations between cocaine's stimulant actions and the environment in which the drug administration occurred.

Given the large body of evidence implicating the amygdaloid complex in the learning of stimulus-reward associations, the effects of quinolinic acid lesions of the amygdala on cocaine-induced conditional locomotion and CPP were examined. Although destruction of the amygdala did not affect basal locomotion, cocaine-induced locomotion or cocaine-induced conditional locomotion, cocaine-induced CPP was completely blocked by the amygdaloid lesions. These data demonstrate that cocaine-induced stimulus-reward conditioning can be differentially affected by lesions of the amygdala.

These studies provide a further understanding of the neurobiology of cocaine's behavioural actions. The implications for the treatment of cocaine abuse are discussed.

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Acknowledgments

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I. INTRODUCTION

Shortly after cocaine became commercially available in Europe and the United States in the 1880's it began to receive endorsements and praise for its action as a tonic for the body and mind (Musto, 1992). At this time the stimulant properties of cocaine were emphasized, while its addictive potential was generally dismissed. Within thirty years, however, the adverse effects of cocaine had become apparent and severe restrictions on its use were enacted. In the United States the recognition of the abuse potential of cocaine culminated in the passage of the Harrison Act of 1914. Cocaine use diminished during the 1920's as the adverse consequences associated with its use became more widely acknowledged (Musto, 1992). As cocaine use began to increase rapidly in the 1970's and early 1980's (Anthony, 1992), it was again suggested by medical professionals that cocaine was a safe, nonaddicting stimulant (Grinspoon and Bakalar, 1980; Van Dyke and Byck, 1982). These assertions were partly the result of an absence of strong empirical evidence regarding the actions of cocaine. At present, clinical and epidemiological research has clearly documented numerous severe consequences of cocaine use (Anthony, 1992; Benowitz, 1992) and it is recognized that cocaine possesses substantial abuse potential. Given that cocaine abuse remains a serious medical and social issue, considerable interest remains in elucidating the actions and effects of this powerful stimulant.

Clinical Characteristics of Cocaine Addiction

Acute administration of cocaine produces a sense of alertness, euphoria and well-being (Jaffe, 1989; Johanson and Fischman, 1989). There are also decreases in hunger and need for sleep. In addition to these behavioural changes, a number of physiological signs occur following cocaine, such as tachycardia, pupillary dilation and elevated blood pressure. Cocaine can also induce paranoia, suspiciousness and overt psychosis, especially following a prolonged "binge" (Gawin and Ellinnwood, 1988; Jaffe, 1989; Satel *et al.*, 1991).

Of those individuals who try intranasal cocaine ("snorting") the National Institute of Drug Abuse estimates that 10 to 15% become abusers (Gawin, 1991). Most cocaine users seeking treatment report that their initial use was intermittent. Eventually, however, episodes of high-dosage use become more frequent until "runs" or binges occur (DSM-III-R; Gawin and Ellinwood, 1988; Jaffe, 1989). Binges, which can last several days, are characterized by the user compulsively readministering cocaine every 10 to 30 minutes. During the binge, the user experiences periods of extreme euphoria, the memories of which will later be contrasted with the mood of the undrugged state and can produce cravings for the drug (Gawin and Ellinwood, 1988; Gawin and Kleber, 1986). Binges tend to be interrupted only when drug toxicity occurs, the user collapses from physical exhaustion or the cocaine supplies are depleted. The termination of the binge is generally followed by an intense and unpleasant "crash".

Clinical findings appear to suggest that cocaine smoking or intravenous administration tend to produce a more rapid progression from infrequent use to cocaine abuse or dependence than intranasal use of cocaine (DSM-III-R; Jaffe, 1989; Gawin and Ellinwood, 1988). This result may reflect the fact that these routes of administration produce a more rapid rise in blood and brain concentrations of cocaine and an intense "rush", followed by a rapid decline in blood and brain concentrations (Jones, 1990).

Psychoactive substance disorders, as defined in the DSM-III-R, are divided into psychoactive substance dependence, and a residual category, psychoactive substance abuse, which is reserved for individuals who exhibit pathological drug use, but fail to meet the criteria for substance dependence. The criteria for psychoactive substance dependence and abuse are presented in Tables 1 and 2, respectively. Most individuals who exhibit high-dose, binge use of cocaine would meet the criteria for cocaine dependence. Unfortunately, not all authors use these terms with reference to the DSM-III-R diagnostic criteria, or they use other terms, such as cocaine addiction, making it difficult to make comparisons between the results of different investigators.

Table 1.

Diagnostic Criteria for Psychoactive Substance Dependence

A. At least three of the following:

1). substance often taken in larger amounts or over a longer period than the person intended.

2). persistent desire or one or more unsuccessful efforts to cut down or control substance use.

3). a great deal of time spent in activities necessary to get the substance (*e.g.* theft), taking the substance, or recovering from its effects.

4). frequent intoxication or withdrawal symptoms when expected to fulfill major role obligations at work, school, or home (*e.g.* does not go to work because hung over, goes to school or work "high", intoxicated while taking care of his or her children), or when substance use is physically hazardous (*e.g.* driving while intoxicated).

5). important social, occupational, or recreational activities given up or reduced because of substance use.

6). continued substance use despite knowledge of having a persistent or recurrent social, psychological, or physical problem that is caused or exacerbated by the use of the substance.

Note: The following items may not apply to cannabis, hallucinogens or phencyclidine

8). characteristic withdrawal symptoms (*e.g.* fatigue, insomnia or hypersomnia, and/or psychomotor agitation for cocaine withdrawal).

9). substance often taken to relieve or avoid withdrawal symptoms.

B. Some symptoms of the disturbance have persisted for at least 1 month, or have occurred repeatedly over a longer period of time.

Adapted from DSM-III-R *Diagnostic and Statistical Manual of Mental Disorders*, ed 3, revised. American Psychiatric Association, Washington, DC, 1987.

Table 2.

Diagnostic Criteria for Psychoactive Substance Abuse

A. A maladaptive pattern of psychoactive substance use indicated by at least one of the following:

1). continued use despite knowledge of having a persistent or recurrent social, occupational, psychological, or physical problem that is caused or exacerbated by the use of the psychoactive substance.

2). recurrent use in situations in which use is physically hazardous (*e.g.* driving while intoxicated).

B. Some symptoms of the disturbance have persisted for at least 1 month, or have occurred repeatedly over a longer period of time.

C. Never met the criteria for psychoactive substance dependence for this substance.

Adapted from DSM-III-R *Diagnostic and Statistical Manual of Mental Disorders*, ed 3, revised. American Psychiatric Association, Washington, DC, 1987.

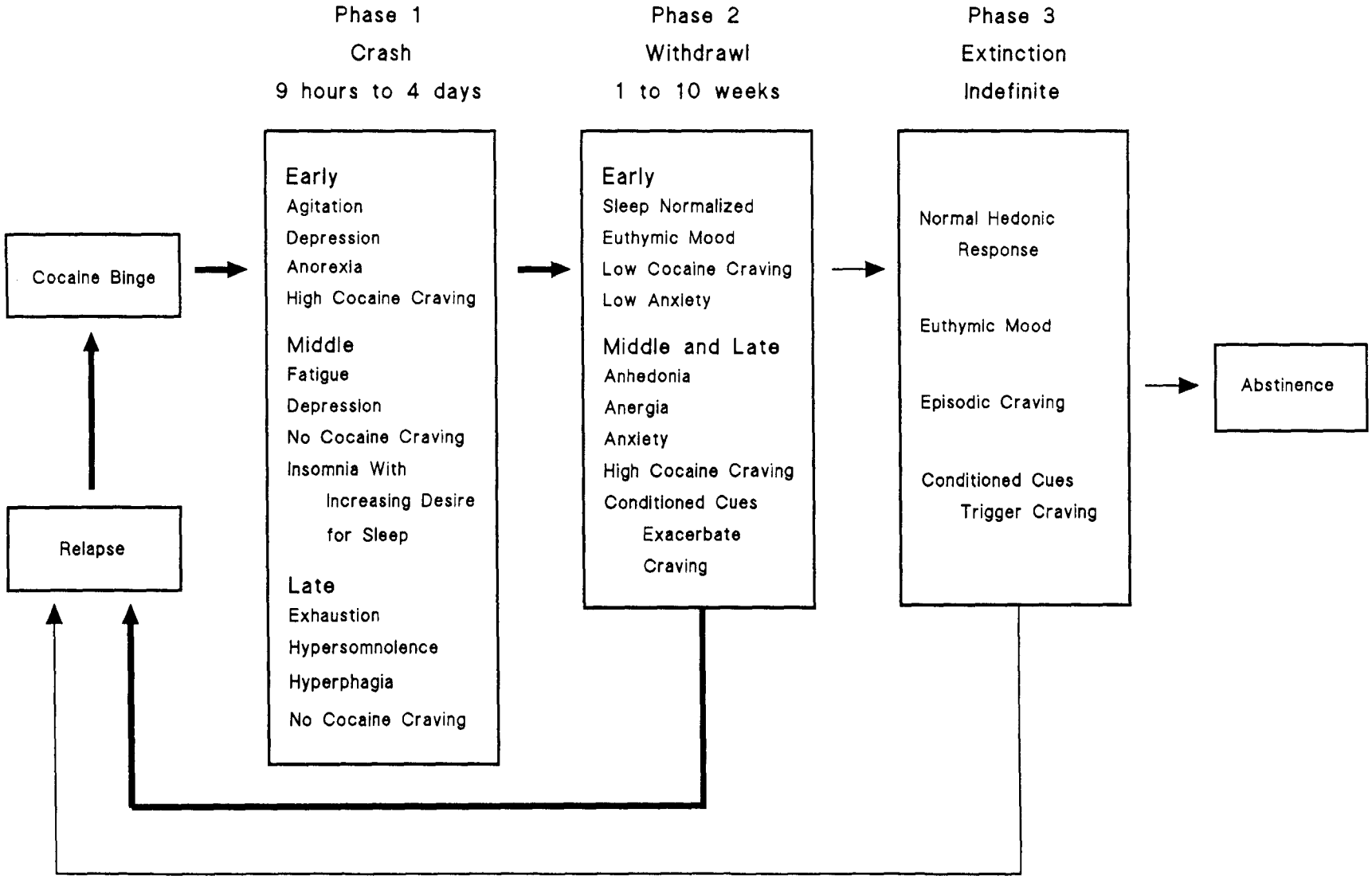
A triphasic syndrome associated with the abstinence from cocaine has been described by Gawin and Kleber (1986). This syndrome is represented schematically in Figure 1. The first phase, the crash, immediately follows the cessation of a cocaine binge, and is characterized by symptoms that vary throughout the crash phase. During the initial period of phase 2, withdrawal, the individual experiences improved mood, normalized sleep and low levels of cocaine craving; however, as this phase proceeds anhedonia, anergia, anxiety and increased cocaine craving, especially in response to stimuli previous associated with cocaine use, become apparent. The third phase, extinction, appears to represent a period of extended vulnerability to relapse, although mood and withdrawal anhedonia have normalized.

A clear understanding of the clinical characteristics of cocaine abuse or dependence is useful for a number of reasons. First, the informed clinician will have greater predictive power regarding the probable course of cocaine abuse for individuals seeking treatment, and hence be capable of formulating more rational treatment programs that specifically address problems associated with the abstinence from cocaine. Second, the relative dirth of empirical clinical data regarding cocaine's abuse potential that contributed to the belief that cocaine was a harmless stimulant will be avoided in the future. Finally, an awareness of the salient features of cocaine abuse will allow basic researchers investigating this topic to consider their findings in a broader context.

Neurobiology of Cocaine-induced Reward

The investigation of the neurobiology of cocaine abuse has proven to be a particularly fruitful endeavor, as a number of animal models have been developed to investigate this topic. For example, the self-administration paradigm (Weeks, 1962) has been used by numerous investigators to examine the neurochemical and neuroanatomical substrates that mediate cocaine's ability to function as a positive reinforcer. The results of a large number of studies using various pharmacological challenges and specific brain lesions

Figure 1. Schematic representation of the phases of the cocaine abstinence syndrome. Duration and intensity of symptoms vary on the basis of binge characteristics and patient history and diagnosis. Binges range from under four hours to six or more days. [Modified from Gawin and Kleber, 1986]


Phase 1
Crash
9 hours to 4 days
Phase 2
Withdrawl
1 to 10 weeks
Phase 3
Extinction
Indefinite
Cocaine Binge
Relapse
Early
Agitation
Depression
Anorexia
High Cocaine Craving
Middle
Fatigue
Depression
No Cocaine Craving
Insomnia With
Increasing Desire
for Sleep
Late
Exhaustion
Hypersomnolence
Hyperphagia
No Cocaine Craving
Early
Sleep Normalized
Euthymic Mood
Low Cocaine Craving
Low Anxiety
Middle and Late
Anhedonia
Anergia
Anxiety
High Cocaine Craving
Conditioned Cues
Exacerbate
Craving
Normal Hedonic
Response
Euthymic Mood
Episodic Craving
Conditioned Cues
Trigger Craving
Abstinence

strongly implicate the mesolimbic dopaminergic pathway in the rewarding effects of cocaine (Fibiger *et al.*, 1992; Fibiger and Phillips, 1987; Johanson and Fischman, 1989). For example, Ritz and colleagues (1987) have reported that although cocaine blocks the reuptake of noradrenaline, dopamine (DA) and serotonin, its action at the DA uptake site appears most directly related to its rewarding effects. This conclusion is in agreement with studies that have demonstrated that low doses of specific DA receptor antagonists increase the rate of responding for intravenous cocaine (De Wit and Wise, 1977; Ettenberg *et al.*, 1982; Roberts and Vickers, 1984; Koob *et al.*, 1987a), while neither α- or β-adrenergic receptor antagonists affect cocaine self-administration (De Wit and Wise, 1977; Wilson and Schuster, 1974; Woolverton, 1987). These results have been interpreted by some investigators to indicate that low doses of DA receptor antagonists produce a partial DA receptor blockade that results in an increase in the rate of responding to maintain the previous level of postsynaptic activation. However, it has also been proposed that "dopamine (receptor) antagonists exert their effect by antagonizing effects of cocaine unrelated to its reinforcement" (Johanson and Fischman, 1989). Recent findings, however, lend additional support for the hypothesis that DA receptor antagonists can affect the self-administration of cocaine in a reward-related manner. Roberts *et al.* (1989) found that a low dose of haloperidol, which had previously been shown to produce an increase in responding for cocaine on a continuous reinforcement schedule (Roberts and Vickers, 1984), produced a significant reduction in the break point for cocaine in the progressive ratio self-administration paradigm, a measure which varies directly with the strength of the rewarding effects of the self-administered drug. Moreover, Bergman and colleagues (1990) have reported that the dose-response curve for cocaine self-administration is shifted to the right by selective D_1 and D_2 receptor antagonists. These data, while consistent with the hypothesis that DA receptor antagonists attenuate the rewarding properties of cocaine, are not readily accounted for by the simple rate-reduction antagonism hypothesis (Johanson and Fischman, 1989). It should be noted, however, that rate of responding has been shown to be

affected by factors independent of the rewarding effects of cocaine, and that conclusions based on changes in the simple rate of responding must be interpreted with caution (Johanson and Fischman, 1989).

The previously discussed results are further supported by studies that have established that 6-hydroxydopamine (6-OHDA) lesions of the mesolimbic dopaminergic pathway, which produce extensive depletions of DA in the nucleus accumbens, produce dramatic suppression in the rate of lever pressing for cocaine (Pettit *et al.*, 1984; Roberts *et al.*, 1977, 1980). This finding does not appear to be the result of a generalized motor deficit or other nonspecific effects, as Dworkin and Smith (1988) have demonstrated that 6-OHDA lesions of the nucleus accumbens suppress the ascending limb of the cocaine self-administration dose-effect curve, while responding for food and water was unaffected. Furthermore, Koob *et al.* (1987b) have reported that the break point for cocaine in the progressive ratio self-administration paradigm was significantly reduced by 6-OHDA lesions of the nucleus accumbens.

Although the aforementioned results suggest that the dopaminergic projection to the nucleus accumbens plays a critical role in the reinforcing effects of cocaine, other observations suggest that additional factors are involved in the self-administration of this psychomotor stimulant. For example, Roberts and Koob (1982) reported that while 6-OHDA lesions of the ventral tegmental area, the origin of the dopaminergic projections to the nucleus accumbens and other limbic structures, produced significant reductions in the rate of cocaine self-administration, there was no significant correlation between the magnitude of the DA depletion in the nucleus accumbens and the change in responding from prelesion rates. Moreover, several rats with considerable depletions of accumbens DA responded at near normal pre-lesion rates for cocaine. The authors speculated that the dopaminergic innervation of other structures, such as the olfactory bulb, frontal cortex or amygdala, or a combination of structures may be critical in the self-administration of cocaine. To this end, Goeders and Smith (1983) reported that rats will self-administer

cocaine directly into the medial prefrontal cortex, but not into the nucleus accumbens. To examine the role of this dopaminergic projection area in the role of systemically administered cocaine, Martin-Iverson *et al.* (1986) investigated the effect of 6-OHDA lesions of the medial prefrontal cortex on the rate and pattern of cocaine self-administration. Although 6-OHDA lesions produced approximately a 95% depletion in DA in the medial prefrontal cortex, no significant effect of these lesions on either the rate or pattern of cocaine self-administration was observed. These results strongly suggest that the dopaminergic innervation of the medial prefrontal cortex is not necessary for the reinforcing effects of intravenously administered cocaine. Given the previously discussed shortcomings of using rate of responding as a measure of the reinforcing properties of cocaine, reexamination of the importance of the medial prefrontal cortex and other DA projection areas is warranted.

The rewarding properties of cocaine have also been extensively investigated using the conditioned place preference (CPP) paradigm. This paradigm is based on the principle that if rewarding or appetitive stimuli are reliably associated with a given environment then there will be an increased tendency to approach or maintain contact with that environment (Carr *et al.*, 1989). Although the findings of self-administration and CPP studies are generally in agreement, it should be noted that these paradigms have divergent theoretical backgrounds and behavioural demands and cannot be assumed necessarily to examine identical phenomena (Wise, 1989). Cocaine has been shown to produce a CPP by numerous investigators (Bardo *et al.*, 1984; Mackey and van der Kooy, 1985; Morency and Beninger, 1986; Nomikos and Spyraki, 1988; Spyraki *et al.*, 1982a, 1987). However, it appears that multiple mechanisms are potentially involved in cocaine-induced CPP, as this effect has been reported to be dependent on dopaminergic transmission under some conditions (Morency and Beninger, 1986; Spyraki et al., 1987), while independent of dopaminergic transmission in other studies (Mackey and van der Kooy, 1985; Morency and Beninger, 1986; Spyraki *et al.*, 1982a). Although these data suggest that DA is not *necessary* for

cocaine-induced CPP following *i.p.* or *s.c.* administration, they cannot be assumed to indicate that dopaminergic transmission is not normally involved in this behaviour. Clearly, an understanding of the apparent DA-independent actions of cocaine in the CPP paradigm will assist in further addressing these discrepancies.

The advent of *in vivo* microdialysis has dramatically increased current understanding of the neurochemical actions of cocaine. The ability of behaviourally relevant doses of cocaine to increase interstitial concentrations of DA in the rat nucleus accumbens has been well established (Bradberry and Roth, 1989; Di Chiara and Imperato, 1988a; Moghaddam and Bunney, 1989a; Pettit and Justice, 1989, 1991). Moreover, Pettit and Justice (1989, 1991) have demonstrated that cocaine self-administration produces significant dose-related increases in interstitial DA in the nucleus accumbens. Interestingly, cocaine has been reported to preferentially increase interstitial DA in the nucleus accumbens, as compared to its effect in the striatum (Di Chiara and Imperato, 1988a) and the medial prefrontal cortex (Moghaddam and Bunney, 1989a). When considered together with the previously discussed behavioural studies, recent microdialysis findings provide further support for the importance of the mesolimbic dopaminergic projection to the nucleus accumbens in the rewarding properties of cocaine.

Summary

Cocaine is presently recognized as a powerful stimulant possessing significant abuse potential, as well as a specific pattern of abuse and withdrawal (DSM-III-R; Gawin, 1991; Jaffe, 1989). A large body of evidence indicates that the mesolimbic DA system plays a fundamental role in the reinforcing properties of cocaine (Di Chiara and Imperato, 1988; Fibiger *et al.*, 1992; Fibiger and Phillips, 1987; Johanson and Fischman, 1989; Roberts *et al.*, 1977; 1989). Despite the growth of both the clinical understanding of cocaine abuse and the neurobiological actions of cocaine, few attempts have been made to integrate the findings of these fields of investigation in a meaningful way. The following studies are a modest attempt to approach this objective.

II. BEHAVIOURAL AND NEUROCHEMICAL INTERACTIONS BETWEEN COCAINE AND BUPRENORPHINE

(A) Introduction

Buprenorphine (BUP) is a synthetic opioid with the pharmacological profile of a mixed agonist-antagonist. It has potent antinociceptive actions (Cowan *et al.*, 1977; Dum and Herz, 1981) with a limited capacity for producing physical dependence (Cowan *et al.*, 1977; Jasinski *et al.*, 1978; Mello and Mendelson, 1980; Yanagita *et al.*, 1981). BUP can also attenuate opiate self-administration in humans (Mello and Mendelson, 1980; Mello *et al.*, 1982) and in non-human primates (Mello *et al.*, 1983).

Recently, it has been reported that BUP suppresses cocaine self-administration by rhesus monkeys (Mello *et al.*, 1989). Based on these data, it was suggested that BUP might be useful in the pharmacotherapy of cocaine abuse. This proposal is supported by a preliminary report by Kosten and colleagues (1989) that indicated that heroin addicts treated with BUP had a significant reduction in cocaine-positive urines, compared to those subjects treated with methadone. However, BUP is a potential drug of abuse, as indicated by epidemiological (Chowdhury and Chowdhury, 1990; Lewis, 1985; O'Connor *et al.*, 1988; Strang, 1985) and primate self-administration studies (Lukas *et al.*, 1986; Mello *et al.*, 1981; Woods, 1977; Young *et al.*, 1984). Moreover, as is the case for a variety of drugs of abuse, BUP decreases the threshold for intracranial self-stimulation (Hubner and Kornetsky, 1988). Cocaine or morphine pretreatment also suppresses responding for cocaine by non-human primates (Balster *et al.*, 1992; Herling *et al.*, 1979; Stretch, 1977), indicating that decreases in the rate of responding in self-administration paradigms can occur for reasons other than decreases in the rewarding properties of the reinforcer. These observations raise the possibility that the BUP-induced decrease in cocaine self-administration reported by Mello *et al.* (1989) was due to a summation of the rewarding properties of these agents. In view

of these considerations, the conditioned place preference (CPP) paradigm was utilized to address two specific questions. First, can BUP produce CPPs? Second, is cocaine-induced CPP affected by BUP?

Although cocaine blocks the reuptake of a variety of biogenic amines, its action at the DA uptake site appears to be most directly related to its rewarding effects (Ritz *et al.*, 1987). This conclusion is consistent with self-administration studies that utilized specific neurotoxic lesions to illustrate that the integrity of the mesolimbic DA pathway to the nucleus accumbens is necessary for cocaine self-administration (Pettit *et al.*, 1984; Roberts *et al.*, 1977; Roberts *et al.*, 1980). Moreover, low doses of specific DA receptor antagonists produce predictable alterations in self-administration of cocaine (De Wit & Wise, 1977; Ettenberg *et al.*, 1982; Roberts & Vickers, 1984; Roberts *et al.*, 1989), and local infusions of the DA receptor antagonist spiroperidol into the nucleus accumbens disrupts cocaine self-administration (Phillips & Broekkamp, 1980). *In vivo* microdialysis studies further suggest that the rewarding properties of cocaine are mediated by increases in interstitial DA in the nucleus accumbens (Di Chiara & Imperato, 1988a; Moghaddam & Bunney, 1989a; Pettit & Justice, 1989, 1991).

Although the association between the rewarding properties of cocaine and the release of DA in the nucleus accumbens is well established, the importance of mesolimbic DA in the rewarding properties of opioids is unclear. Ettenberg *et al.* (1982) reported that pretreatment with the neuroleptic α-flupenthixol produced a compensatory increase in cocaine self-administration, but had no effect on heroin self-administration. In agreement with this finding, 6-OHDA lesions, which reduced cocaine self-administration, had no lasting effect on heroin self-administration (Pettit *et al.*, 1984). However, other findings suggest that the DA projections from the ventral tegmental area (VTA) to the nucleus accumbens may play a role in the rewarding effects of opioids. First, it has been reported that infusions of opioids directly into the VTA are rewarding, as assessed by self-administration (Bozarth and Wise, 1981a; Welzl *et al.*, 1989) and CPP (Bals-Kubik *et al.*,

1993; Phillips and LePiane, 1980, 1982). Second, heroin-induced CPPs are attenuated by neuroleptics (Bozarth & Wise, 1981b; Spyraki *et al.*, 1983). Third, infusions of opioid receptor antagonists directly into the VTA decrease the rewarding properties of systemic heroin (Britt and Wise, 1983). Finally, results from *in vivo* microdialysis studies demonstrate that μ receptor agonists, such as morphine, methadone, fentanyl and [D-Ala^2, *N*-methyl-Phe^4, Gly^5-ol]-enkephalin (DAMGO), increase interstitial DA concentrations in the nucleus accumbens (Di Chiara and Imperato, 1988b; Spanagel *et al.*, 1990). Because BUP increases DA turnover at behaviourally relevant doses (Cowan *et al.*, 1976), it is possible that the effect of BUP on cocaine self-administration (Mello *et al., 1989*) may be the result of interactions between these agents on DA release in the nucleus accumbens. To determine if BUP alters the effects of cocaine on interstitial DA concentrations, *in vivo* microdialysis studies were also undertaken.

(B) Materials and Methods

Subjects

Subjects were 268 male Long Evans rats (Charles River, Quebec), weighing 270-350 g at the beginning of the experiment. The rats were group housed (four per cage), on a 12:12 h light:dark cycle (lights on 07:00), with food and water available *ad libitum*. All subjects were handled daily for one week prior to initiation of the experiment. All experimental procedures were conducted at approximately the same time each day, during the animal's light phase.

Apparatus

Place preference conditioning was conducted in four identical shuttle boxes (78 x 25 cm, 35 cm high). Each box was divided into two compartments (36 x 25 cm) joined by a tunnel (6 x 8 x 8 cm) that could be closed at both ends by guillotine doors. The two compartments differed in the appearance of the walls and the type of floor (solid brown walls and a 1.2 cm wire mesh floor versus walls with black and white strips 1 cm wide and a floor of parallel bars spaced 1.2 cm apart). Translucent Plexiglas lids allowed a moderate amount of light from overhead incandescent lights to enter each compartment. Each shuttle box was mounted on a fulcrum, allowing its position to be detected by microswitches. The time spent in each compartment and the number of crosses between compartments was recorded with dedicated electronic equipment.

Procedure

The procedure for CPP consisted of three phases: habituation, conditioning and test. During the three day habituation phase, rats were placed in one of the compartments of the shuttle box (hereafter referred to as the start side), following a counterbalanced design. Following placement in the start side, and with both guillotine doors raised, the rats were given access to both compartments during the 900 s trial. During each trial, the time spent in each compartment and the number of crosses between compartments were recorded. The conditioning phase was conducted over the next eight days. On days 1, 3, 5 and 7, rats were given drug injections and immediately confined to the non-start side for 30 min. On alternate days, rats were injected with saline and confined to the start side for 30 min. On the test day, each rat was placed in the start-side and given access to both compartments; the time spent in each compartment and the number of crosses between compartments were recorded.

Cocaine-Induced CPP

The first experiment was conducted to determine the dose-response relationship for cocaine in the CPP paradigm. Animals were randomly assigned to one of four groups (n=12 per group), that received 0, 1.25, 2.5 or 5.0 mg/kg cocaine.

BUP-Induced CPP

The dose response relationship for BUP in the CPP paradigm was determined in this experiment. Animals were randomly assigned to one of nine groups (n=12 per group) that received 0, 0.005, 0.01, 0.03, 0.075, 0.15, 0.3, 0.6 or 0.9 mg/kg BUP.

Cocaine & BUP, CPP Experiments

Based on the dose-response data obtained in the first two experiments, two studies were conducted to examine the effects of combinations of cocaine and BUP in the CPP paradigm. In the first of these studies, animals were randomly assigned to one of four groups (n=12 per group) that received either vehicle + vehicle, cocaine + vehicle, vehicle + BUP or cocaine + BUP. Cocaine and BUP were given in doses of 1.5 and 0.01 mg/kg, respectively. The second study of this experiment used the same design as the first, except that cocaine and BUP were given in doses of 5.0 and 0.075 mg/kg, respectively.

Cocaine & BUP, Dialysis Experiments

Rats were anaesthetized with sodium pentobarbital (50 mg/kg *i.p.*), mounted in a stereotaxic instrument, and a vertical microdialysis probe was implanted into the nucleus accumbens (AP: +3.6 mm; ML: -1.5 mm; DV: -8.2 mm from dura; relative to bregma; Pellegrino *et al.*, 1979). The microdialysis probe was a variant of the concentric vertical design (outer diameter = 250 μm, molecular weight cut-off = 6000 Dalton; Spectra Por dialysis fibre). The active surface was 2 mm in length, and was 0.2 mm from the tip of the probe. Recovery of DA, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid

(HVA) and 5-hydroxyindoleacetic acid (5-HIAA) was measured *in vitro* at 37^{0}C and at a flow rate of 5 μl/min. The *in vitro* recoveries (%) for this probe were (mean $\pm$ SD, n=6): 3.5 $\pm$ 0.4 (DA), 4.0 $\pm$ 0.5 (DOPAC), 3.7 $\pm$ 0.4 (HVA) and 4.3 $\pm$ 0.5 (5-HIAA).

Following surgery, the rats were individually housed for 48 h prior to testing. On the test day, after a stable baseline had been established (not more than 5% variation for 3 consecutive samples), each rat was injected with either cocaine + saline, BUP + saline, cocaine + BUP or saline + saline. Microdialysis samples were taken for five hours post-injection. Histological verification of the probe placement was conducted following testing.

Microdialysis experiments were conducted on-line (Damsma *et al.*, 1990), such that the microdialysis probe was perfused at 5 μl/min (Harvard Apparatus perfusion pump); the resulting dialysate was directed to the sample loop (100 μl) of an injector (Valco) through the outlet tubing (PE-10, Clay Adams). Samples were automatically injected into a high performance liquid chromatographic (HPLC) system with electrochemical detection every 20 min. The perfusion fluid contained: NaCl (147 mM), KCl (3.0 mM), $CaCl_2$ (1.3 mM), $MgCl_2$ (1.0 mM), and sodium phosphate (1.5 mM, pH 7.3).

The concentrations of DA, DOPAC, HVA and 5-HIAA in the dialysate were determined with HPLC in conjunction with electrochemical detection. Separation of DA and the acid metabolites was achieved by reversed-phase liquid chromatography (150 x 4.6 mm, Nucleosil 5 C_{18}, Chrompack). The flow rate of the mobile phase [0.1 M acetic acid adjusted to pH 4.1 with solid sodium acetate, 0.01 mM EDTA, 0.35 - 0.5 mM octanesulfonic acid (Kodak) and 10 % methanol] was 1.85 ml/min. Detection of the amines was achieved by the sequential oxidation and reduction of the samples by a coulometric detection system (coulometric electrode = +0.4 V; amperometric electrode = -0.2 V; ESA Inc.). The acid metabolites were quantified by their oxidation at the coulometric electrode, while DA was measured at the subsequent amperometric electrode. The detection limit of this assay was approximately 5 fmol/injection for DA and 20 fmol/injection for DOPAC, 5-HIAA and HVA.

Drugs

Cocaine hydrochloride (Sigma) and buprenorphine hydrochloride (Schering) were dissolved in isotonic saline and were injected intraperitoneally (*i.p.*). In both the cocaine and BUP CPP experiments, drugs were injected in a volume of 3 ml/kg; in the combination experiments (cocaine and BUP), drugs were injected in a volume of 1 ml/kg. Doses of cocaine are expressed as weight of salt, while those of buprenorphine are expressed as the base.

Statistical Analysis

Trend analysis was used to evaluate the results from the cocaine and BUP CPP experiments. Data were weighted based on the dose of the drug. Within-group comparisons were then conducted using the Bonferroni *t* statistic to assess which doses produced significant CPP. An initial analysis of variance illustrated that the preconditioning values for the time spent on the non-start side did not differ between groups [$F(20,231)=0.30$, $p=0.999$], justifying the use of within-subject comparisons. The results from the combination CPP experiments (cocaine and BUP) were also analyzed using the Bonferroni *t* statistic. A Reverse Helmert analysis was conducted to further examine some of the CPP data. The data from the dialysis experiments (% values), from time 0 to 300 min, were evaluated using a two-way analysis (Treatment X Time) of variance with repeated measures (Geisser-Greenhouse adjustment of *d.f.*). Percentage values of dialysate concentrations were based on an average of the three samples prior to the injection of drug. Comparisons of differences between groups at specific time points were made using the Bonferroni *t* statistic. All statistics were performed using SPSS:X version.3 software.

(C) Results

Cocaine-induced CPP

The results of this experiment indicate that the time spent in the drug-paired (non-start) side was directly related to the dose of cocaine (Figure 2). Specifically, a significant linear dose-response function was observed [$F(1,44)=7.26$, $p<0.02$]. Within-group comparisons illustrated that only the 5.0 mg/kg group spent significantly more time on the drug-paired side of the apparatus [$t(11)=2.6$, $p<0.05$].

BUP-induced CPP

BUP elicited a dose-related CPP (Figure 3). The data were best fit by a cubic dose-response function [$F(1,99)=13.88$, $p<0.001$], although a linear dose-response function was also observed [$F(1,99)=4.83$, $p<0.05$]. Within-group comparisons indicated that at 0.03 mg/kg, 0.075 mg/kg, 0.15 mg/kg and 0.3 mg/kg BUP produced significant CPP [$t(11)=5.28$, 4.54, 5.78, 3.61, respectively, $p<0.001$].

Cocaine & BUP, CPP Experiments

Consistent with the data from the previous experiments, low doses of BUP (0.01 mg/kg) or cocaine (1.5 mg/kg) did not by themselves produce CPP (Figure 4). However, a within-group comparison indicated that animals pretreated with a combination of BUP and cocaine did exhibit CPP [$t(11)=2.63$, $p<0.05$]. This finding was replicated using another group of animals [$t(11)=2.55$, $p<0.05$].

CPP was produced by moderate doses of cocaine (5.0 mg/kg) [$t(11)=2.89$, $p<0.01$] and BUP (0.075 mg/kg) [$t(11)=4.09$, $p<0.001$], as well as by a combination of these drugs [$t(11)=6.25$, $p<0.001$] (Figure 5). A Reverse Helmert analysis of the three treatment conditions indicated that, while the cocaine and BUP treatment groups did not differ

Figure 2. The effects of cocaine on the time spent in the drug-paired (non-start) compartment before (□) and after (■) conditioning (n = 12/group). Values represent the mean ± SEM. * indicates a significant within-group difference (p<0.05) of pre- versus post-conditioning scores.

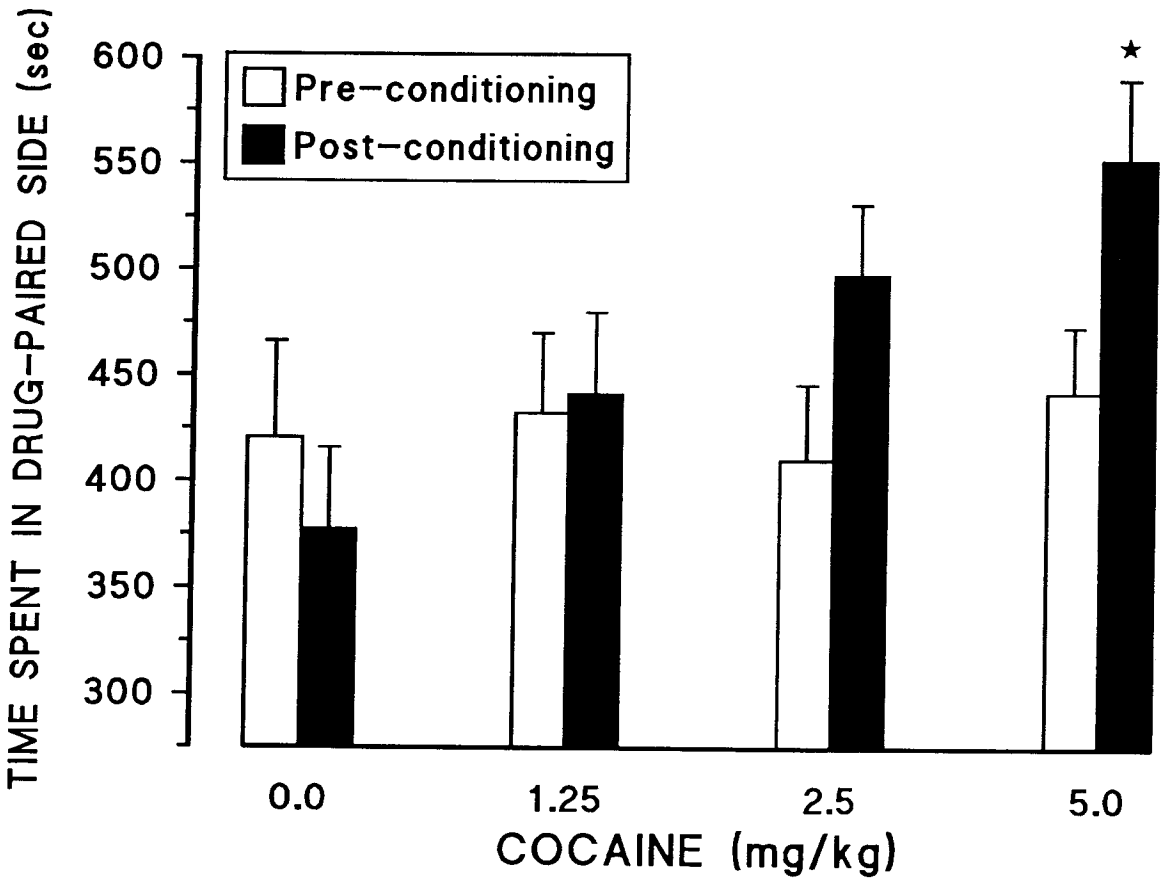

TIME SPENT IN DRUG-PAIRED SIDE (sec)
600
550
500
450
400
350
300
Pre-conditioning
Post-conditioning
★
0.0
1.25
2.5
5.0
COCAINE (mg/kg)

Figure 3. The effects of buprenorphine on the time spent in the drug-paired (non-start) compartment before (□) and after (■) conditioning (n = 12/group). Values represent the mean ± SEM. * indicates a significant within-group difference ($p<0.05$) of pre- versus post-conditioning scores.

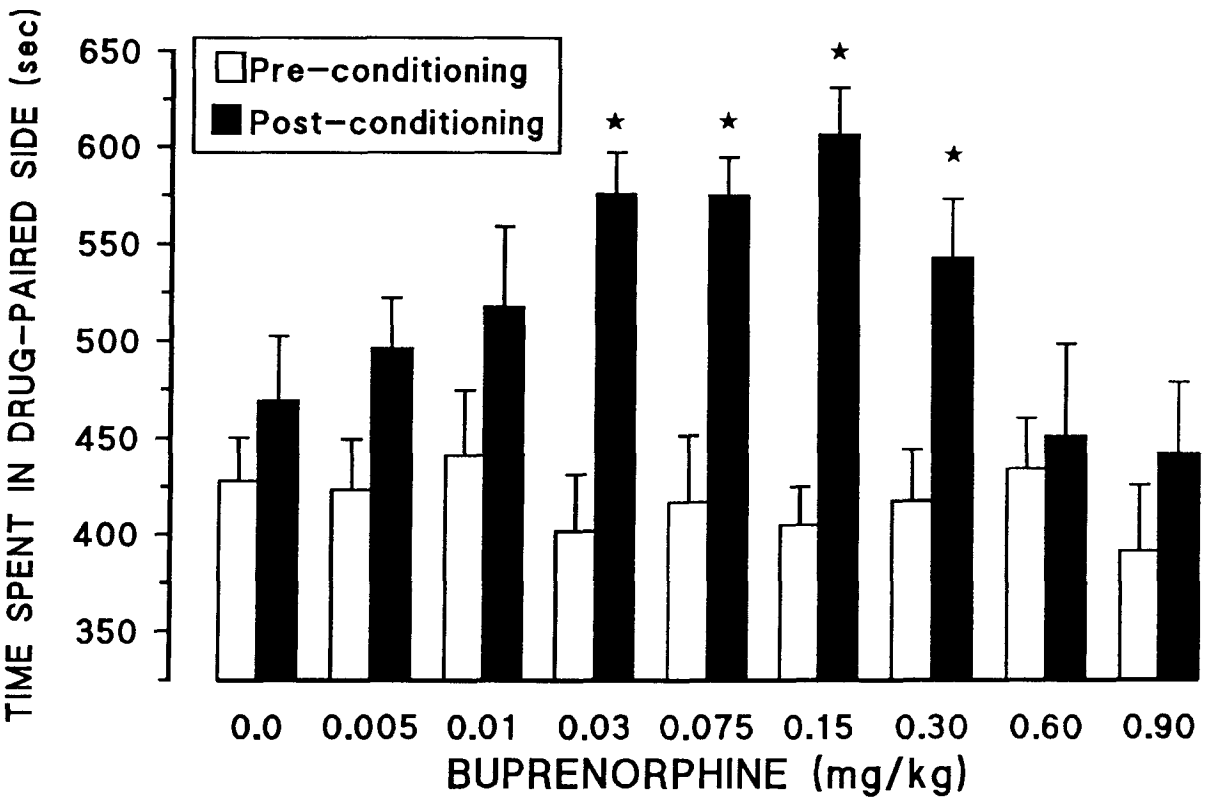

TIME SPENT IN DRUG-PAIRED SIDE (sec)
650
600
550
500
450
400
350
Pre-conditioning
Post-conditioning
★
★
★
★
0.0
0.005
0.01
0.03
0.075
0.15
0.30
0.60
0.90
BUPRENORPHINE (mg/kg)

Figure 4. The effects of saline, cocaine (1.5 mg/kg), BUP (0.01 mg/kg), and cocaine (1.5 mg/kg) + BUP (0.01 mg/kg) on the time spent in the drug-paired (non-start) compartment before (□) and after (■) conditioning (n = 12/group). Values represent the mean ± SEM. * indicates a significant within-group difference (p<0.05) of pre- versus post-conditioning scores.

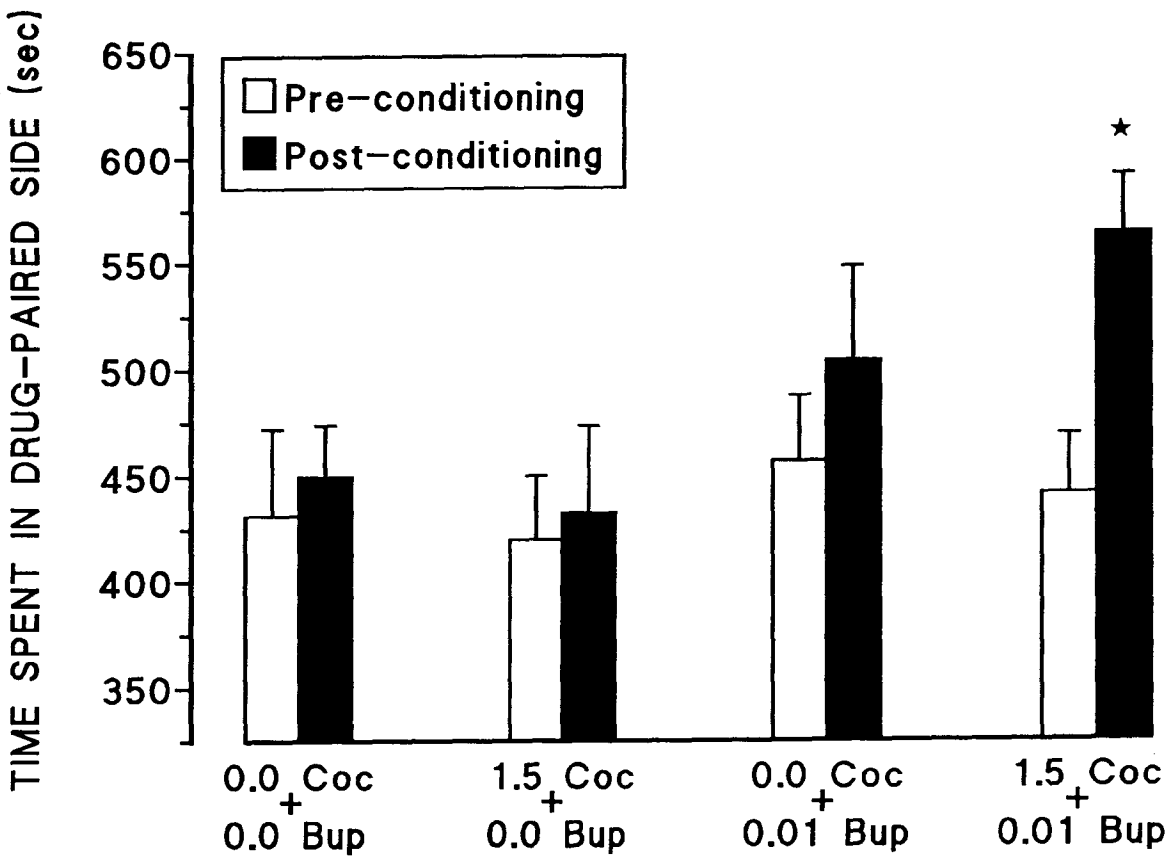
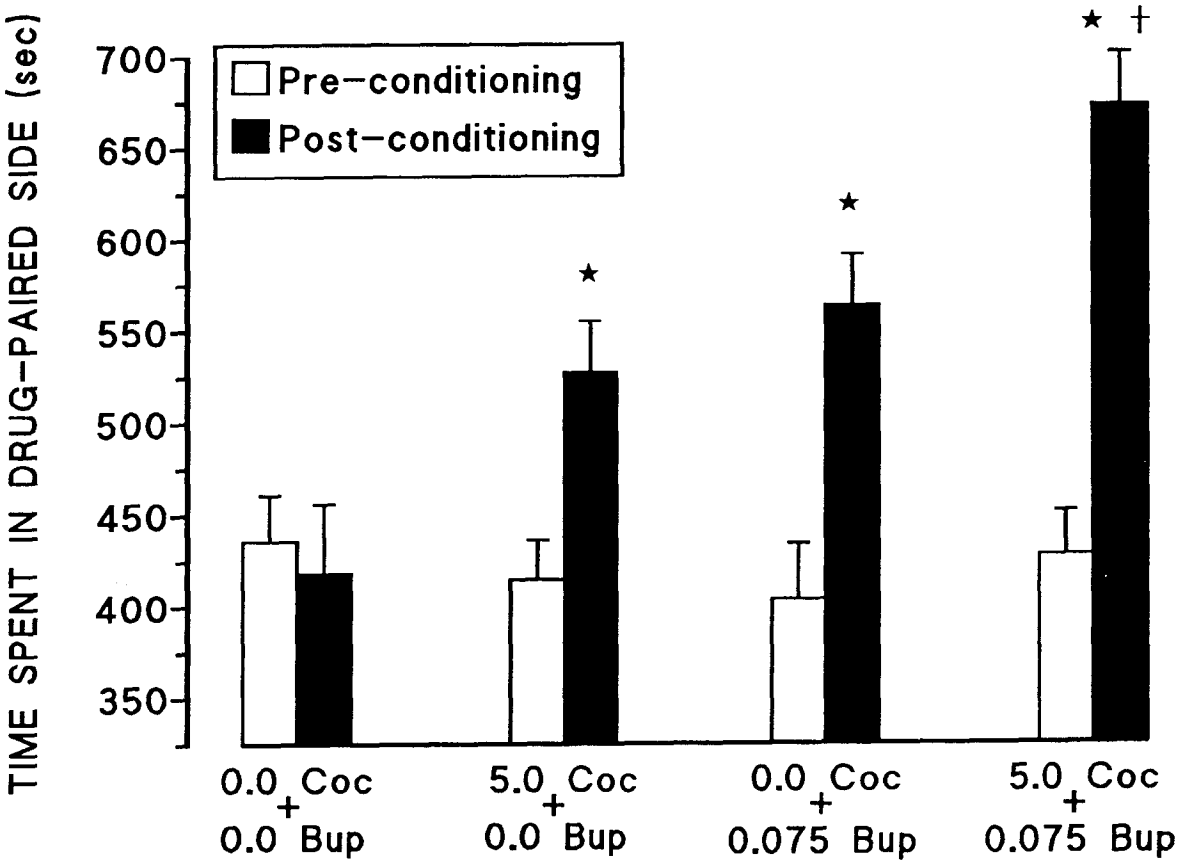

TIME SPENT IN DRUG-PAIRED SIDE (sec)
650
600
550
500
450
400
350
Pre-conditioning
Post-conditioning
★
0.0 Coc + 0.0 Bup
1.5 Coc + 0.0 Bup
0.0 Coc + 0.01 Bup
1.5 Coc + 0.01 Bup

Figure 5. The effects of saline, cocaine (5.0 mg/kg), BUP (0.75 mg/kg), and cocaine (5.0 mg/kg) + BUP (0.75 mg/kg) on the time spent in the drug-paired (non-start) compartment before (□) and after (■) conditioning (n = 12/group). Values represent the mean ± SEM. * indicates a significant within-group difference ($p<0.05$) of pre- versus post-conditioning scores. † indicates a significant difference ($p<0.05$) between the combination group (cocaine + BUP) and the cocaine and the BUP treatment groups, as determined by a reverse Helmert analysis.


TIME SPENT IN DRUG-PAIRED SIDE (sec)
700
650
600
550
500
450
400
350
Pre-conditioning
Post-conditioning
★
★
★ †
0.0 Coc + 0.0 Bup
5.0 Coc + 0.0 Bup
0.0 Coc + 0.075 Bup
5.0 Coc + 0.075 Bup

significantly from each other [F(1,33)=0.75], the combined treatment was significantly different from the individual treatments [F(1,33)=5.29, $p<0.05$].

Cocaine & BUP, Dialysis Experiments

The average baseline output of DA (± SEM, n=16) was 1.84 fmol/min ± 0.48, and did not differ significantly between groups [F(3,12)=0.68]. The average basal outputs (± SEM, n=16) for DOPAC, HVA and 5-HIAA were 533 ± 65, 297 ± 42, and 245 ± 54 fmol/min, respectively. Basal values of DOPAC [F(3,12)=1.53], HVA [F(3,12)=0.18] and 5-HIAA [F(3,12)=0.69] did not differ significantly between the groups.

Cocaine produced a rapid increase in interstitial concentrations of DA [F(3.29,19.74)=11.76, $p<0.001$] that returned to baseline within 120 to 160 min (Figure 6). Cocaine also significantly decreased DOPAC [F(2.70,16.23)=4.85, $p<0.05$] and HVA [F(2.77,16.65)=4.68, $p<0.05$] without affecting the serotonin metabolite, 5-HIAA [F(2.41,12.05)=1.45]. All statistical comparisons are made against saline injected controls (data not shown).

Interstitial DA was gradually increased by BUP [F(3.99,23.92)=9.80, $p<0.001$], reaching approximately 200% of baseline values five hours post injection (Figure 7). Interstitial concentrations of DOPAC [F(3.17,19.04)=5.21, $p<0.01$] and HVA [F(3.32,19.92)=15.73, $p<0.001$] were also increased by BUP, although the effect on DOPAC was not sustained over the five hour test period. Interstitial 5-HIAA was unaffected by BUP [F(2.60, 15.62)=1.63].

Co-administration of cocaine and BUP produced a rapid increase in the interstitial concentration of DA that returned to baseline within 120 to 160 min [F(2.45,14.73)=8.80, $p<0.005$] (Figure 8). DA concentrations showed a further modest increase after 180 min. DOPAC [F(3.12,18.72)=12.18, $p<0.001$] and HVA [F(2.96,17.76)=14.89, $p<0.001$] were also significantly altered by the drug combination, as compared to saline injected controls. The DA metabolites showed a transient decrease, followed by a gradual increase over the

Figure 6. The effects of cocaine (5.0 mg/kg) + saline on microdialysis output of DA (o), DOPAC (□), HVA (△) and 5-HIAA (◊). Cocaine was administered at time 0. Values represent the group mean (n = 4) ± SEM. Percentage values of dialysate concentrations were based on an average of the three samples prior to the injection of drug.

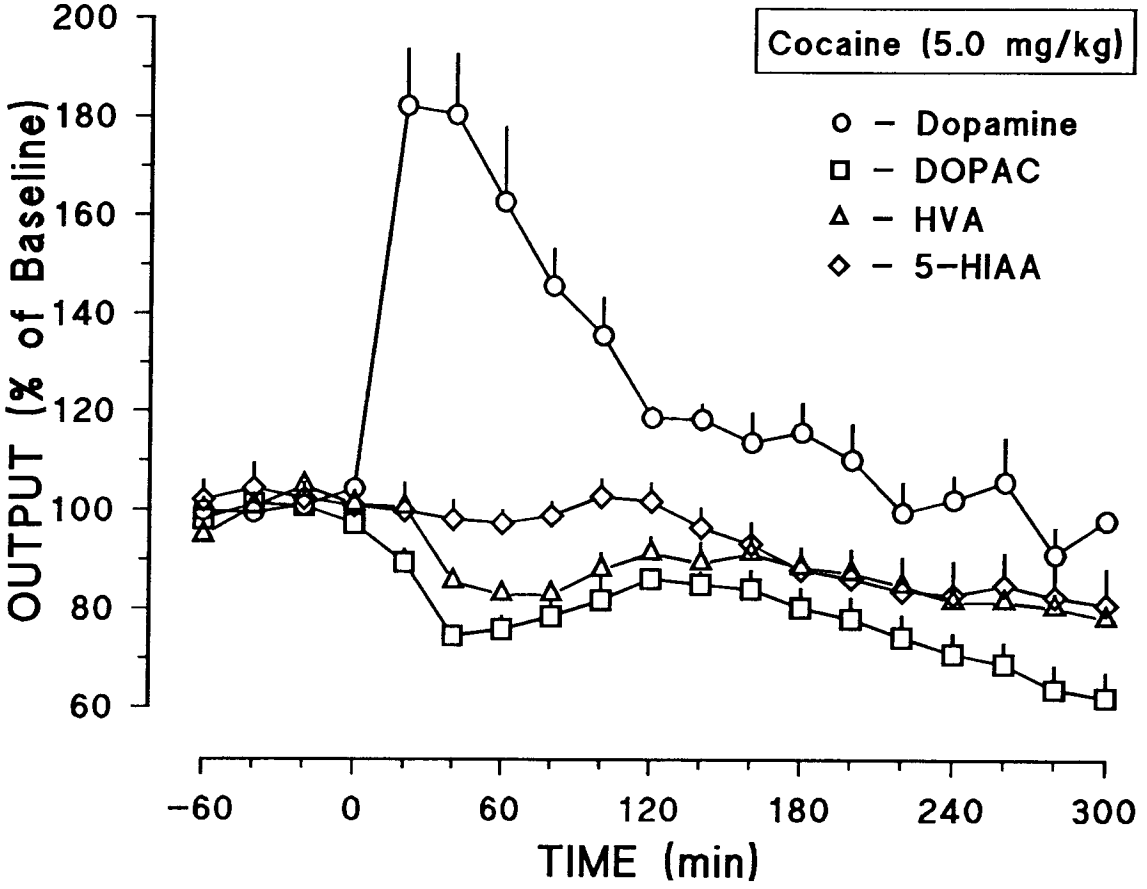

Cocaine (5.0 mg/kg)
○ – Dopamine
□ – DOPAC
△ – HVA
◇ – 5-HIAA
OUTPUT (% of Baseline)
200
180
160
140
120
100
80
60
-60
0
60
120
180
240
300
TIME (min)

Figure 7. The effects of BUP (0.01 mg/kg) + saline on microdialysis output of DA (o), DOPAC (□), HVA (△) and 5-HIAA (◊). BUP was administered at time 0. Values represent the group mean (n = 4) ± SEM. Percentage values of dialysate concentrations were based on an average of three samples prior to the injection of drug.

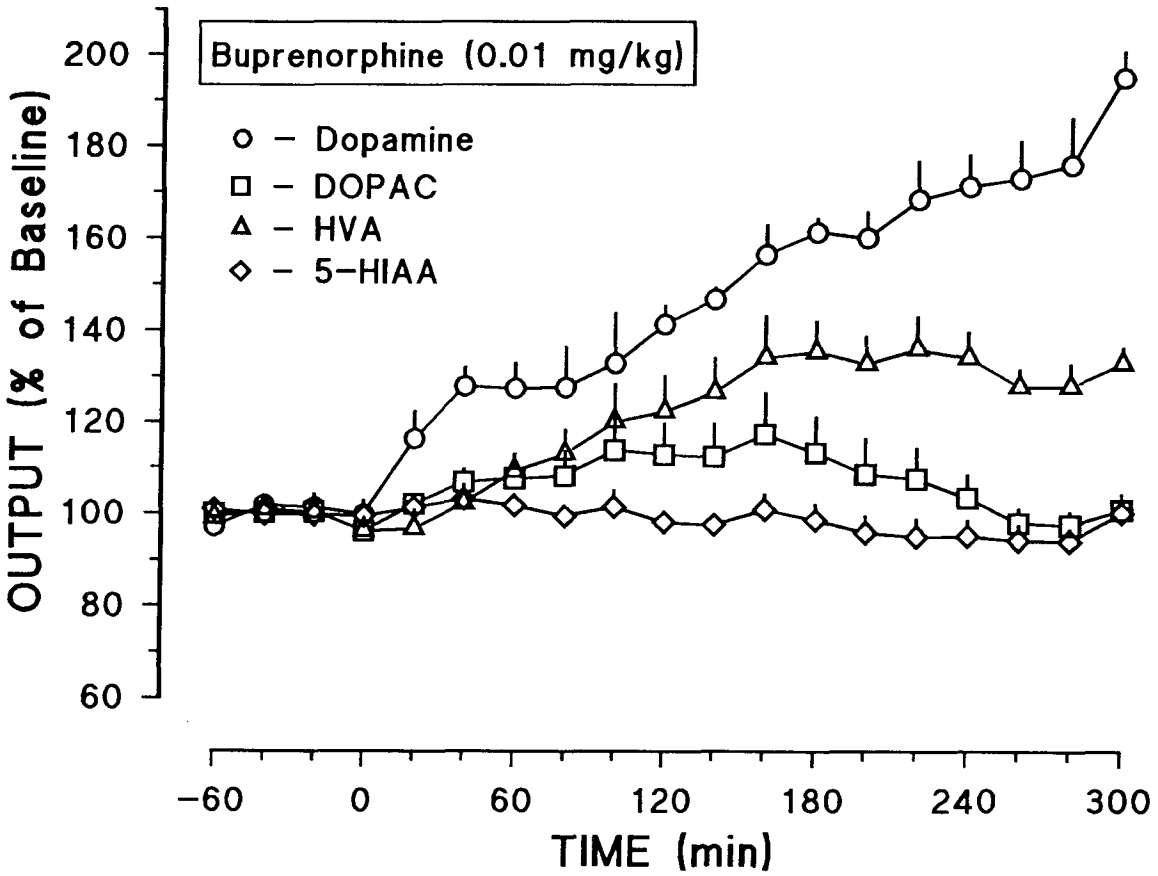

Buprenorphine (0.01 mg/kg)
○ – Dopamine
□ – DOPAC
△ – HVA
◇ – 5-HIAA
OUTPUT (% of Baseline)
200
180
160
140
120
100
80
60
−60
0
60
120
180
240
300
TIME (min)

Figure 8. The effects of the combination of cocaine (5.0 mg/kg) and BUP (0.01 mg/kg) on microdialysis output of DA (o), DOPAC (□), HVA (△) and 5-HIAA (◊). The drugs were administered at time 0. Values represent the group mean (n = 4) $\pm$ SEM. Percentage values of dialysate concentrations were based on an average of three samples prior to the injection of drug.

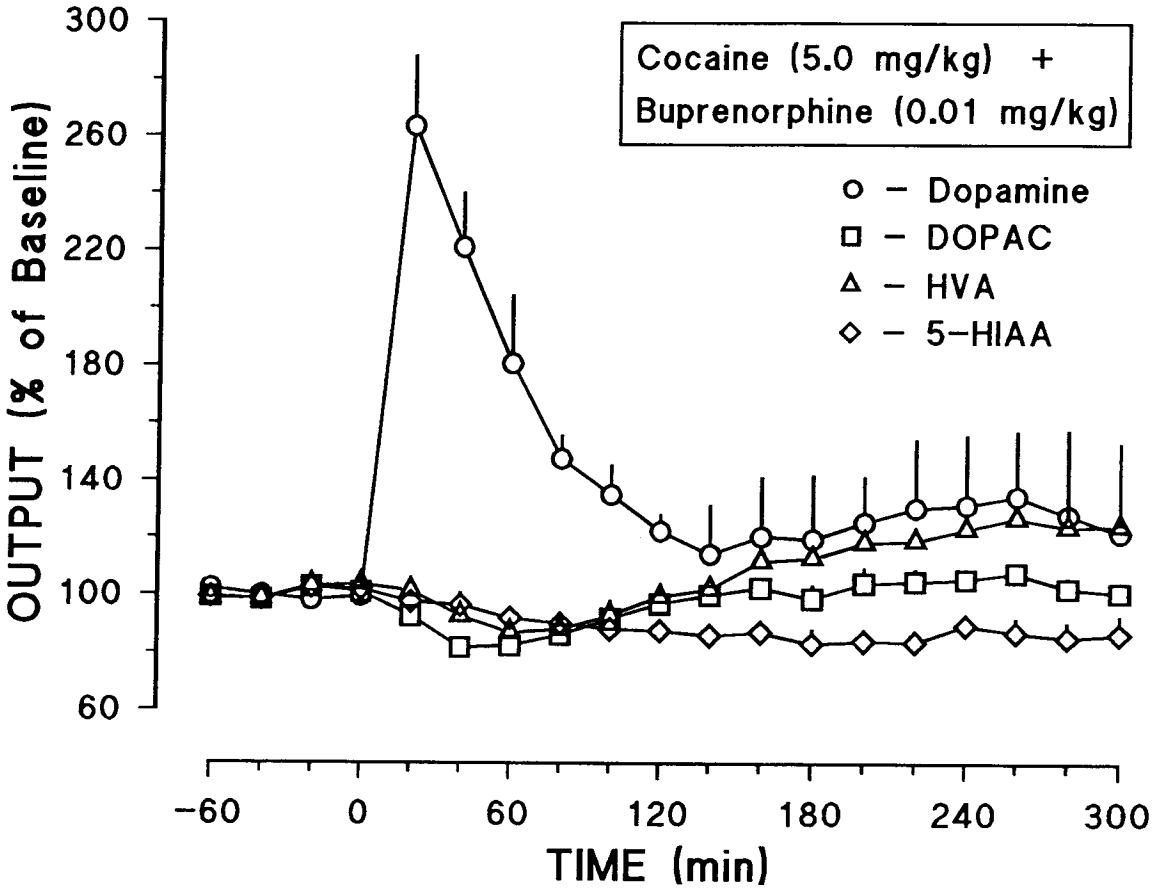
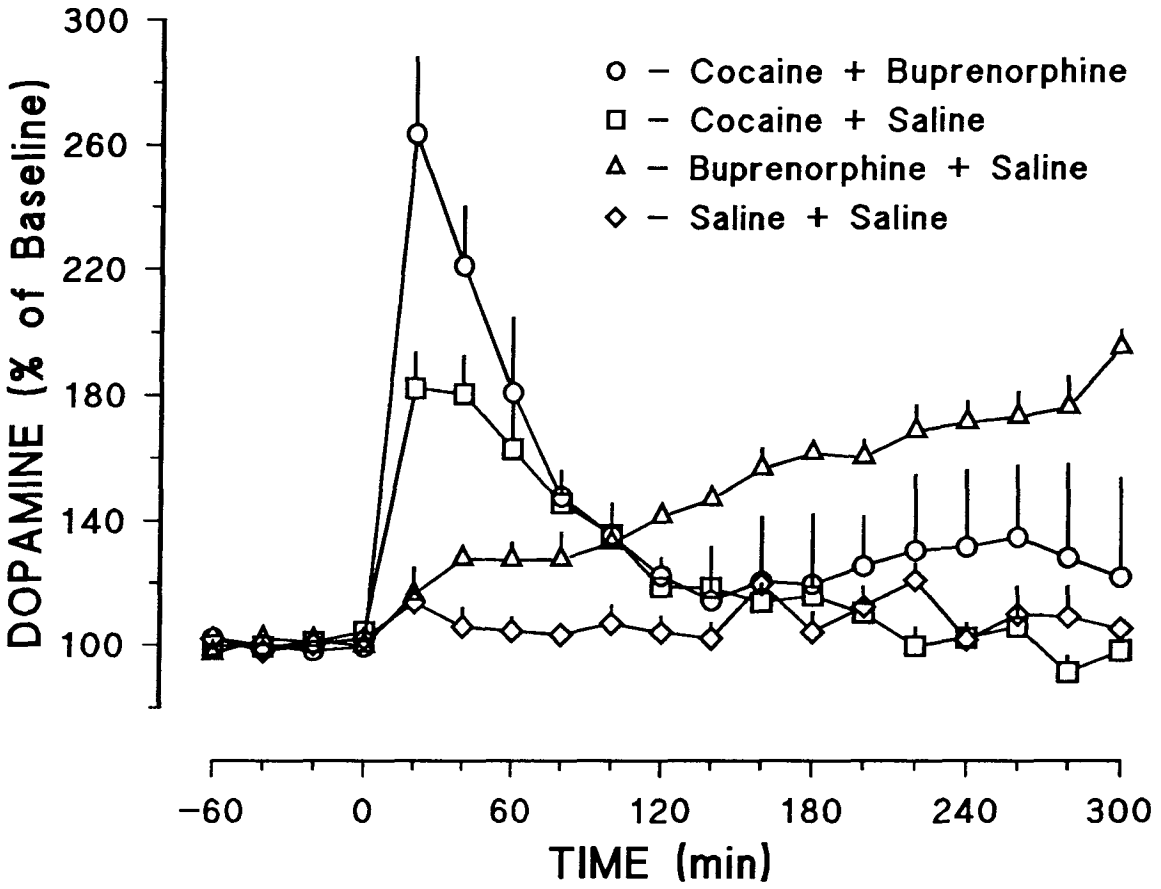

Cocaine (5.0 mg/kg) +
Buprenorphine (0.01 mg/kg)
o – Dopamine
□ – DOPAC
△ – HVA
◇ – 5-HIAA
OUTPUT (% of Baseline)
300
260
220
180
140
100
60
-60
0
60
120
180
240
300
TIME (min)

Figure 9. A summary of the effects of saline + saline (◊), cocaine (5.0 mg/kg) + saline (□), BUP (0.01 mg/kg) + saline (△) and cocaine (5.0 mg/kg) + BUP (0.01 mg/kg) (○) on microdialysis output of DA. Drugs were administered at time 0. Values represent the group mean (n = 4) ± SEM. Percentage values of dialysate concentrations were based on an average of three samples prior to the injection of drug.


300
260
220
180
140
100
DOPAMINE (% of Baseline)
o – Cocaine + Buprenorphine
□ – Cocaine + Saline
△ – Buprenorphine + Saline
◇ – Saline + Saline
-60
0
60
120
180
240
300
TIME (min)

remainder of the session. This profile encompasses the individual effects that were previously observed for both cocaine and BUP alone. 5-HIAA was unaffected by the administration of the drug combination [$F(3.07,18.39)=1.21$].

The combination of cocaine and BUP resulted in a significantly larger peak effect (20 min) on interstitial DA than was observed after cocaine [$t(6)=2.99$, $p<0.05$] (Figure 9). Although the cocaine-BUP group continued to have larger average concentrations of DA than the cocaine-saline group over the subsequent 40 min (two samples), these differences failed to reach significance. The combination of cocaine and BUP also produced effects on interstitial concentrations of DOPAC and HVA that were different from the effect of cocaine alone. Analyses of individual time points indicated that DOPAC was significantly higher in the combination group than in the cocaine group from 160 min [$t(6)=3.92$, $p<0.01$] to the end of the session [$t(6)=5.83$, $p<0.005$]. HVA showed a similar profile, but the difference between the groups did not become significant until 180 min [$t(6)=3.38$, $p<0.05$] and then remained significant until the end of the session [$t(6)=4.68$, $p<0.005$]. DA concentrations also appeared to differ between the cocaine-BUP and the cocaine-saline groups after three hours; however, these differences failed to reach statistical significance.

(D) Discussion

The first experiment confirmed that cocaine can produce a CPP (Morency & Beninger, 1987; Mucha *et al.*, 1982; Spyraki *et al.*, 1987). Moreover, cocaine-induced CPP was directly related to the dose administered.

The CPP elicited by BUP was also dose-dependent, with maximal effects occurring between 0.03 and 0.15 mg/kg. In so far as the CPP is predictive of abuse liability (Carr *et al.*, 1989), these results are consistent with previous reports which have indicated that BUP may have abuse potential (Hubner and Kornetsky, 1988; Lukas *et al.*, 1986; Mello, *et al.*,

1981; Young *et al.*, 1984). BUP produces complete generalization in rats trained to discriminate μ-agonists (Colpaert, 1978), suggesting that BUP possesses μ-like discriminative effects. Given that other μ-agonists reliably produce CPP (Finlay *et al.*, 1988; Mucha & Herz, 1985; Shippenberg *et al.*, 1993; Spyraki *et al.*, 1983), the finding that BUP induces CPP is not unexpected. The inverted U-shaped function obtained for BUP-induced CPP may reflect the agonist-antagonist properties of this drug. The analgesic actions of BUP show a similar profile of action, with maximal effects occurring at 0.5 mg/kg (Dum & Herz, 1981; Sadée *et al.*, 1982). Although the data from the present CPP experiment may be due to the agonist-antagonist actions of BUP, other explanations exist. Due to its unusually slow dissociation from opioid receptors and its high degree of lipophilicity, BUP has a long duration of action (Hambrook & Rance, 1976; Lewis, 1985; Schulz & Herz, 1976). At the higher doses of BUP, behaviourally relevant amounts of the drug may have remained in the animals for extended periods. Specifically, the animals may have been unable to make the discrimination between the BUP-paired and the saline-paired compartments if BUP was present in behaviourally relevant amounts 24 h later, during the next day's (*i.e.* saline training day's) pairing. This hypothesis may be supported by the finding that rhesus monkeys responding for intravenous infusions of BUP and saline on alternate days failed to respond preferentially for BUP (Mello *et al.*, 1981). However, if three days of saline were interposed between the BUP sessions, the monkeys took significantly more BUP than saline. These previous self-administration results illustrate that the potentially prolonged behavioural effects of BUP should be considered when interpreting results obtained with this long acting opioid.

The results from the drug combination experiments indicate that cocaine and BUP can interact synergistically to produce a CPP. Thus, subthreshold doses of BUP and cocaine, themselves incapable of eliciting CPP, produced a significant CPP when given in combination. In addition, moderate doses of cocaine and BUP that were individually capable of eliciting CPP interacted to produce a significantly larger CPP. Taken together,

these data suggest that BUP can increase the rewarding properties of cocaine. Although this finding is contrary to the recent interpretations offered by Mello *et al.* (1989), other results support this conclusion (Hubner and Kornetsky, 1988). Previous self-stimulation studies have shown a clear synergistic interaction between psychomotor stimulants and opiates (Hubner *et al.*, 1987; Izenwasser & Kornetsky, 1989), which appears to be related to a DA-opioid interaction (Izenwasser & Kornetsky, 1989). Subjects in a clinical study also reported a greater degree of euphoria following the administration of *d*-amphetamine and morphine, than when receiving either drug alone (Jasinski & Nutt, 1972). Given the results of the present study, and previous studies that indicate BUP has rewarding properties, it is reasonable to expect that, like other opiates, BUP can augment the rewarding effects of psychomotor stimulants.

Following the completion of this study a number of investigators have published the results of both preclinical and clinical investigations regarding cocaine-BUP interactions. In support of the present findings, it has been demonstrated that although BUP does not itself possess cocaine-like discriminative-stimulus properties, it can potentiate the discriminative-stimulus effects of cocaine (Dykstra *et al.*, 1992; Kamien and Spealman, 1991). Dykstra *et al.* (1992) reported that BUP increased cocaine-appropriate responding by rats following a low dose of cocaine in a discrimination paradigm, while Kamien and Spealman (1991) observed that BUP shifts the full cocaine dose-effect curve to the left in monkeys discriminating between cocaine and saline. Taken as a whole, these data suggest that BUP can potentiate the discriminative-stimulus properties of cocaine. The neurochemical interactions between cocaine and BUP observed in the present study may provide the mechanism for this behavioural effect.

Mello and colleagues (1990, 1992, 1993) have replicated their finding that BUP suppresses cocaine self-administration. These authors report that this effect is relatively specific for cocaine because food reinforced responding, although significantly reduced by BUP, is not consistently reduced in all subjects and because many subjects become tolerant

to BUP's effect on food-maintained responding. Despite the reproducible nature of the BUP-induced suppression of cocaine self-administration, previously discussed issues regarding the interpretation of these findings have not been adequately addressed in these subsequent studies. Specifically, a decrease in responding cannot be assumed to reflect a decrease in the rewarding effects of cocaine, as rate of responding is not determined solely by these effects of the drug (Johanson and Fischman, 1989). This point is exemplified by the fact that cocaine, amphetamine or morphine pretreatment suppress responding for cocaine by non-human primates (Balster *et al.*, 1992; Herling *et al.*, 1979; Stretch, 1977). Other investigators have also documented that BUP suppresses responding for intravenous or inhaled cocaine (Carroll and Lac, 1992; Carroll *et al.*, 1992; Winger *et al.*, 1992). However, the results of these studies illustrated that BUP suppresses behaviour maintained by several drug reinforcers, as well as non-drug reinforcers. Moreover, Winger *et al.* (1992) demonstrated that BUP did not shift the cocaine dose-response curve to the right, as would be expected if BUP were antagonizing the effects of cocaine. Rather, BUP produced a generalized suppression of responding across the cocaine dose-response curve in this paradigm, a response similar to that observed for heroin. Although the mechanism responsible for the BUP-induced suppression of responding is unknown, it is apparent that the decrease in responding for intravenous cocaine cannot be assumed to necessarily reflect a decrease in the rewarding effects of cocaine (Johanson and Fischman, 1989)

Two recent studies have re-examined the ability of BUP to affect cocaine-induced CPP. Suzuki *et al.* (1992) reported that the acute administration of 0.5 mg/kg BUP suppressed cocaine-induced CPP, while Kosten *et al.* (1991) observed that the chronic administration of 0.5 mg/kg of BUP twice a day also reduced cocaine-induced CPP. As discussed previously, following the administration of large doses of BUP, behaviourally relevant amounts of the drug may have remained in the animals for extended periods of time (Hambrook & Rance, 1976; Lewis, 1985; Schulz & Herz, 1976). Specifically, the animals may have been unable to make the discrimination between the cocaine-paired and

the saline-paired compartments if BUP was present in behaviourally relevant amounts throughout testing.

In addition to the large number of preclinical studies examining cocaine-BUP interactions, clinical trials have directly investigated the effect of BUP on the subjective responses of human subjects to cocaine challenge. In an initial study, Mendelson *et al.* (1991) reported that BUP maintenance (4 or 8 mg/day, sublingually) completely blocked the subjective effects of 10 mg of intravenous morphine, as well as significantly reducing subjects' reported craving for heroin. In contrast, the effect of BUP on cocaine craving and the subjective response to a cocaine challenge were variable. Some subjects reported that BUP diminished the intensity and quality of the cocaine challenge dose, while other subjects reported that the BUP maintenance enhanced the subjective effects of the cocaine. In a subsequent study, it was reported that subjective responses to cocaine were diminshed following maintenance on 4 mg/day BUP, while subjects treated with 8 mg/day of BUP reported that cocaine challenges were more intense and prolonged, as well as being of higher quality (Teoh *et al.*, 1992). Mendelson and colleagues (1992) recently found that BUP (4 mg/kg, sublingually) suppressed the acute cocaine-induced stimulation of both adrenocorticotropin hormone (ACTH) and euphoria. Taken as a whole, these preliminary clinical studies suggest that the subjective responses to cocaine may be differentially effected by BUP treatment in a dose related manner. It is noteworthy that these human studies compared the same subjects across time (*i.e.* before and after BUP treatment) and did not utilize control subjects.

A number of clinical studies have also directly examined the effect of BUP on cocaine abuse. Two pilot studies provided evidence that BUP was highly efficacious in reducing cocaine abuse in heroin abusers, as evidenced by a decrease in cocaine positive urines (Gastfriend *et al.*, 1992; Kosten *et al.*, 1989). Despite these promising initial results, two double-blind, controlled clinical trials of BUP for the treatment of opioid and cocaine dependence have demonstrated that BUP is no more effective than methadone in reducing

cocaine abuse (Johnson *et al.*, 1992; Kosten *et al.*, 1992). Given that methadone maintenance is not an effective treatment for cocaine abuse (Chambers *et al.*, 1972; Kosten *et al.*, 1986, 1987a, 1987b), the use of BUP in the pharmacotherapy of cocaine abuse remains questionable.

The *in vivo* microdialysis experiments were designed to provide neurochemical data that could potentially explain the positive interaction observed between cocaine and BUP in the CPP drug-combination experiments. Specifically, these experiments provided information regarding the actions of cocaine, BUP, and a combination of these drugs on interstitial concentrations of DA and its metabolites in the nucleus accumbens. Cocaine produced an 82% mean peak increase in interstitial concentrations of DA that returned to baseline within two to three hours. The DA metabolites, DOPAC and HVA, were significantly decreased by cocaine. This profile is similar to that reported in previous dialysis experiments (Kalivas & Duffy, 1990; Maissonneuve *et al.*, 1990). Other dialysis experiments examining the effects of systemic cocaine have reported minimal effects on DOPAC and HVA (Di Chiara & Imperato, 1988a; Hurd *et al.*, 1989); these discrepancies may be the result of factors such as the amount of time between probe implantation and testing, and differences in the Ca^{2+} concentration in the dialysis perfusion fluid. Both tissue and dialysate concentrations of the DA metabolites are elevated 24 hr post-implantation (Reiriz *et al.*, 1989; Zis *et al.*, 1991), raising the possibility that the results from the former studies which were conducted 24 hr post-implantation contained an implantation artifact. Moreover, the earlier experiments were performed using perfusion fluids that contained high, non-physiological Ca^{2+} concentrations (2.3 - 3.5 mM), which have been shown to influence both the basal amounts of DA recovered as well as the effects of pharmacological manipulations (Moghaddam & Bunney, 1989b; Westerink *et al.*, 1988).

BUP produced a considerably different neurochemical profile than cocaine. Interstitial DA concentrations increased gradually over the test period, and reached 200% of basal values 5 hours post-injection. DOPAC and HVA were also increased by BUP, but the

effect on DOPAC was shorter lasting. The gradual onset of action of BUP may be due to the slow association kinetics of this drug (Schulz & Herz, 1976). In a study of the human pharmacology of BUP, it was reported that the peak miotic effect occurred six hours post-injection (Jasinski *et al.*, 1978).

It is important to note that BUP has high affinity for both μ and κ opiate receptors (Lewis, 1985), and that μ- and κ-agonists have opposing actions on DA release in the nucleus accumbens (Di Chiara & Imperato, 1988b). The present study suggests that low doses of BUP act predominantly at μ opiate receptors to increase the release of DA in this structure. Electrophysiological studies have shown that morphine increases the frequency of firing of VTA-DA neurons (Nowycky *et al.*, 1978; Glysing & Wang, 1983); therefore, the increase in interstitial DA following BUP administration is probably due to an increase in the firing rate of the DA neurons in the VTA. The increase in DOPAC and HVA after BUP-treatment also suggests an increase in activity in these neurons.

The combination of cocaine and BUP produced a 163% increase in interstitial DA concentrations in the first sample after drug administration. This effect was approximately double that seen for cocaine + saline (82%), providing neurochemical evidence that BUP can enhance cocaine-induced increases in interstitial DA in the nucleus accumbens. This enhancement is not readily accounted for by simple additivity, as BUP + saline produced an increase in interstitial DA (20%) that was not significantly different from saline + saline in the first sample after drug administration. These neurochemical data provide a potential explanation for the synergism observed in the CPP studies. Cocaine increases interstitial DA by blocking reuptake into presynaptic terminals (Nomikos *et al.*, 1990; Richelson & Pfenning, 1984). BUP, like other opiates, probably increases the firing rate of dopaminergic neurons (Nowycky *et al.*, 1978; Glysing & Wang, 1983), thereby resulting in increased DA release. BUP had only a minor effect (20%) on interstitial concentrations of DA shortly after injection, probably due to a small increase in the firing rate of VTA dopaminergic neurons. An increase in the firing rate would, however, be expected to have a larger effect

on interstitial concentrations of DA if reuptake was compromised, as occurs following cocaine administration. An additional finding of potential interest is the apparent diminution of the long-term effects of BUP on interstitial DA concentrations in the drug combination group, as evident in Figure 8. Although cocaine may affect the long-term BUP-induced release of DA, the large standard error present in the combination group at these later time points caution against this speculation without further study.

It is unlikely that the larger effect on DA seen in the combination group was due to simple metabolic interactions between BUP and cocaine. First, cocaine is metabolized by liver and plasma cholinesterases (Vitti & Boni, 1985), while BUP is largely inactivated by conjugation with glucuronic acid (Rance & Shillingford, 1976). Second, if BUP decreased the metabolism of cocaine then it would be expected that cocaine concentration would decrease more gradually, resulting in a more prolonged effect on DA. An examination of the time course of the DA concentrations after these two treatments shows that only the first few time points differed (Figure 9).

The present data do not support the view that BUP may be useful in the pharmacotherapy of cocaine abuse because it antagonizes the reinforcing properties of this stimulant (Mello *et al.* 1989, 1990, 1992). Differences in the doses of BUP used in the present study and those of Mello and colleagues may explain these different conclusions. Previous research indicates that BUP has dose-related agonist-antagonist properties (Cowan *et al.*, 1977; Dum & Herz, 1981; Yanagita, 1981); therefore, different doses of this drug could result in different, even opposite effects. The present experiment concentrated primarily on low to moderate doses, while Mello *et al.* (1989, 1990, 1992) examined only high doses of BUP. Although it is clear that the doses used in the combination experiments of the present investigation were in the agonist range, it is uncertain where the doses used by Mello *et al.* (1989, 1990, 1992) fall on the dose-response curve for BUP. Nevertheless, if the doses of BUP used by Mello *et al.* (1989, 1990, 1992) were in the agonist portion of the dose-response curve, then an alternative explanation for their results can be proposed.

Specifically, a summation of the rewarding properties of cocaine and BUP could equally well account for the findings of Mello *et al.* (1989, 1990, 1992), and such an interpretation is clearly supported by the present results, as well as the finding that BUP can potentiate the discriminative-stimulus effects of cocaine (Kamien and Spealman, 1991; Dykstra *et al.*, 1992). It is also noteworthy that morphine produces a decrease in the rate of responding for cocaine (Stretch, 1977). Given that "speedballs" (cocaine + heroin or other opioids) are a popular form of illicit drug use (Kosten *et al.*, 1987) and that subjects in a clinical study reported a greater degree of euphoria following the administration of *d*-amphetamine and morphine than when they received either drug alone (Jasinski & Nutt, 1972), it is reasonable to assume that morphine-induced decreases in responding for cocaine are due to a summation of the rewarding properties of these two drugs. Decreases in the self-administration of cocaine following BUP may similarly be due to additive effects of BUP- and cocaine-induced reward.

(E) Notes

Note 1: In Experiments I and II *in vivo* microdialysis has been used to measure the interstitial concentrations of DA, DOPAC, HVA and 5-HIAA. Because the efflux of DA has been shown to be TTX-sensitive and Ca^{2+}-dependent under appropriate conditions (Benveniste and Hüttemeier, 1990; Brown *et al.*, 1991; Santiago and Westerink, 1990), it is widely accepted that dialysate concentrations of DA reflect activity-dependent neuronal release.

Note 2: The concentrations of DA and the metabolites (fmol/min) presented in Experiments I and II are dialysate values uncorrected for probe recovery.

III. COCAINE-INDUCED CONDITIONED LOCOMOTION: ABSENCE OF ASSOCIATED INCREASES IN DOPAMINE RELEASE

(A) Introduction

An important aspect of the behavioural properties of cocaine involves the classical conditioning of its unconditioned neurochemical effects with specific environmental stimuli (Barr *et al.*, 1983; Stewart *et al.*, 1984; Tatum and Seevers, 1929). This property of cocaine is of major significance with respect to its abuse potential, as intense craving can be evoked by stimuli previously associated with the act of taking the drug (Gawin, 1991; Johanson and Fischman, 1989; O'Brien *et al.*, 1992). The magnitude of these conditioned cravings can be overwhelming and can result in previously abstinent abusers resuming the use of cocaine.

Stewart *et al.* (1984) have proposed that stimuli associated with the administration of opiates or stimulants can come to elicit neural states that are similar to those produced by the drugs themselves. Given the large body of evidence implicating the mesolimbic DA system in the reinforcing properties of these drugs of abuse (Di Chiara and Imperato, 1988a; Fibiger and Phillips, 1987; Lyness *et al.*, 1979; Roberts *et al.*, 1977; Roberts *et al.*, 1989) it is possible that conditioned stimuli associated with drug administration may also produce increases in dopaminergic transmission. Earlier studies have provided evidence for conditioned dopaminergic activity with a variety of drugs, such as morphine, amphetamine and apomorphine (Lal *et al.*, 1976; Perez-Cruet, 1976; Shiff, 1982). However, Barr *et al.* (1983) reported that presentation of environmental stimuli associated with the injection of cocaine elicited behaviours similar to the drug itself, but noted an absence of conditional neurochemical changes in the DA metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) or homovanillic acid (HVA). Other investigators have also failed to observe conditioned dopaminergic activity following conditioning with a variety of agents such as fentanyl (Finlay et al., 1988), morphine (Walter and Kuschinsky, 1989), and apomorphine (Möller,

1987). Although these recent studies reported an absence of conditional neurochemical effects, all found significant conditioned behavioural changes.

One shortcoming of the previous neurochemical studies is that changes in dopaminergic transmission were inferred from changes in tissue concentrations of DA metabolites. Although this approach can be useful, it also possesses certain limitations and potential confounds (Commissiong, 1987). The advent of *in vivo* microdialysis has allowed a more direct assessment of the release of DA. The present study utilized this *in vivo* technique to determine if environmental stimuli paired with cocaine administration produce increases in DA release in the nucleus accumbens.

(B) Materials and Methods

Subjects and Drugs

Subjects were 61 male Long Evans rats (Charles River, Quebec), weighing 250 - 350 g at the beginning of the experiments. The rats were group housed (2 - 4 per cage), on a 12-hr light/12-hr dark cycle (lights on 08:00), with food and water available *ad libitum*. All subjects were handled periodically for one week prior to the experiments. All experimental procedures were conducted at approximately the same time each day, during the animal's light phase.

Both cocaine hydrochloride (10 mg/kg, dissolved in isotonic saline , BDH) and 0.9 % saline were injected *i.p.* in a volume of 1 ml/kg. The dose of cocaine is expressed as the weight of the salt.

Apparatus

Locomotor Activity (Experiment 2)

Five circular (61 cm diameter) activity cages (BRS/LVE), each transected by six infra-red beams, were used to measure locomotor activity. Photocell beam interruptions

occurring more than 0.5 s apart were recorded with a NOVA IV (Data General) minicomputer equipped with MANX (GC Controls) software and interface.

Conditioning Box (Experiment 3)

The "conditioning environment" for this experiment was a Plexiglas cage (34 x 26 x 36 cm) with 2 cm wide black and white vertical stripes on the walls and a floor of parallel metal bars (1 cm apart). A black Plexiglas lid , with a small stimulus light (CM-47, Chicago Miniature Lamp) mounted in its center, was held 1 cm above the top of the conditioning box. The space between the box and the lid was designed to allow free movement of the dialysis inlet and outlet tubings during testing. A tray of corncob bedding (bed-o' cobs, The Andersons) was placed beneath the floor of the cage, and was replaced between each rat. The conditioning box was placed within a sound attenuating chamber equipped with a continuously operating fan.

Locomotor Activity (Experiment 4)

The conditioning box for this experiment was a Plexiglas cage (40 x 31 x 42 cm) with red acetate (Behnsen's Graphic Supplies) covering the lower portion (21 cm) of the box and 1 cm wide black and white horizontal stripes covering the upper portion (21 cm) of the box. The bottom of the cage was filled (3 cm deep) with cat litter (99% Dust-Free, Kitty Litter Brand). No lid was used. A buzzer (SMB-24, Star Microtronics, 15 V, 60-70 dB) was continuously activated while the rat was in the box.

This box was placed within a Digiscan Animal Activity Monitor [model RXYZCM(16); Omnitech Electronics, Inc.] to measure locomotor activity in 10 min blocks corresponding to the 10 min dialysate samples.

Surgery and microdialysis

Rats were anesthetized with sodium pentobarbital (60 mg/kg, *i.p.*), mounted in a stereotaxic instrument, and a vertical microdialysis probe was implanted into the nucleus accumbens (AP: +3.6 mm; ML: -1.5 mm; DV: -8.2 mm from dura; relative to bregma; Pellegrino *et al.*, 1979). The microdialysis probe was a variant of the concentric vertical design (outer diameter = 320 μm, molecular weight cut-off < 60,000 Dalton; AN69, Hospal). The active surface was 2 mm and was 0.2 mm from the tip of the probe.

After surgery, the rats were individually housed for 48 hr before testing. On the test day, the appropriate experimental manipulations were performed on each rat after a stable baseline had been established (not more than 10% variation for three consecutive samples). Histological verification of the probe placement was conducted after testing. Specifically, rats were administered a lethal dose of pentobarbital (120 mg/kg, *i.p.*) and transcardially perfused with 4% formalin. Coronal sections (50 μm) were collected and stained with cresyl violet.

Microdialysis experiments were conducted on-line as described in Chapter II, except that samples were automatically injected into a HPLC system with electrochemical detection every 10 min.

Biochemical Assay

The concentrations of DA, DOPAC, HVA and 5-HIAA were determined with HPLC in conjunction with electrochemical detection utilizing the same experimental protocol as described in Chapter II.

Procedure

The first experiment involved the determination of the acute neurochemical effects of saline and cocaine on DA and its metabolites in the nucleus accumbens using *in vivo* microdialysis. On the test day, subjects were injected with saline (1 ml/kg, *i.p.*), followed

60 min later by cocaine (10 mg/kg, *i.p.*). Microdialysis sampling continued for 3 hr after the administration of cocaine.

The classical conditioning of the locomotor stimulant effects of cocaine with a specific environment was examined in the second experiment. Thirty rats were randomly assigned to 1 of 3 groups: conditioned, pseudoconditioned or control. Conditioned subjects were injected with cocaine (10 mg/kg, *i.p.*) and then placed into one of the circular activity cages (as previously described) for 30 min. After the training session, subjects were returned to their homecages, where they were injected with saline (1 ml/kg, *i.p.*) 4 hr later. Pseudoconditioned rats were exposed to an identical procedure except that the order of administration of cocaine and saline was reversed. Specifically, these subjects were injected with saline prior to being placed in the activity cages and later with cocaine in their homecages. Control subjects were exposed to the same procedure as the other groups, except that they were injected with saline in both environments and never received cocaine. Each subject was assigned to a particular locomotor cage for the duration of the experiment. Training was conducted daily for 7 days at approximately the same time each day. On the test day (48 hr after the final training session), subjects were placed in the locomotor cages and activity was monitored for 30 min. No injections were given on the test day.

The third experiment examined if the previous training regimen, which had been shown to produce conditioned locomotion, also produced changes in indices of dopaminergic transmission. Subjects were randomly assigned to either conditioned or pseudoconditioned groups, and trained following the same procedure as in Experiment 2. The only notable difference was that a different cage (as described above) was used. Three to 4 hrs after the final injection of the training procedure, animals were anesthetized and implanted with a vertical microdialysis probe into the nucleus accumbens. On the test day (approximately 45 hr after the final training session), dialysis was initiated while the rats remained in their "post-operative" home cages. After a stable baseline had been obtained, each subject was

moved to the testing room and placed in the conditioning box, where it remained for 50 min.

In order to validate the results of the previous two experiments, an additional conditioning experiment was undertaken in which both behaviour and neurochemistry were monitored simultaneously during the exposure of rats to the previously conditioned environment. The training procedure and surgery for this experiment was similar to the third experiment, with the exception of the different conditioning box (as noted above). On the test day (approximately 45 hrs after the final training session), baseline dialysis samples were collected while the subject remained in its homecage. After a stable baseline had been obtained, the rat was placed into the conditioning box for 50 min. Behaviour was monitored throughout the period the rat remained in the conditioning box.

Statistical Analysis

The data from the dialysis experiments (percent values) were evaluated using a univariate analysis of variance with repeated measures (Huynh-Feldt adjustment of degrees of freedom). Between group differences for both the dialysis data from Experiments 3 and 4 and the locomotor data from Experiments 2 and 4 were evaluated with a two-way analysis of variance with repeated measures (Huynh-Feldt adjustment of degrees of freedom). Additional comparisons of differences in locomotor counts between groups at specific time points were made using the Bonferoni *t* statistic. Comparisons of absolute concentrations (fmol/min) of DA, DOPAC, HVA and 5-HIAA between the different experimental groups were made using a univariate analysis of variance.

(C) Results

The average baseline output of DA (± SEM., n = 29) was 6.20 ± 0.51 fmol/min and did not differ significantly between the various experimental groups [$F(4,24) = 1.00$] (Conditioned subjects (n=13): 6.5 ± 0.9 fmol/min, pseudoconditioned subjects (n=12): 5.4 ± 0.6 fmol/min, acute cocaine subjects (n=4): 7.7 ± 1.4 fmol/min). The average basal output (± SEM, n = 29) for DOPAC, HVA and 5-HIAA were 683 ± 49, 377 ± 29 and 261 ± 14 fmol/min, respectively. Basal values of DOPAC [$F(4,24) = 2.67$], HVA [$F(4,24) = 1.85$] and 5-HIAA [$F(4,24) = 1.80$] did not differ significantly between experimental groups. Figure 10 is a photomicrograph of a typical histological section (50 μm, cresyl violet stain) illustrating the placement of the dialysis probe within the nucleus accumbens.

Cocaine produced a rapid increase in interstitial concentrations of DA [$F(10.02,30.07) = 67.21$, $p < 0.001$] that returned to baseline within 140 to 180 min (Figure 11). Cocaine also significantly decreased DOPAC [$F(1.92, 5.77) = 9.89$, $p < 0.02$], HVA [$F(4.00, 12.00) = 16.16$, $p < 0.001$] and 5-HIAA [$F(2.09, 6.28) = 8.82$, $p < 0.02$]. The dramatic increase in interstitial DA produced by cocaine is contrasted by the lack of effect of saline administration [$F(2.78, 8.33) = 0.88$]. Saline also failed to affect DOPAC [$F(3.83, 11.48) = 0.64$], HVA [$F(1.84, 5.52) = 0.78$] or 5-HIAA [$F(6.00, 18.00) = 1.44$].

The conditioning procedure employed in Experiment 2 produced a notable conditional behavioural effect (Figure 12). A significant difference in locomotor counts between the conditioned and pseudoconditioned groups is demonstrated by significant group [$F(1,18) = 12.6$, $p < 0.005$] and group x time [$F(2.00,36.00) = 6.25$, $p < 0.01$] effects. The conditioned group exhibited significantly more locomotor counts during the first [$t(18) = 3.82$, $p < 0.005$] and second [$t(18) = 3.43$, $p < 0.005$] 10 min periods. This conditioned effect is directly contrasted by the lack of a significant group [$F(1,18) = 0.00$] or group x time [$F(2.00,36.00) = 0.51$] difference between the pseudoconditioned and control groups.

Figure 10. Photomicrograph of a coronal section (50 μm) through the nucleus accumbens of a rat implanted with a dialysis probe. **Inset:** Schematic representation of the location of the dialysis probe.

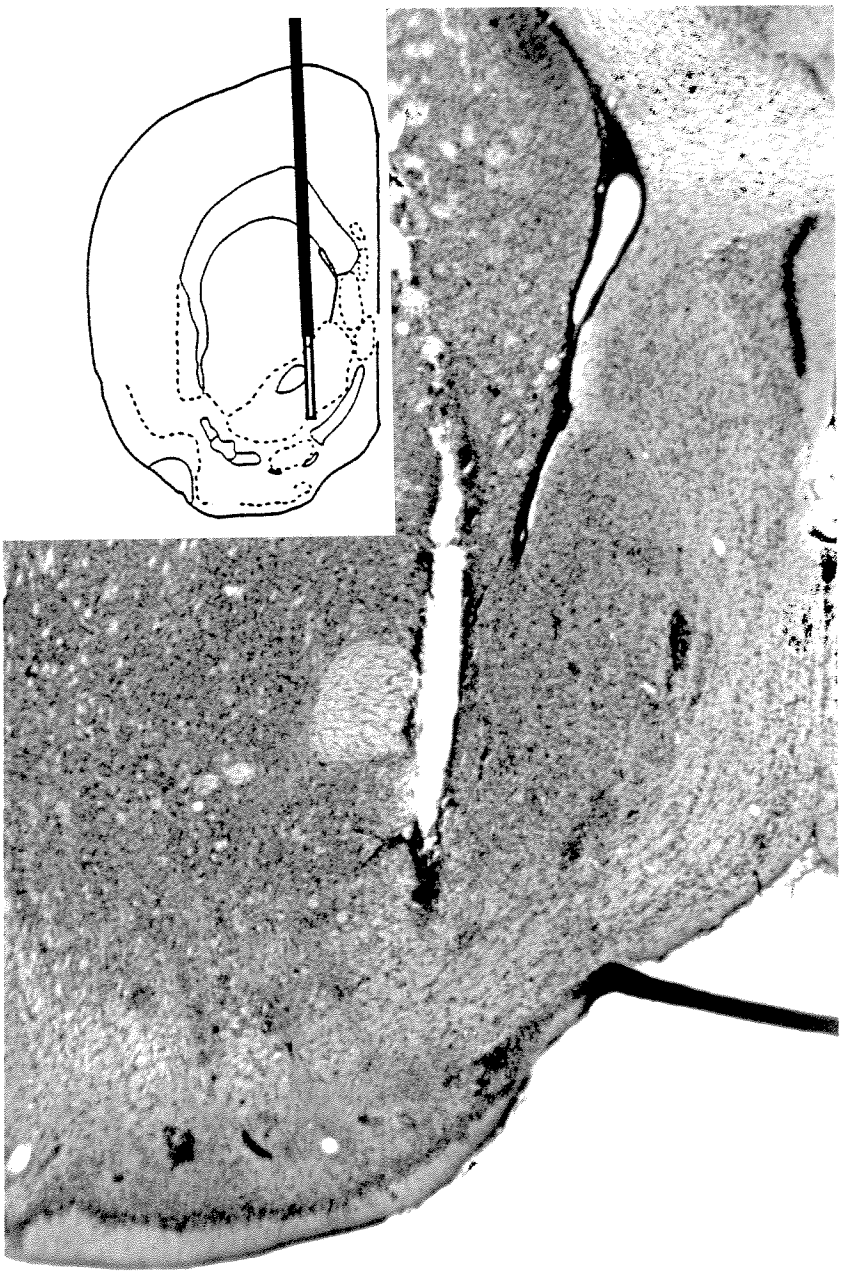


Figure 11. The effect of acute administration of saline (1 ml/kg) and cocaine (10 mg/kg, *i.p.*) on the microdialysis output of DA (o), DOPAC (□), HVA (△) and 5-HIAA (◊) in the nucleus accumbens (n=4). Values represent the group mean ± SEM. Percentage values of dialysate concentrations were based on an average of three samples before the injection of saline.

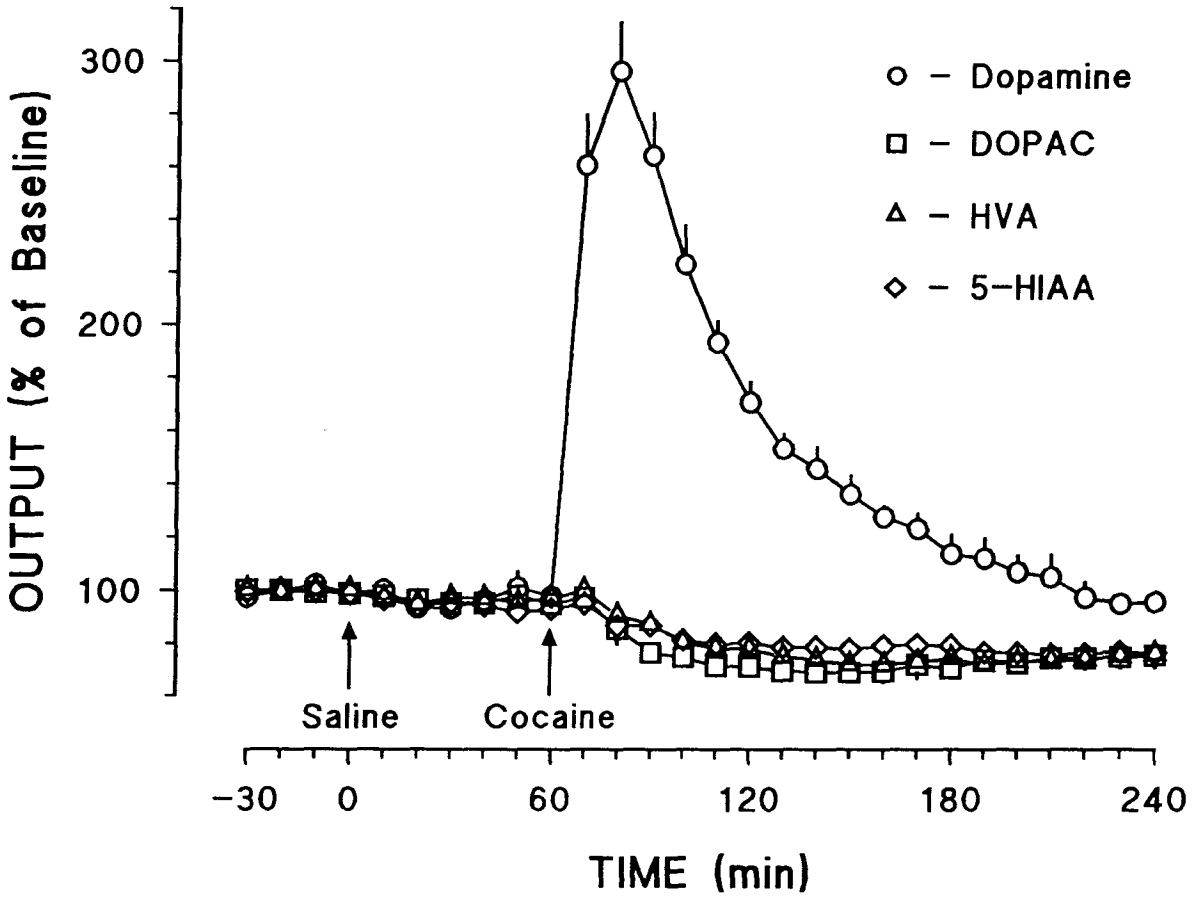
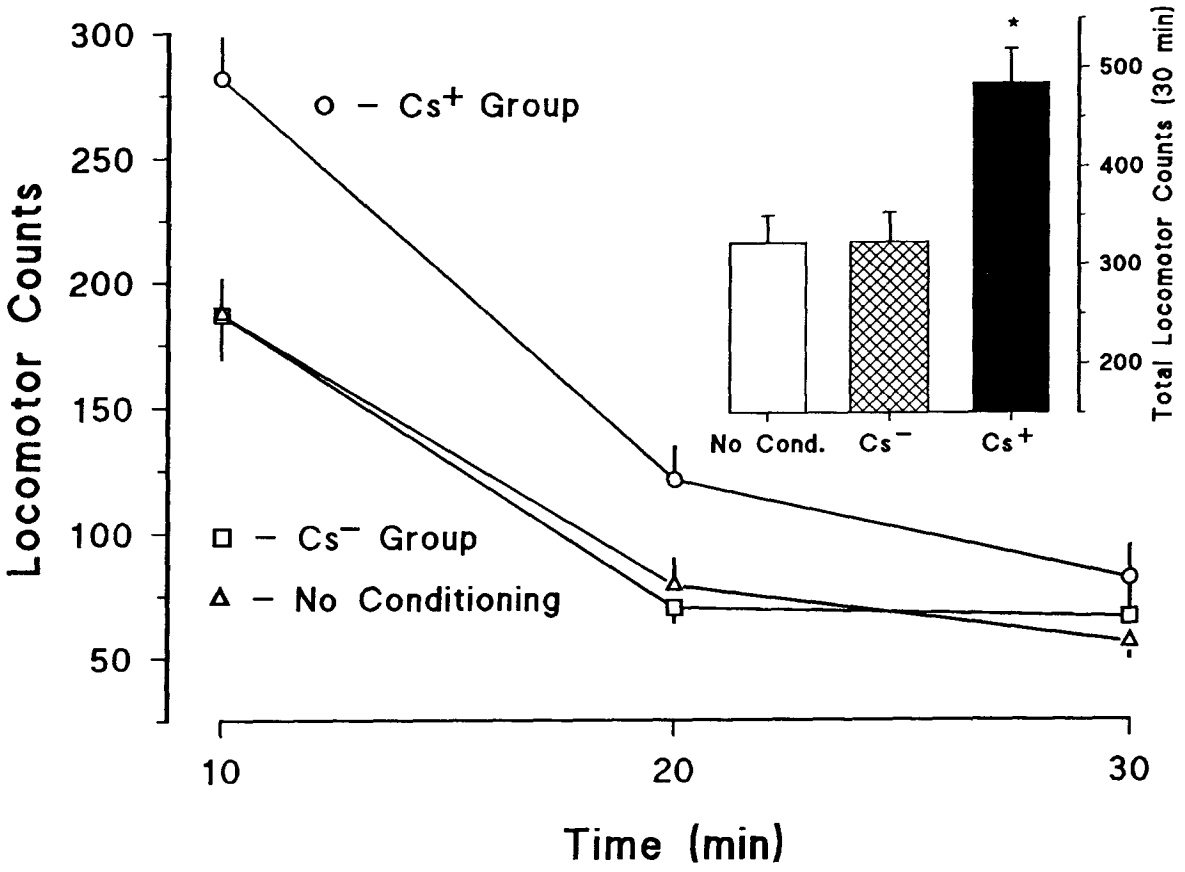

300
200
100
OUTPUT (% of Baseline)
○ – Dopamine
□ – DOPAC
△ – HVA
◇ – 5-HIAA
Saline
Cocaine
−30
0
60
120
180
240
TIME (min)

Figure 12. Locomotor counts of conditioned (Cs^+), pseudoconditioned (Cs^-) and control subjects following one week of conditioning with cocaine (10 mg/kg, *i.p.*). Testing occurred 48 hrs after the last training session, and was conducted in the same apparatus as used for the training procedure. Values represent the group mean ± SEM (n=10/group). **Inset:** Total number of locomotor counts for the conditioned, pseudoconditioned and control subjects for the 30 min test period. * $p < 0.05$ compared to pseudoconditioned controls.


300
250
200
150
100
50
Locomotor Counts
o – Cs+ Group
□ – Cs− Group
△ – No Conditioning
10
20
30
Time (min)
*
500
400
300
200
Total Locomotor Counts (30 min)
No Cond.
Cs−
Cs+

Although the data from Experiment 2 indicated that the conditioning paradigm produced significant conditional changes in behaviour, the exposure of conditioned subjects to the cocaine-paired environment did not produce a change in interstitial DA [$F(4.97,54.71) = 0.40$] (Figure 13-A), DOPAC [$F(3.54,38.89) = 0.47$] or HVA [$F(2.72,29.9) = 0.30$] (Figure 13-B) that differed from the pseudoconditioned controls. The limited increase in DA observed in both groups (approximately 10%) failed to reach significance [$F(4.97,54.71) = 0.90$]; however, a significant increase in both DOPAC [$F(3.54, 38.89) = 4.17$, $p < 0.01$] and HVA [$F(3.15, 34.70) = 2.93$, $p < 0.05$] was observed in both groups.

The data from the fourth experiment strongly substantiated the results from the previous two experiments. Exposure of conditioned subjects to the drug-paired environment did not produce a change in interstitial DA that was significantly different from the pseudoconditioned controls [$F(4.87, 48.65) = 0.80$] (Figure 14-A). Moreover, neither DOPAC [$F(4.09, 40.94) = 1.22$] nor HVA [$F(5.00, 50.00) = 0.64$] differed between the conditioned or pseudoconditioned groups (Figure 14-B). Although no group x time differences were present, significant effects over time were observed for DA [$F(4.87, 48.65) = 10.12$, $p < 0.001$], DOPAC [$F(4.09, 40.94) = 9.83$, $p < 0.001$] and HVA [$F(5.00, 50.00) = 7.05$, $p < 0.001$]. The lack of neurochemical differences between the conditioned and pseudoconditioned subjects is contrasted by the significant group [$F(1, 10) = 11.14$, $p < 0.01$] and group x time [$F(1.73, 17.27) = 5.59$, $p < 0.05$] effects observed for the locomotor activity of these subjects (Figure 15). The conditioned subjects exhibited significantly more locomotor activity than the pseudoconditioned controls during the first [$t(10) = 2.81$, $p < 0.05$] and third [$t(10) = 4.34$, $p < 0.05$] 10 min periods of the test session.

Figure 13. Experiment 3 **A.** Interstitial concentrations of DA in the nucleus accumbens prior to and during exposure to an environment paired with cocaine (Cs^+ group, n=6) or saline (Cs^- group, n=7). **B.** Interstitial concentrations of DOPAC and HVA in the nucleus accumbens prior to and during exposure to an environment paired with cocaine (Cs^+ group, n=6) or saline (Cs^- group, n=7). Values represent the group mean $\pm$ SEM. Percentage values of dialysate concentrations were based on an average of three samples before exposure to the conditioned environment.

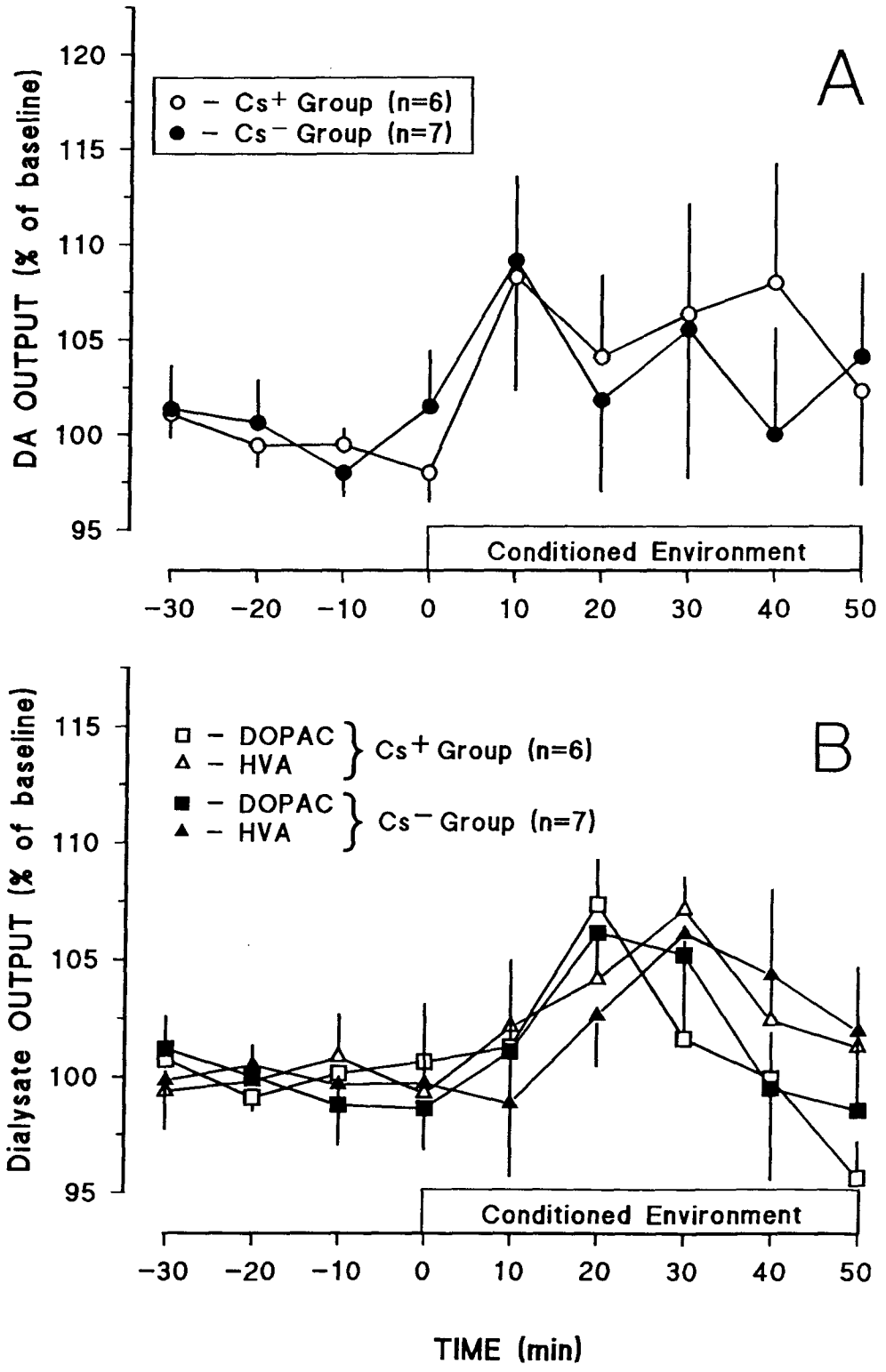

A
○ – Cs+ Group (n=6)
● – Cs− Group (n=7)
DA OUTPUT (% of baseline)
120
115
110
105
100
95
Conditioned Environment
−30 −20 −10 0 10 20 30 40 50
B
□ – DOPAC
△ – HVA
Cs+ Group (n=6)
■ – DOPAC
▲ – HVA
Cs− Group (n=7)
Dialysate OUTPUT (% of baseline)
115
110
105
100
95
Conditioned Environment
−30 −20 −10 0 10 20 30 40 50
TIME (min)

Figure 14. Experiment 4 **A.** Interstitial concentrations of DA in the nucleus accumbens prior to and during exposure to an environment paired with cocaine (Cs^+ group, n=6) or saline (Cs^- group, n=6). **B.** Interstitial concentrations of DOPAC and HVA in the nucleus accumbens prior to and during exposure to an environment paired with cocaine (Cs^+ group, n=6) or saline (Cs^- group, n=6). Values represent the group mean $\pm$ SEM. Percentage values of dialysate concentrations were based on an average of three samples before exposure to the conditioned environment.

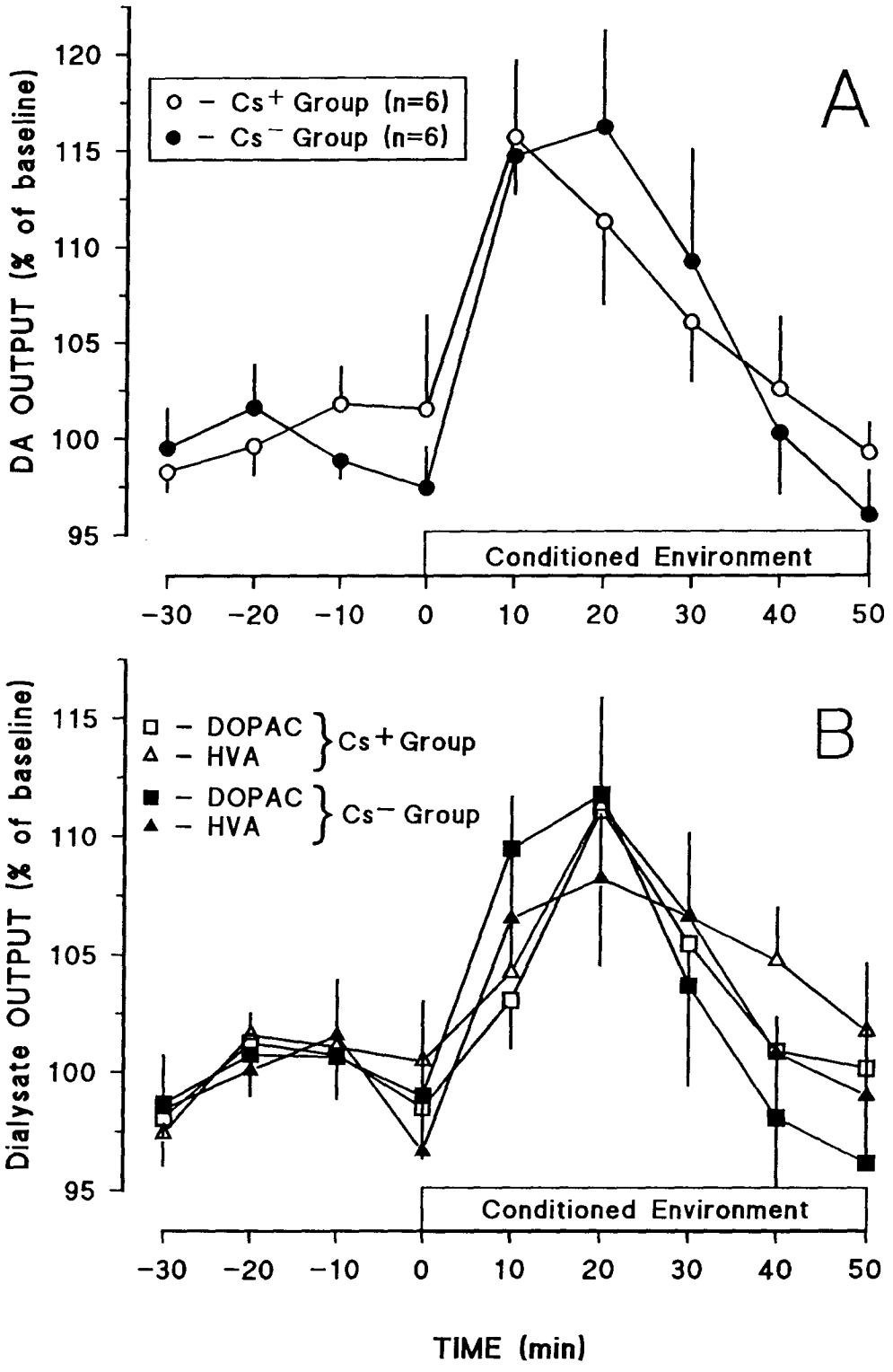
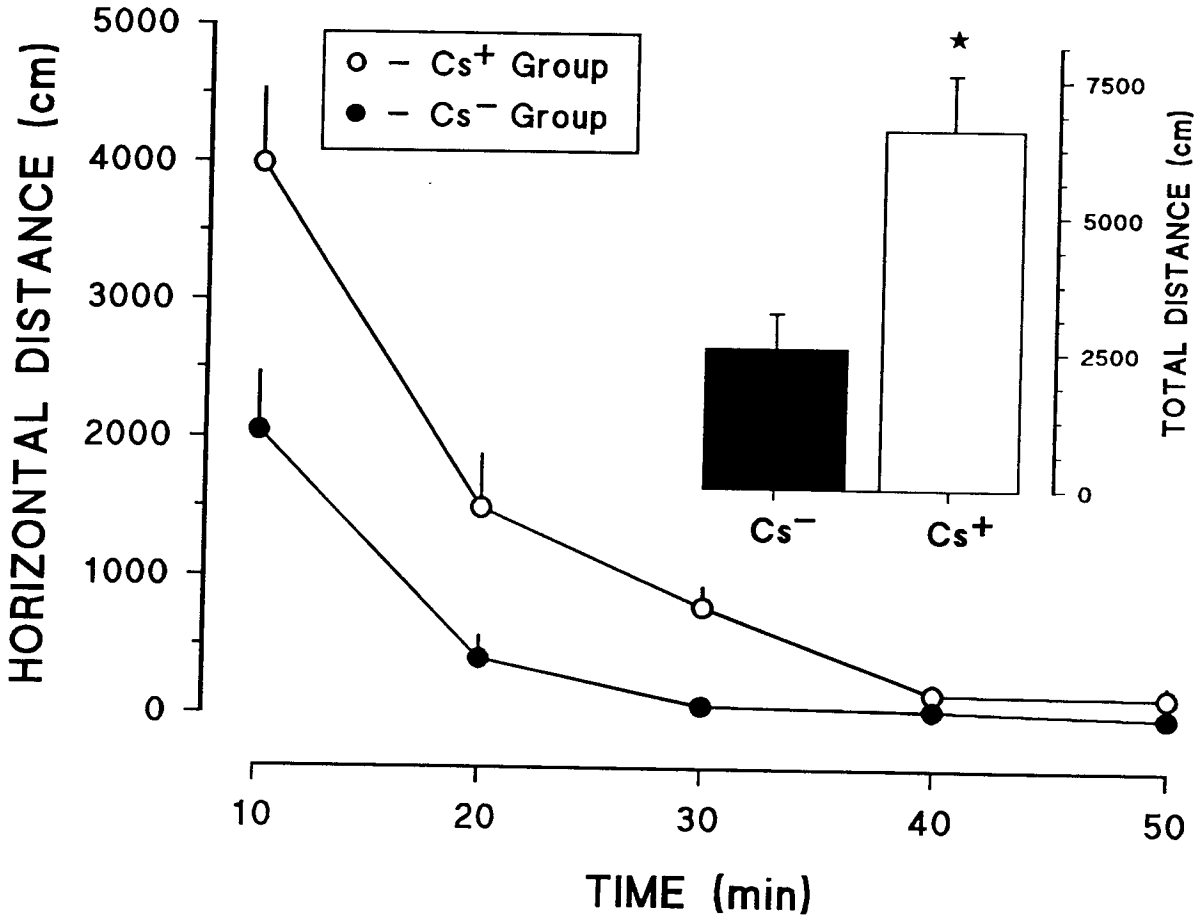

A
o – Cs+ Group (n=6)
● – Cs− Group (n=6)
DA OUTPUT (% of baseline)
120
115
110
105
100
95
Conditioned Environment
−30 −20 −10 0 10 20 30 40 50
B
□ – DOPAC
△ – HVA
Cs+ Group
■ – DOPAC
▲ – HVA
Cs− Group
Dialysate OUTPUT (% of baseline)
115
110
105
100
95
Conditioned Environment
−30 −20 −10 0 10 20 30 40 50
TIME (min)

Figure 15. Locomotor activity (horizontal distance) of conditioned (Cs^+, n=6) and pseudoconditioned (Cs^-, n=6) subjects from Experiment 4. Values represent the group mean $\pm$ SEM. **Inset:** Total horizontal distance for the conditioned and pseudoconditioned subjects for the 30 min test period. * $p < 0.05$ compared to pseudoconditioned controls.


5000
4000
3000
2000
1000
0
HORIZONTAL DISTANCE (cm)
o – Cs+ Group
● – Cs− Group
10
20
30
40
50
TIME (min)
★
7500
5000
2500
0
TOTAL DISTANCE (cm)
Cs−
Cs+

(D) Discussion

The first experiment was designed to assess the unconditioned neurochemical effects of cocaine. The findings of this experiment confirm that cocaine produces an increase in interstitial DA concentrations in the nucleus accumbens (Chapter II; Di Chiara and Imperato, 1988a; Kalivas and Duffy, 1990). The ability of cocaine to increase dopaminergic transmission appears to be critically involved in its reinforcing properties (De Wit and Wise, 1977; Ritz *et al.*, 1987; Roberts *et al.*, 1977; Roberts *et al.*, 1989). Although it has been suggested that the dopaminergic projection to the medial prefrontal cortex may also play an important role in the reinforcing properties of intracranially administered cocaine (Goeders and Smith, 1983), a variety of behavioural and neurochemical studies suggests that the dopaminergic innervation of the nucleus accumbens is necessary for the reinforcing properties of systemically administered cocaine (Martin-Iverson *et al.*, 1986; Moghaddam and Bunney, 1989a; Roberts *et al.*, 1977).

Cocaine also produced decreases in the DA metabolites, DOPAC and HVA, and this is consistent with the proposed neurochemical actions of cocaine (Heikkila *et al.*, 1975; Nomikos *et al.*, 1990). These decreases are thought to be a direct result of the inhibition of DA uptake, resulting in less DA being available for intraneuronal metabolism by monoamine oxidase (Brown *et al.*, 1991; Roffler-Tarlov *et al.*, 1971; Soares-da-Silva and Garrett, 1990). Although some earlier dialysis studies reported limited effects of cocaine on these DA metabolites (Di Chiara and Imperato, 1988a; Hurd and Ungerstedt, 1989), the present findings are consistent with our previous results (Chapter II), as well as recent data from other laboratories (Kalivas and Duffy, 1990; Maissonneuve *et al.*, 1990).

The results of the second experiment confirmed that behavioural effects of cocaine can be classically conditioned to environmental stimuli (Barr *et al.*, 1983; Beninger and Herz, 1986; Tatum and Seevers, 1929). Specifically, when subjects were tested in the environment that had previously been paired with cocaine they exhibited significantly more locomotor

activity than subjects that had received cocaine in their homecage. The classical conditioning of a drug to environmental cues is not unique to cocaine and has been reported with other stimulants, as well as with opiates (Beninger and Hanh, 1983; Carey, 1992; Möller *et al.*, 1987; Stewart *et al.*, 1984; Walter and Kuschinsky, 1989). This type of conditioning has important clinical significance because conditioned cues can evoke intense craving, and these conditioned cravings play a substantial role in the relapse into cocaine use (Gawin, 1991; O'Brien *et al.*, 1992).

Given the demonstration of the classical conditioning of cocaine to environmental stimuli, as well as the large unconditioned effect of cocaine on interstitial DA in the nucleus accumbens, the third experiment was designed to determine if the conditional change in behaviour was associated with an increase in DA release. No evidence for conditioned dopaminergic activity was found, as the exposure of conditioned subjects to the cocaine-paired environment failed to produce a change in interstitial DA or its metabolites, DOPAC or HVA, that was significantly different from pseudoconditioned controls. Although the 10 % increase in DA for the two groups of subjects failed to reach significance, the increases in DOPAC and HVA were significant. The increase in the DA metabolites suggests that exposure to the conditioning environment did influence these dopaminergic neurons; however, the lack of differentiation between the groups indicates that the increase was not related to the conditioning.

The final experiment served as a validation and extension of Experiment 3, in which neurochemistry and behaviour were simultaneously monitored in the experimental subjects on the test day. In agreement with the results of the previous experiment, exposure of the conditioned subjects to the cocaine-paired environment did not produce an increase in DA, DOPAC or HVA that differed from the pseudoconditioned subjects. This negative neurochemical finding is directly contrasted by the significantly greater amount of locomotor behaviour exhibited by the conditioned subjects. Taken as a whole, these data suggest that there is a clear dissociation between the behavioural and the neurochemical

responses of the two groups of subjects. Both the conditioned and pseudoconditioned groups exhibited a significant increase in DA, DOPAC and HVA when they were placed in the conditioning apparatus, suggesting that dopaminergic tone was increased. However, the finding that this increase did not differ between the groups indicate that this effect is unrelated to the conditioning. One difference between the findings of Experiments 3 and 4 is that there was a significant increase in DA in the later. Although the increase in DA was similar in both studies (approximately 10% in Experiment 3 and 15% in Experiment 4), the larger standard error in Experiment 3 resulted in the failure of this effect to reach significance. It is also important to note that the magnitude of the increase in DA, or its metabolites, is of minor interest, as it is the hypothesized difference between the conditioned and pseudoconditioned subjects that is of primary concern.

The present data do not support the hypothesis that conditioned stimuli associated with cocaine arouse similar neural states as the drug itself. The first caveat about this conclusion is that absence of evidence cannot be equated with evidence of absence, and it is possible that microdialysis is not sufficiently sensitive to detect small and/or transient neurochemical changes associated with cocaine-conditioning. It is also possible that a small subset of dopaminergic projections to the accumbens is responsible for the conditional change in behaviour, and that these discrete changes are being masked by the surrounding unresponsive neurons. An additional possibility is that, despite the fact that the nucleus accumbens has been strongly implicated in stimulant-induced locomotor activity (Delfs *et al.*, 1990; Joyce and Koob, 1981; Kelly et al., 1975), dopaminergic projections to other structures may also play an important role. Although the present data cannot fully discount these possibilities, the results of other neurochemical and pharmacological studies, discussed below, cast doubt on these alternative interpretations.

It is unlikely that microdialysis is not sensitive enough to detect behaviourally relevant changes in DA, as data from this laboratory has illustrated that the consumption of a palatable meal (Nomikos *et al.*, in preparation) and sexual behaviour (Pfaus *et al.*, 1990)

increase interstitial DA in the nucleus accumbens in a robust and reliable fashion. Moreover, a significant increase in DA in the final conditioning experiment was reported. Although this increase was similar in the conditioned and pseudoconditioned groups, it does illustrate the sensitive nature of the presently employed microdialysis procedure. A similar magnitude increase in accumbens DA has been reported by Nomikos *et al.* (in preparation) as a result of transfering a rat from one cage to another. The increase in DA and its metabolites observed in the present experiments may also simply reflect this manipulation.

The present data are supported by a previous neurochemical study which failed to find a difference in tissue DA turnover between conditioned and pseudoconditioned subjects upon presentation of a cocaine conditioned environment (Barr *et al.*, 1983). Other investigators have also failed to find changes in dopaminergic transmission associated with conditional changes in behaviour following conditioning with a variety of agents such as fentanyl (Finlay *et al.*, 1988), morphine (Walter and Kuschinsky, 1989), or apomorphine (Möller *et al.*, 1987). Although Barrett and Nader (1990) have recently concluded that "... there appears to be a reasonable amount of evidence to indicate that neurochemical changes can be conditioned using Pavlovian procedures", important aspects of some of the early neurochemical studies that gave rise to this conclusion are flawed. For example, an elevation in tissue HVA levels over control values has been taken as evidence of a conditioned increase in DA metabolism in all of the studies that reported this effect (Lal *et al.*, 1976; Perez-Cruet, 1976; Shiff, 1982). Although tissue concentrations of DA metabolites can provide an indirect measure of dopaminergic transmission, this is of questionable value in the absence of concomitant information about DA concentrations (Commissiong, 1985; Westerink, 1985). This limitation aside, an additional factor that limits the value of the data of both Perez-Cruet (1976) and Lal *et al.* (1976) is that these studies lacked adequate data demonstrating that their conditioning procedures affected behaviour. Specifically, there was no evidence that conditioning had occurred. Taken as a whole, the neurochemical evidence for conditioned dopaminergic activity is weak.

In addition to the absence of convincing neurochemical support for a dopaminergic role in the cocaine-induced conditional locomotion, a number of investigators have reported that while DA receptor antagonists block both the acute unconditioned behavioural effects of cocaine and the development of environment-specific conditioning, they are much less effective in attenuating the conditioned locomotor effects of cocaine and other psychomotor stimulants (Beninger and Hanh, 1983; Beninger and Herz, 1986; Carey, 1992; Weiss *et al.*, 1989). It is noteworthy that pimozide also fails to block the conditioned response to food-paired stimuli (Horvitz and Ettenberg, 1991). It should be noted, however, that some investigators have suggested that the mesolimbic DA system is involved in the conditional response to stimulants (Drew and Glick, 1990; Gold *et al.*, 1988). Gold *et al.* (1988) demonstrated that the conditioned locomotor response to amphetamine was significantly attenuated by 6-OHDA lesions of the nucleus accumbens when the lesion was made prior to or following the conditioning procedure. The authors interpret their results to indicate that the mesolimbic DA system may be responsible for both the unconditioned and conditioned locomotor responses to psychomotor stimulant drugs. This conclusion, however, is not justified given that these lesions also produced significant depletions in noradrenaline. Moreover, the effect of these lesions on catecholamine concentrations in regions other than the nucleus accumbens were not reported. Given the lack of specificity of these lesions it cannot be assumed that the attenuation of conditioned locomotion was the result of DA depletion in the nucleus accumbens. Drew and Glick (1990) have reported that both D_1 and D_2 DA receptor antagonists can attenuate amphetamine-induced conditioned circling. However, the absence of pseudoconditioned or control subjects in this study make it impossible to determine if conditioning had occured. Taken as a whole, the previous pharmacological results, together with the present *in vivo* neurochemical data, strongly suggest that although the development of cocaine-induced environment-specific conditioning is DA-dependent, the neurochemical events associated with the expression of the conditioned response are not.

Given the clinical relevance of conditioned cocaine cues, the elucidation of the neurobiology of this phenomenon is of obvious importance. Furthermore, the availability of a pertinent animal model makes this a feasible and worthwhile avenue of investigation. Investigations of the role of the amygdala in drug-induced environment-specific conditioning may prove to be particularly fruitful. For example, Post *et al.* (1988) have reported that although lesions of the amygdala (electrolytic and 6-OHDA) did not affect cocaine-induced hyperactivity they greatly attenuated environment-specific cocaine sensitization. Moreover, a role for the amygdala in the development of stimulus-reward associations has been demonstrated in a variety of experimental paradigms (Cador *et al.*, 1989; Everitt *et al.*, 1991; Hiroi and White, 1991a; Robbins *et al.*, 1989).

IV. EVIDENCE FOR CONDITIONAL NEURONAL ACTIVATION FOLLOWING EXPOSURE TO A COCAINE-PAIRED ENVIRONMENT: ROLE OF FOREBRAIN LIMBIC STRUCTURES

(A) Introduction

As discussed previously, the classical conditioning of cocaine's behavioural effects with specific environmental stimuli is an important aspect of its actions (Barr *et al.*, 1983; Stewart *et al.*, 1984; Tatum and Seevers, 1929). This property of cocaine is of major significance with respect to its abuse potential, as intense craving can be evoked by stimuli previously associated with the act of taking the drug (Gawin, 1991; Johanson and Fischman, 1989; O'Brien *et al.*, 1992). Given the large body of evidence implicating the mesolimbic DA system in the reinforcing properties of drugs of abuse (Di Chiara and Imperato, 1988a; Fibiger and Phillips, 1987; Lyness *et al.*, 1979; Roberts *et al.*, 1977, 1989) it is possible that conditioned stimuli associated with drug administration may also produce increases in dopaminergic transmission (Stewart *et al.*, 1984). However, the data from Chapter III failed to provide evidence for this hypothesis. Specifically, the presentation of an environment that had been repeatedly paired with cocaine administration did not produce an increase in interstitial DA concentrations in the nucleus accumbens that was significantly greater than that observed in pseudoconditioned controls. This negative neurochemical finding was sharply contrasted by the significantly greater amount of locomotor behaviour exhibited by the conditioned subjects. Previous investigators have also reported an absence of evidence for conditioned dopaminergic activity following conditioning with a variety of agents such as cocaine (Barr *et al.*, 1983), fentanyl (Finlay *et al.*, 1988), morphine (Walter and Kuschinsky, 1989) and apomorphine (Möller *et al.*, 1987). Although the findings of these studies indicated an absence of conditional DA release, all found significant conditioned behavioural changes.

Recent studies have demonstrated that cocaine increases neuronal expression of Fos, the product of the proto-oncogene *c-fos*, within the striatum and nucleus accumbens (Graybiel *et al.*, 1990; Young *et al.*, 1991), in agreement with the importance of these structures in many of the behavioural properties of cocaine. The transient expression of Fos in response to a variety of physiological and pharmacological manipulations suggests that *c-fos* induction may be used as a marker of neuronal activation (Dragunow and Robertson, 1987; Fu and Beckstead, 1992; Hunt *et al.*, 1987; Morgan and Curran, 1991; Robertson *et al.*, 1989, 1991; Rusak *et al.*, 1990). It has therefore been proposed that Fos immunohistochemistry might be utilized as a cellular metabolic marker, similar to 2-deoxyglucose (Dragunow and Faull, 1989; Hunt *et al.*, 1987; Morgan and Curran, 1989; Sagar *et al.*, 1988). Given the ability of cocaine to increase Fos expression and the proposed use of this proto-oncogene product as a marker of neuronal activation, the present study utilized Fos immunohistochemistry to determine if environmental stimuli paired with cocaine administration produce increases in Fos expression in the basal ganglia and various limbic regions.

(B) Materials and Methods

Subjects and Drugs

Subjects were 28 male Long Evans rats (Charles River, Quebec), weighing 300 - 400 g at the beginning of the experiments. The rats were group housed (3 per cage), on a 12-hr light/12-hr dark cycle (lights on 08:00), with food and water available *ad libitum*. All subjects were handled periodically for one week prior to the experiments. All experimental procedures were conducted at approximately the same time each day, during the animals' light phase.

Both cocaine hydrochloride (10 mg/kg, dissolved in isotonic saline , BDH) and 0.9 % saline were injected *i.p.* in a volume of 1 ml/kg. The dose of cocaine is expressed as the weight of the salt.

Apparatus and Behavioural Procedure

Four circular (61 cm diameter) activity cages (BRS/LVE), as described in Chapter III, were used to measure locomotor activity.

The first experiment involved the characterization of the acute effects of cocaine (10 mg/kg) and saline on locomotor behaviour and Fos expression in the rat forebrain. Rats were randomly assigned to one of the activity cages and habituated for 90 min on four consecutive days. One day following the final habituation period rats were injected with either cocaine (n=4) or saline (n=4) and immediately placed into the activity cage, and locomotor counts were monitored for 90 min. Following the test session, the subjects were deeply anesthetized with sodium pentobarbital (100 mg/kg, *i.p.*) and transcardially perfused with isotonic saline followed by 4% paraformaldehyde in phosphate-buffered (0.1 M) saline.

The effect of the presentation of an environment that had previously been paired with cocaine administration on locomotor behaviour and Fos expression in the rat forebrain was examined in the second experiment. Rats were randomly assigned to 1 of 3 groups: conditioned (n=7), pseudoconditioned (n=7) or control (n=6). Conditioned subjects were injected with cocaine (10 mg/kg, *i.p.*) and then placed into one of the circular activity cages for 30 min. After the training session, subjects were returned to their homecages, where they were injected with saline (1 ml/kg, *i.p.*) 4 hr later. Pseudoconditioned rats were exposed to an identical procedure except that the order of administration of cocaine and saline was reversed. Specifically, these subjects were injected with saline prior to being placed in the activity cages and later with cocaine in their homecages. Control subjects were exposed to the same procedure as the other groups, except that they were injected with saline in both environments and never received cocaine. Each subject was assigned to a

particular locomotor cage for the duration of the experiment. Training was conducted daily for 10 consecutive days at approximately the same time each day. On the test day (48 hr after the final training session), subjects were placed in the locomotor cages (no injection given) and activity was monitored for 90 min. Following the test session, the subjects were deeply anesthetized with sodium pentobarbital (100 mg/kg, *i.p.*) and transcardially perfused with isotonic saline followed by 4% paraformaldehyde in phosphate-buffered (0.1 M) saline.

Fos Immunohistochemistry

Following a 24 - 48 hr postfixative period, 30 μm sections were cut from each brain using a Vibratome (Pelco). The sections were washed (10 min) three times with 0.02 M phosphate buffer (PB) before being incubated in phosphate buffered 0.3% hydrogen peroxide for 10 min to remove endogenous peroxidase activity. Sections were then washed three times in PB and incubated with the Fos primary antisera (1:2000 dilution; also contained 0.3% Triton-X and 0.02% Na azide in PB) for 48 hr. A polyclonal sheep antibody (Cambridge Research Biochemicals; CRB OA-11-823) directed against residues 2 - 16 of the N-terminal region of the Fos protein was used in the present studies. The sections were then washed three times in PB and incubated with a biotinylated rabbit anti-sheep secondary antibody (Vector Laboratories; 1:500 dilution; also contained 0.3% Triton-X in PB) for 1 hr. Sections were washed three times in PB before being incubated with 0.3% Triton-X and 0.5% avidin-biotinylated horseradish peroxidase complex (Vector Laboratories) in PB for 1 hr. Sections were then washed two times with PB and once with an acetate buffer (0.1 M, pH 6.0) before being visualized by the glucose oxidase-DAB-nickel method (Shu et al., 1988). The reaction was terminated by washing the sections in acetate buffer (0.1 M, pH 6.0). Sections were mounted on chrome-alum coated slides, dehydrated and prepared for microscopic examination. A more detailed description of this technique has been published previously (Robertson and Fibiger, 1992). It should be noted that immunoblot analysis (CRB technical data sheet) indicates that the CRB antibody recognizes Fos (62 kDa) as well

as other Fos-related antigens (48/49 and 70 kDa). However, the delayed onset of the Fos-related antigens (Morgan and Curran, 1989) would suggest that Fos is responsible for the immunoreactivity quantified in the present study. The time chosen for perfusion (90 min after the initiation of all experimental manipulations) approximately coincides with the peak levels of Fos antigens (Morgan and Curran, 1989).

Quantification of Fos Positive Cells

Fos expression within the cingulate cortex, claustrum, piriform cortex, nucleus accumbens, dorsomedial striatum, lateral septal nucleus and paraventricular nucleus of the thalamus was quantified by counting the number of Fos-positive nuclei within a 510 x 510 μm grid placed over the area at 107 X magnification. The number of Fos-positive nuclei within the amygdala was also quantified; the grid for these counts was 1290 x 1290 μm at 43 X magnification. Camera lucida drawings illustrate the specific regions sampled and AP position of the sections used (Figure 16). In addition to the aforementioned regions, the number of Fos-positive nuclei within the lateral habenula was also examined. However, due to rostro-caudal variation of the sections available from this region it was not deemed appropriate to quantify the data in the same manner as was used for the other regions. To increase the reliability of the quantification, cell counts were made from two separate sections of each region from a given subject. In addition, duplicate counts of each section were conducted by two independent observers. This procedure, therefore, resulted in a total of four determinations of the number of Fos-positive nuclei within a specified region for each subject. The average of these four determinations was utilized for the subsequent statistical analysis.

Statistical Analysis

Between group differences in locomotor counts and the number of Fos-positive nuclei within specified regions from the conditioning experiment subjects were evaluated using univariate analyses of variance. Post hoc comparisons between control, pseudoconditioned and conditioned subjects were conducted using Tukey's HSD. Univariate analyses of variance were also conducted to assess locomotor differences and the number of Fos-positive nuclei within specified regions between acute saline- and cocaine-treated subjects.

(C) Results

The brain areas quantified in the present study reflect those areas that were determined to have positive responses to the acute administration of cocaine or the presentation of the cocaine-paired environment. These initial observations were performed by a "blind" observer who scanned coronal sections throughout the forebrain to determine regions that exhibited increased Fos-immunoreactivity. No other forebrain regions exhibited reliable responses to the present treatments.

As expected, compared to saline treated controls the acute administration of cocaine produced a significant increase in locomotor counts [$F(1,6)=45.42$, $p<0.005$] (Figure 17). This behavioural effect was accompanied by significant increases in the number of Fos-positive nuclei in the cingulate cortex [$F(1,6)=10.69$, $p<0.05$], claustrum [$F(1,6)=50.26$, $p<0.001$], piriform cortex [$F(1,6)=49.98$, $p<0.001$], nucleus accumbens [$F(1,6)=82.81$, $p<0.001$], dorsomedial striatum [$F(1,6)=40.83$, $p<0.005$], lateral septum [$F(1,6)=134.32$,

Figure 16. Camera lucida drawings of representative sections used for the counting of Fos-positive nuclei in the cingulate cortex (**1**), claustrum (**2**), piriform cortex (**3**), nucleus accumbens (**4**), dorsomedial striatum (**5**), lateral septum (**6**), paraventricular nucleus of the thalamus (7) and the amygdala (**8**). The area of quantification for areas 1 - 7 was 510 x 510 μm. The quantification of the amygdala (8) encompassed a 1290 x 1290 μm area.

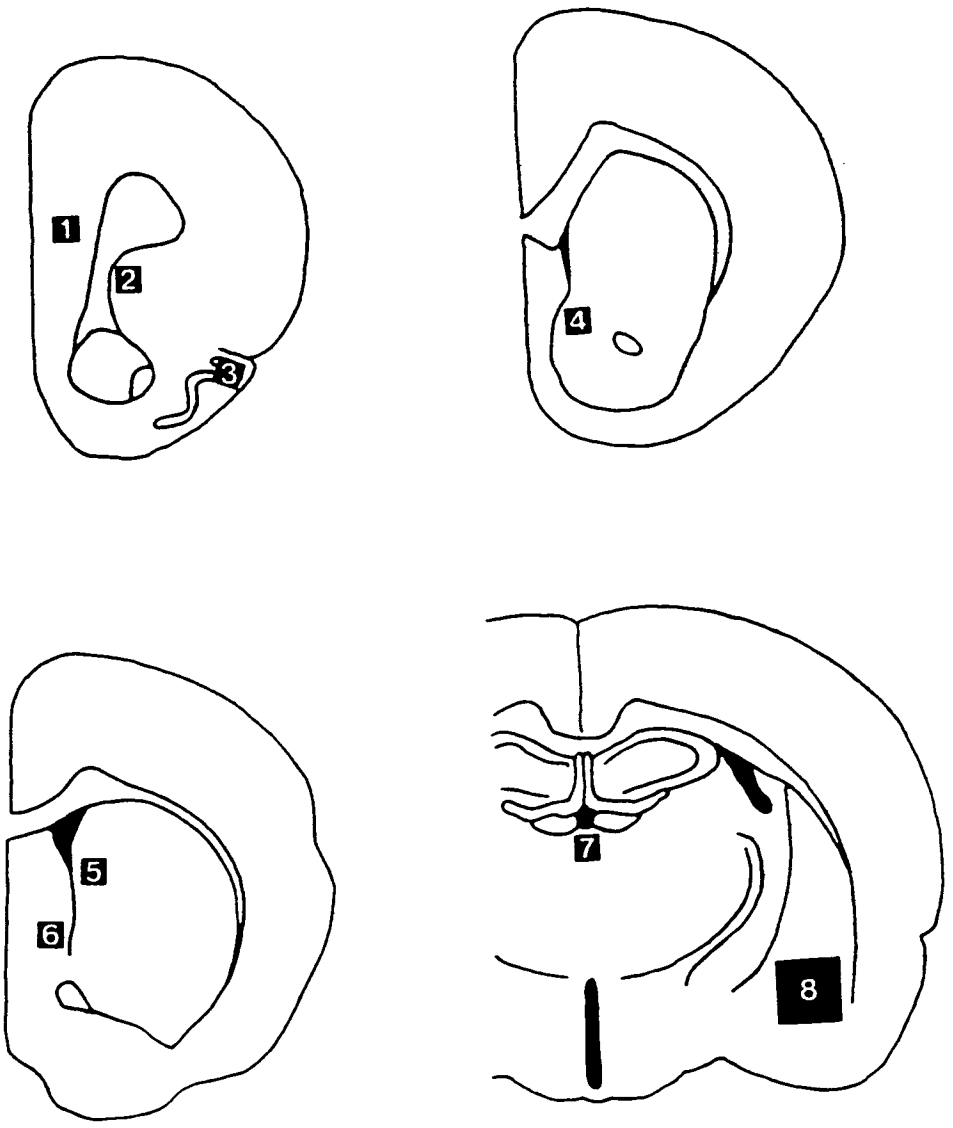

1
2
3
4
5
6
7
8

Figure 17. Total number of locomotor counts for control (n=6), pseudoconditioned (Cs^-, n=7) and conditioned (Cs^+, n=7) subjects following 10 days of conditioning with cocaine (10 mg/kg, *i.p.*). Testing occurred 48 hr after the last training session, and was conducted in the same apparatus as used for the training procedure. Locomotor counts are also shown for unconditioned subjects that received acute injections of saline (1 ml/kg, n=4) or cocaine (10 mg/kg, n=4). Values represent the group mean ± SEM. * $P < 0.005$ compared to pseudoconditioned controls. † $P < 0.005$ compared to saline treated controls.

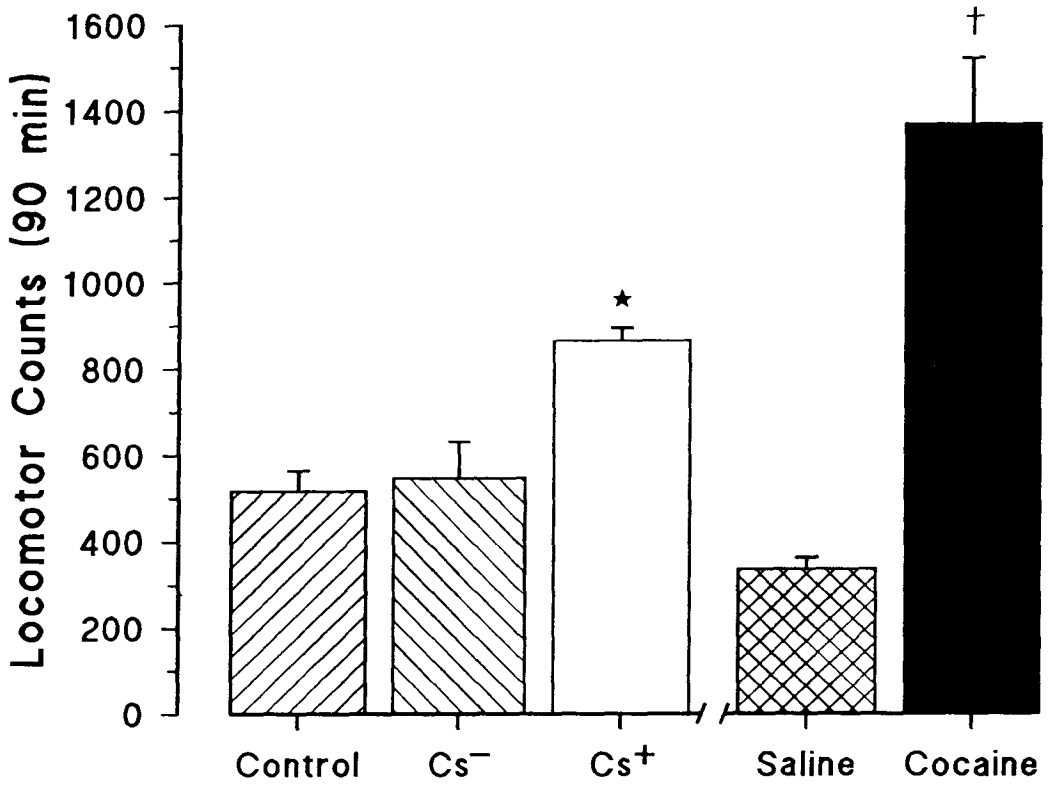

Locomotor Counts (90 min)
1600
1400
1200
1000
800
600
400
200
0
★
†
Control
Cs⁻
Cs⁺
Saline
Cocaine

Figure 18. Number of Fos-positive nuclei within a 510 x 510 μm area in the cingulate cortex, claustrum, piriform cortex and nucleus accumbens for control (n=6), pseudoconditioned (Cs^-, n=7), conditioned (Cs^+, n=7), saline treated (n=4) and cocaine treated (n=4) subjects. Values represent the group mean $\pm$ SEM. * $P < 0.05$ compared to pseudoconditioned controls. † $P < 0.05$ compared to saline treated controls.

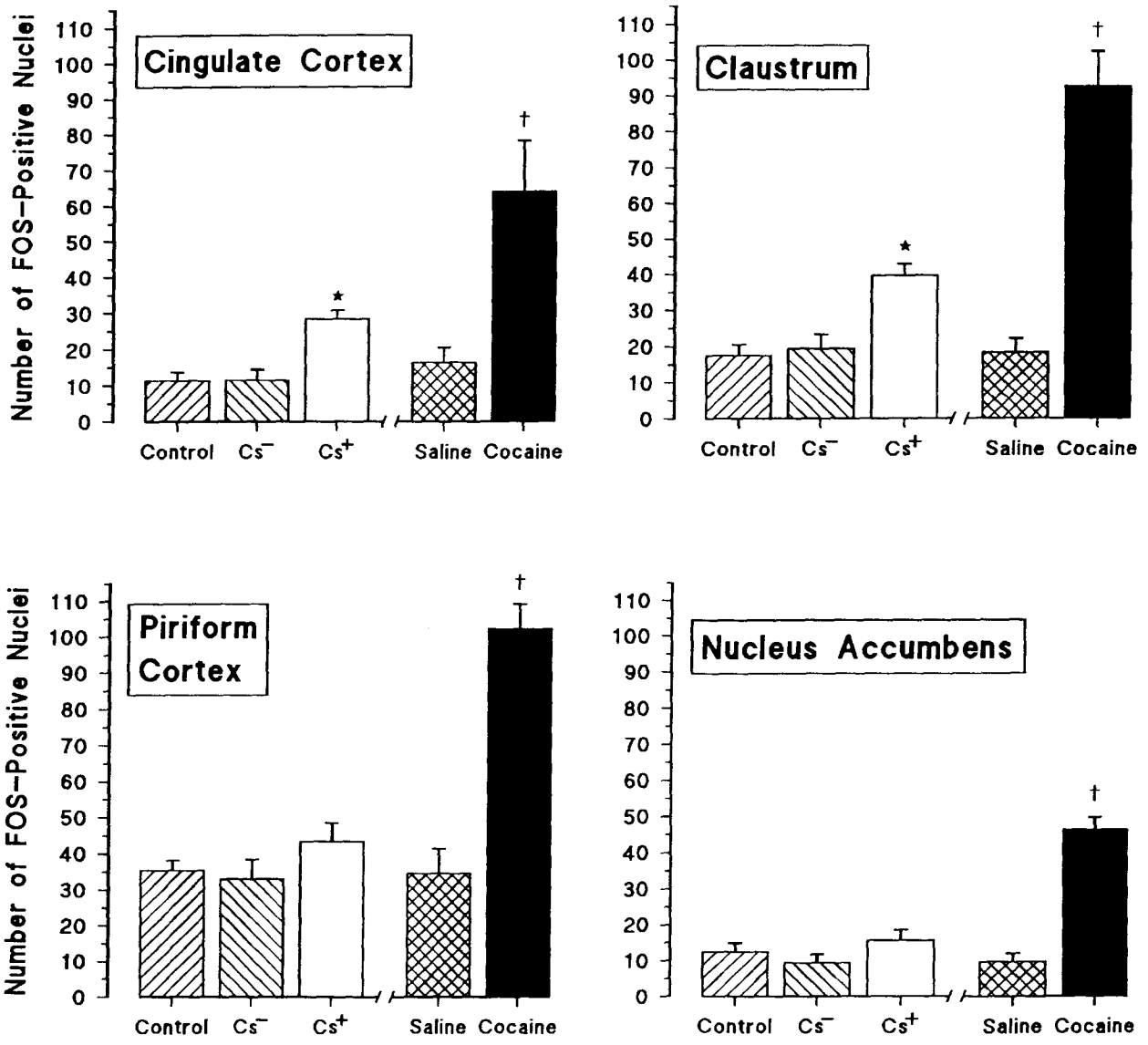
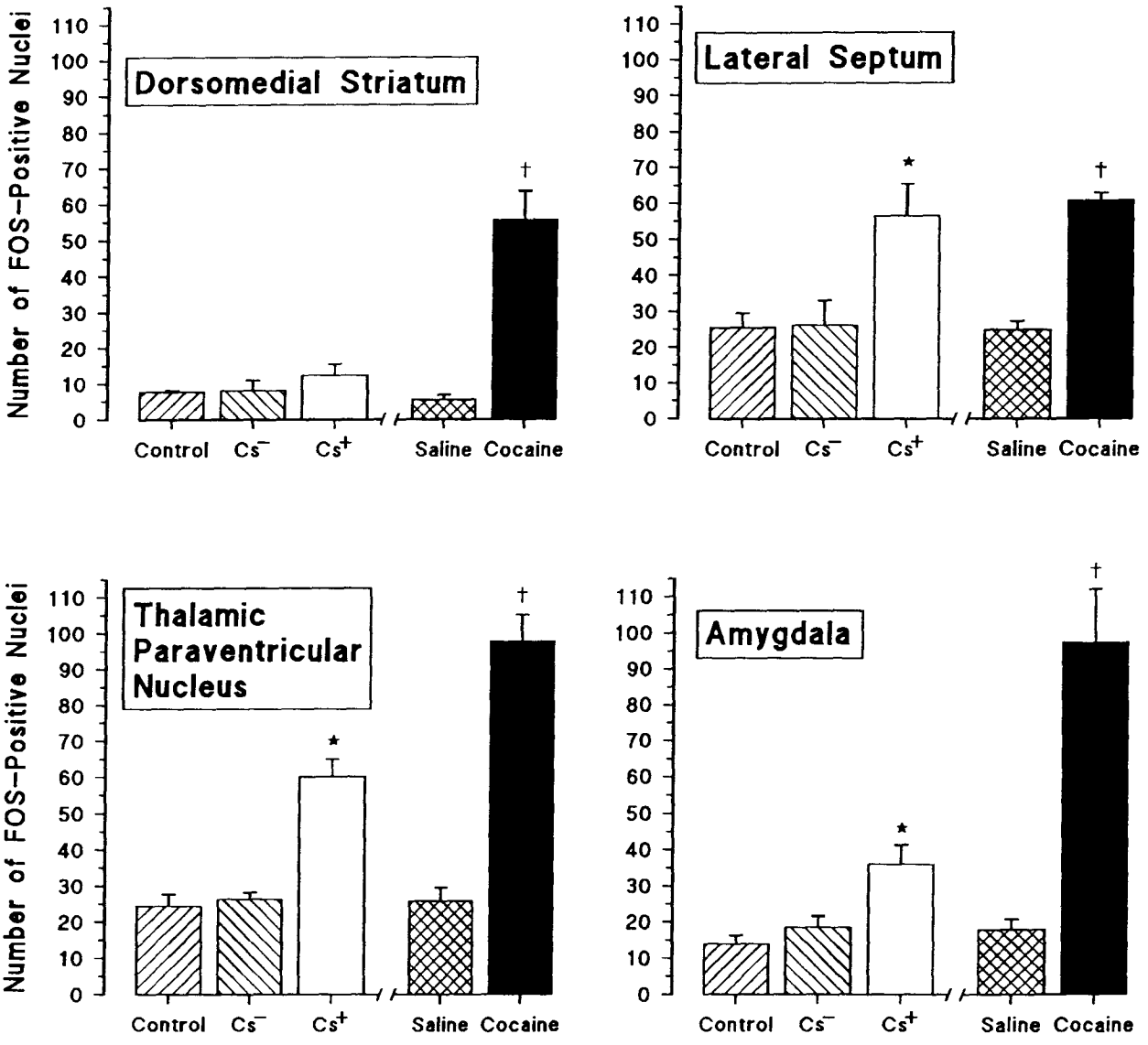

Number of FOS-Positive Nuclei
Cingulate Cortex
Claustrum
Piriform Cortex
Nucleus Accumbens
Control
Cs−
Cs+
Saline
Cocaine
0
10
20
30
40
50
60
70
80
90
100
110
*
†

Figure 19. Number of Fos-positive nuclei within a 510 x 510 μm area in the dorsomedial striatum, lateral septum and paraventricular nucleus of the thalamus and within a 1290 x 1290 μm area in the amygdala for control (n=6), pseudoconditioned (Cs^-, n=7), conditioned (Cs^+, n=7), saline treated (n=4) and cocaine treated (n=4) subjects. Values represent the group mean $\pm$ SEM. * $P < 0.05$ compared to pseudoconditioned controls. † $P < 0.05$ compared to saline treated controls.


Number of FOS-Positive Nuclei
Dorsomedial Striatum
Lateral Septum
Thalamic Paraventricular Nucleus
Amygdala
Control
Cs−
Cs+
Saline
Cocaine
*
†

$p<0.001$], paraventricular nucleus of the thalamus [$F(1,6)=80.46$, $p<0.001$] and amygdala [$F(1,6)=28.3$, $p<0.005$], as compared to saline treated controls (Figures 18, 19, 20 and 21).

The conditioning procedure employed in the present study produced a clear conditional behavioural effect [$F(2,17)=10.35$, $p<0.005$] (Figure 17). Post hoc comparisons demonstrated that conditioned subjects exhibited significantly more locomotor counts than either control or pseudoconditioned controls ($p<0.05$). This conditioned effect stands in contrast to the lack of a significant group difference between the pseudoconditioned and control subjects (Figure 17).

In addition to producing a significant behavioural effect, the conditioning procedure resulted in increased Fos expression within specific brain regions (Figures 18, 19, 20 and 21). Significant group effects were observed for the cingulate cortex [$F(2,17)=14.19$, $p<0.0005$], claustrum [$F(2,17)=12.56$, $p<0.0005$], lateral septal nucleus [$F(2,17)=6.05$, $p<0.02$], paraventricular nucleus of the thalamus [$F(2,17)=32.12$, $p<0.0001$] and amygdala [$F(2,17)=8.33$, $p<0.005$]. Post hoc comparisons revealed that conditioned subjects exhibited significantly more Fos-positive nuclei than control or pseudoconditioned subjects in the cingulate cortex, claustrum, lateral septal nucleus, paraventricular nucleus of the thalamus and amygdala ($p<0.05$), while no differences were observed between control and pseudoconditioned subjects (Figures 18 and 19). In contrast, the number of Fos-positive nuclei within the nucleus accumbens [$F(2,17)=1.51$], dorsomedial striatum [$F(2,17)=1.04$] and piriform cortex [$F(2,17)=1.29$] did not differ significantly between the control, pseudoconditioned or conditioned subjects.

Although the conditioned subjects exhibited an enhanced locomotor response, the magnitude of this effect was considerably less than that observed following an acute injection of cocaine [$F(1,9)=18.68$, $p<0.005$] (Figure 17). In accordance with this behavioural difference, conditioned subjects exhibited significantly fewer Fos-positive nuclei than animals treated acutely with cocaine in the cingulate cortex [$F(1,11)=11.29$, $p<0.01$], claustrum [$F(1,11)=40.45$, $p<0.001$], piriform cortex [$F(1,11)=46.38$, $p<0.001$], nucleus

Figure 20. Photomicrographs of Fos-immunoreactivity in the cingulate cortex from representative saline (A) and cocaine (B) treated subjects, as well as pseudoconditioned (C) and conditioned (D) subjects. Scale Bar = 100 μm.

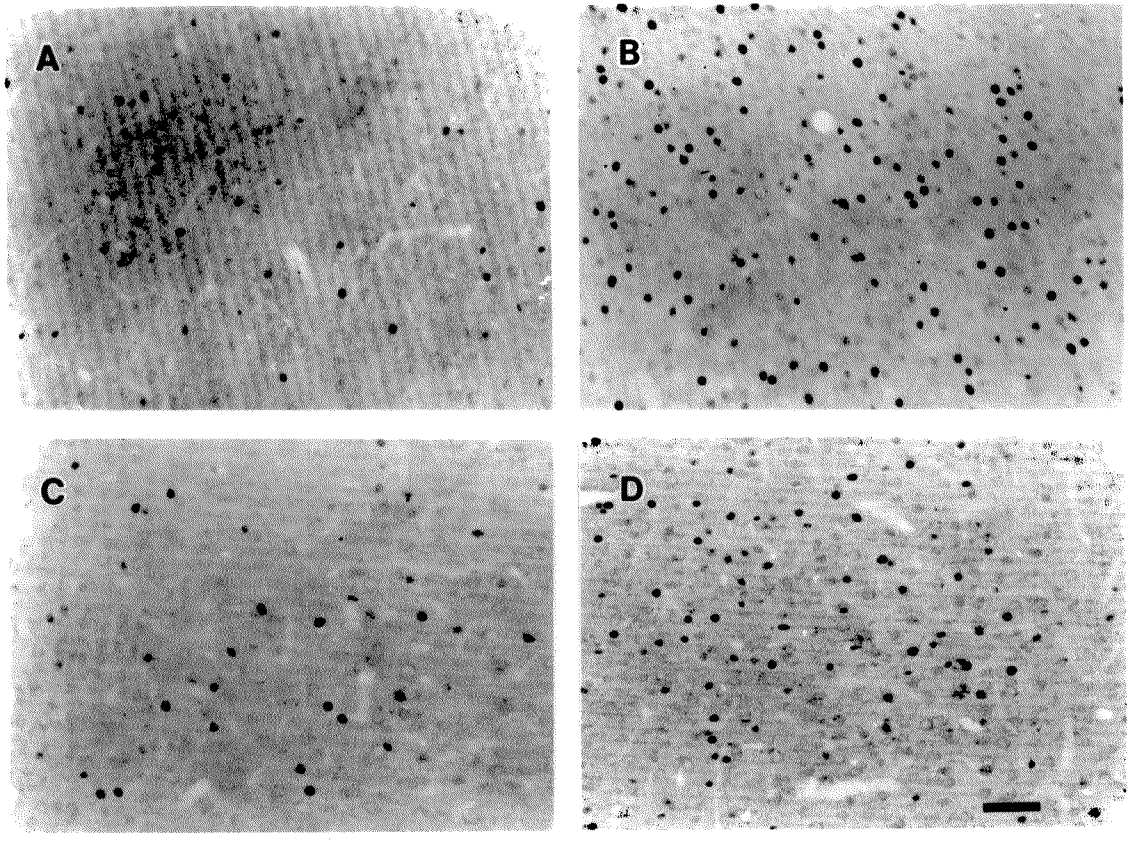
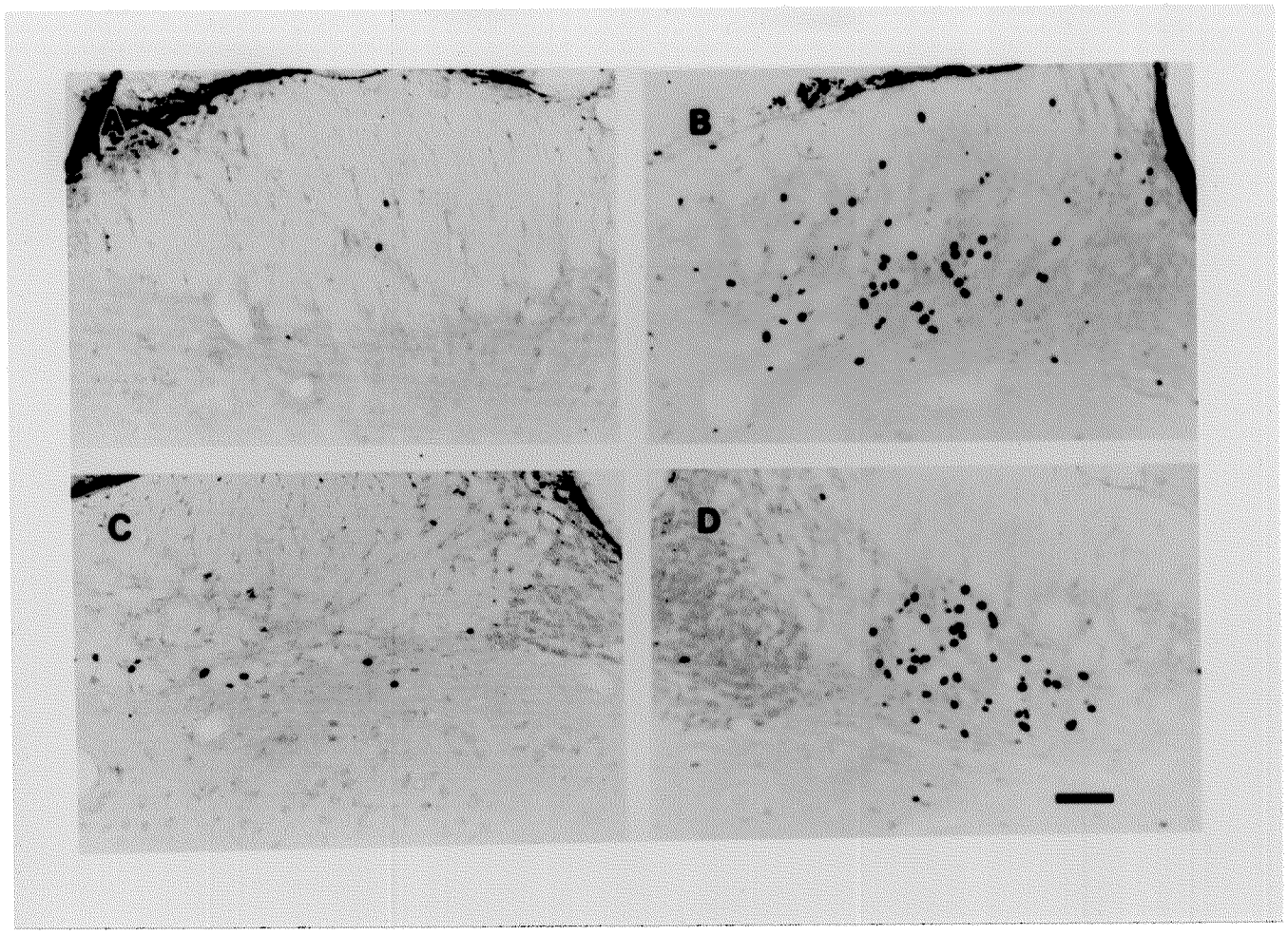

A
B
C
D

Figure 21. Photomicrographs of Fos-immunoreactivity in the lateral habenula from representative saline (A) and cocaine (B) treated subjects, as well as pseudoconditioned (C) and conditioned (D) subjects. Scale Bar = 100 μm.


A
B
C
D

accumbens [$F(1,11)=44.89$, $p<0.001$], dorsomedial striatum [$F(1,11)=37.82$, $p<0.001$], paraventricular nucleus of the thalamus [$F(1,11)=20.61$, $p<0.005$] and amygdala [$F(1,11)=22.66$, $p<0.005$] (Figures 18 and 19). Interestingly, the number of Fos-positive nuclei within the lateral septal nucleus was equivalent between the conditioned and cocaine treated subjects [$F(1,11)=0.13$] (Figure 19).

It is noteworthy that the number of Fos-positive nuclei within the cingulate cortex [$F(1,8)=1.23$], claustrum [$F(1,8)=0.02$], piriform cortex [$F(1,8)=0.01$], nucleus accumbens [$F(1,8)=0.58$], dorsomedial striatum [$F(1,8)=2.89$], lateral septal nucleus [$F(1,8)=0.01$], paraventricular nucleus of the thalamus [$F(1,8)=0.08$] or amygdala [$F(1,8)=1.08$] were not significantly different between control subjects from the conditioning experiment and subjects acutely injected with saline (Figures 18 and 19).

In addition to the aforementioned regions, compared to saline treated controls cocaine produced an increase in the number of Fos-positive nuclei in the medial portion of the lateral habenula (Figure 21). Conditioned subjects also exhibited a large and robust increase in Fos expression within this area, while virtually no Fos-positive nuclei were observed within this region of the pseudoconditioned controls. Due to variations in the anterior-posterior coordinates of the sections obtained from this region, reliable quantification was not possible. However, all of the acute cocaine and conditioned subjects that were examined displayed reliable increases in Fos expression, while none of the control, pseudoconditioned or saline treated subjects exhibited substantial Fos expression in this structure.

(D) Discussion

In agreement with earlier studies (Graybiel *et al.*, 1990; Young *et al.*, 1991), acute administration of cocaine elevated Fos-immunoreactivity in the striatum and nucleus accumbens. In addition, cocaine enhanced Fos-immunoreactivity in the cingulate cortex, claustrum, piriform cortex, lateral septal nucleus, paraventricular nucleus of the thalamus, medial portion of the lateral habenula and amygdala. The results of the second experiment confirmed that the unconditioned locomotor stimulatory effects of cocaine can be classically conditioned to environmental stimuli (Barr et al., 1983; Beninger and Herz, 1986; Tatum and Seevers, 1929). Moreover, this conditioning was accompanied by changes in Fos immunoreactivity within specific limbic regions, suggesting a conditional increase in neuronal activity within these areas and that these limbic regions are involved in mediating the conditioned behaviour.

The ability of cocaine to increase Fos expression in the striatum and nucleus accumbens is thought to be mediated through the activation of D_1 DA receptors (Graybiel *et al.*, 1990; Young *et al.*, 1991). This finding may be anticipated from the ability of cocaine to increase dopaminergic transmission (Chapters II and III; Di Chiara and Imperato, 1988a; Pettit and Justice, 1991) and the heavy dopaminergic innervation of the nucleus accumbens and striatum (Björklund and Lindvall, 1984). The present results illustrate that cocaine also produces increases in Fos in the cingulate cortex, claustrum, piriform cortex, lateral septal nucleus, paraventricular nucleus of the thalamus, medial portion of the lateral habenula and amygdala. Although each of these structures receive at least a modest dopaminergic innervation (Björklund and Lindvall, 1984), it is possible that the increase in Fos-positive nuclei within some or all of these regions is not dopaminergically mediated. Specifically, cocaine blocks the uptake of noradrenaline and serotonin, in addition to its well studied actions on DA (Richelson and Pfenning, 1984; Ritz *et al.*, 1987). Furthermore, the presence of noradrenergic and serotonergic projections to many of these areas precludes the

assumption that the observed effects were dopaminergically mediated (Moore and Card, 1984; Steinbusch, 1984). It is noteworthy that a recent study has provided evidence that clozapine-induced increases in Fos expression within the nucleus accumbens are DA-dependent, while increases in the cingulate cortex and lateral septum are not (Robertson and Fibiger, 1992). Finally, it is also possible that cocaine-induced activation of Fos expression in some areas was one or more synapses "downstream" from one of the primary sites of action of cocaine. Studies utilizing selective neurotoxins and receptor antagonists would help elucidate the neurotransmitter(s) involved in the cocaine-induced increases in Fos expression within these limbic regions.

In agreement with previous studies, the results from the second experiment demonstrate that the behavioural effects of cocaine can be classically conditioned to environmental stimuli (Barr *et al.*, 1983; Beninger and Herz, 1986; Tatum and Seevers, 1929). The classical conditioning of a drug to environmental cues is not unique to cocaine and has been reported with other stimulants, as well as with opiates (Beninger and Hahn, 1983; Carey, 1992; Möller *et al.*, 1987; Stewart *et al.*, 1984; Walter and Kuschinsky, 1989). The clinical significance of this drug-environment conditioning is considerable, as conditioned cues can evoke intense cravings, which play a substantial role in the relapse into cocaine use (Gawin, 1991; O'Brien *et al.*, 1992).

Cocaine-induced conditioned locomotion was accompanied by a conditional increase in Fos expression within specific limbic nuclei. Given the proposed use of Fos immunohistochemistry to map functional pathways in the brain (Dragunow and Faull, 1989; Hunt *et al.*, 1987; Morgan and Curran, 1989; Sagar *et al.*, 1988) and the ability of various behavioural and physiological manipulations to increase *c-fos* expression in the central nervous system (Campeau *et al.*, 1991; Chastrette *et al.*, 1991; Hunt *et al.*, 1987; Rusak *et al.*, 1990; Sharp *et al.*, 1991) the present findings may reflect at least a portion of the nuclei and regions involved in the conditioned response. It is noteworthy that a recent report has also demonstrated a conditioned activation of *c-fos*. Campeau *et al.* (1991) have reported

that conditioned fear produces dramatic increases in *c-fos* expression within the amygdala, a result supported by previous electrophysiological findings (Applegate *et al.*, 1982; Pascoe and Kapp, 1985).

The claustrum or orbitofrontal area and the cingulate cortex, both components of the prefrontal cortex (Groenwegen, 1988), exhibited an increase in Fos expression in conditioned subjects when they were exposed to the environment in which they had previously received cocaine. This finding is fully compatible with previous electrophysiological and lesion studies that demonstrate the importance of the cingulate cortex in classical conditioning (Buchanan and Powell, 1982; Gabriel *et al.*, 1980; Gabriel and Sparenborg, 1987; Powell *et al.*, 1990). Moreover, the diverse afferent connections of the prefrontal cortex from limbic, as well as sensory areas, allow for the potential integration of multimodal sensory information necessary to produce the associations involved in conditioning (Groenwegen *et al.*, 1990; Lopez da Silva, 1990). Although both the acute administration of cocaine and the presentation of the cocaine-paired environment produced increases in Fos-positive nuclei in the claustrum and cingulate cortex, it is not possible to ascertain from the present data if these different treatments produce their effects by the activation of the same afferent projections.

Conditional Fos expression was also observed in the lateral septal nucleus. Interestingly, this was the only area examined where the magnitude of the conditioned response was equivalent to the acute drug effect (Figure 19). This unique response may suggest a primary role for the lateral septum in the conditioned behaviour. With afferents from the amygdala, ventral tegmental area, and hippocampus and major efferent pathways to the mammillary bodies, lateral hypothalamus and medial septum, the lateral septum exhibits connectivity that is consistent with its role in the formation of associations between affective states and environmental stimuli (Krettek and Price, 1978; Raisman, 1966; Swanson and Cowan, 1979). Consistent with this proposal, electrophysiological studies have demonstrated that the activity of neurons in the lateral septal nucleus is sensitive to the

presentation of conditioned stimuli (Thomas, 1988; Thomas *et al.*, 1991; Thomas and Yadin, 1980). Based on these and other findings, it has been hypothesized that the lateral septum is involved in the inhibition of aversive affective states (Thomas, 1988). In view of the present findings, future examination of the potential role of this structure in appetitive classical conditioning is warranted.

Given the large body of data suggesting that various amygdaloid nuclei participate in Pavlovian conditioning (Applegate *et al.*, 1982; Dunn and Everitt, 1988; Kapp *et al.*, 1981; Mishkin and Aggleton, 1981; Pascoe and Kapp, 1985; Roozendaal *et al.*, 1990), it is not surprising that a conditional increase in Fos-positive nuclei was observed within this structure in the present study. These data are also consistent with the proposed role of the amygdala in the development of stimulus-reward associations, as demonstrated in a variety of experimental paradigms (Cador *et al.*, 1989; Everitt *et al.*, 1991; Hiroi and White, 1991a; Robbins *et al.*, 1989). Furthermore, Post *et al.* (1988) have reported that although lesions of the amygdala (electrolytic and 6-OHDA) did not affect cocaine-induced hyperactivity they greatly attenuated environment-specific cocaine sensitization. Given these previous findings, the present results are fully consistent with the proposed role of the amygdala in conditioning in general, and in stimulus-reward associations in particular.

One shortcoming of the present data is that Fos-immunoreactivity was not localized to specific amygdaloid nuclei. Although the area of quantification was centered around the central and basolateral nuclei, the present histological material was not suitable for the unequivocal delineation of the boundaries of the amygdaloid nuclei. Given the anatomical and physiological diversity of the specific amygdaloid nuclei, future studies should examine the role of particular nuclei.

Two additional areas that exhibited a conditional increase in Fos-immunoreactivity were the lateral habenula and the paraventricular nucleus of the thalamus. Although the absence of quantifiable data precludes any strong conclusions being put forward regarding the effect observed in the lateral habenula, it is noteworthy that it has recently been

reported that the medial portion of the lateral habenula exhibits an increase in Fos-immunoreactivity in response to restraint stress (Chastrette *et al.*, 1991). Interestingly, the distribution of Fos-positive nuclei observed in this study (Figure 21) and that of Chastrette *et al.* (1991) corresponds to the dopaminergic innervation of the lateral habenula (Phillipson and Pycock, 1982). Given the proposed role of this nucleus in a feedback loop from the frontal cortex to the ventral tegmental area (Lisoprawski *et al.*, 1980; Phillipson and Pycock, 1982) and its purported significance in motivated behaviour (Sutherland, 1982), further examination of its role in conditioned behaviour is warranted.

A variety of stressors have been shown to increase Fos-immunoreactivity in the paraventricular nucleus of the thalamus (Chastrette *et al.*, 1991; Sharp *et al.*, 1991). The present data indicate that this midline thalamic nucleus is also responsive to the presentation of an appetitive conditioned stimulus. Although the functional significance of the paraventricular nucleus is unclear, it is apparent that it is not a "nonspecific" nucleus (Bentivoglio *et al.*, 1991). The dopaminergic innervation of the paraventricular nucleus and its dense efferent projections to the amygdala, cingulate cortex and the nucleus accumbens are consistent with a "limbic" role for this midline thalamic nucleus and are in agreement with its hypothesized role in learning and memory (Bentivoglio *et al.*, 1991).

Although the conditional activation of Fos expression was observed within many of the regions that were affected by acute cocaine, the nucleus accumbens, striatum and piriform cortex did not exhibit an increased number of Fos-positive nuclei in the conditioned subjects. The absence of conditional Fos expression within the nucleus accumbens supports our results from Chapter II that demonstrated that cocaine-induced conditioned locomotion is not accompanied by an increase in DA release in this structure. Despite the fact that the nucleus accumbens has been strongly implicated in stimulant-induced locomotor activity (Delfs *et al.*, 1990; Joyce and Koob, 1981; Kelly *et al.*, 1975), dopaminergic projections to other limbic structures may play a critical role in environment-specific conditioned locomotion. This possibility is consistent with the conditional increase

in Fos expression in the aforementioned limbic structures, such as the cingulate cortex, lateral septum and amygdala, that have been demonstrated to participate in classical conditioning and to receive dopaminergic innervation (Björklund and Lindvall, 1984). Although a number of investigators have reported that DA receptor antagonists are ineffective in attenuating the conditioned locomotor effects of cocaine and other psychomotor stimulants (Beninger and Hahn, 1983; Beninger and Herz, 1986; Weiss *et al.*, 1989), the antagonists used in these studies are primarily directed against the D_2 receptor, leaving the possibility that DA could be acting through D_1 receptors.

Irrespective of the potential dopaminergic involvement in the conditional behavioural effect, the present data indicate that the neurobiological substrates for a conditioned response can differ from those of the unconditioned response. As noted previously, the dopaminergic projection to the nucleus accumbens appears to be necessary for the unconditioned locomotor effects of cocaine (Delfs *et al.*, 1990; Joyce and Koob, 1981; Kelly *et al.*, 1975) and the acute reinforcing effects of cocaine and other psychomotor stimulants (Lyness *et al.*, 1979; Roberts *et al.*, 1977, 1980; Pettit *et al.*, 1984). However, the present findings fail to provide evidence of increased Fos expression in the nucleus accumbens associated with the conditioned response. This absence of effect occurs in spite of the large number of other regions that displayed a conditioned response and the finding that this nucleus exhibits a robust increase in Fos expression in response to cocaine itself. This result is in general agreement with previous neurochemical studies that have reported no evidence for an increase in dopaminergic transmission in the nucleus accumbens in response to the presentation of a cocaine-paired environment (Chapter III; Barr *et al.*, 1983). The present results, together with the previous neurochemical data, strongly suggest that although the development of cocaine-induced environment-specific conditioning is dependent on the dopaminergic projection to the nucleus accumbens, the expression of the conditioned response is not. Taken as a whole, it appears that cocaine-induced environment-specific locomotion is not simply reflective of an increase in dopaminergic transmission to the

nucleus accumbens, but is associated with increased neuronal activation within various forebrain limbic structures known to be critically involved in emotion and learning. Consequently, the results of the present study suggest that this specific form of conditioning involves similar neural circuits as other forms of learning (Mishkin and Aggleton, 1981).

V. DIFFERENTIAL EFFECTS OF EXCITOTOXIC LESIONS OF THE AMYGDALA ON COCAINE-INDUCED CONDITIONED LOCOMOTION AND CONDITIONED PLACE PREFERENCE

(A) Introduction

A significant clinical feature of cocaine abuse is the occurrence of environmentally cued craving (Gawin, 1991; O'Brien et al., 1992). These conditioned cravings are the product of the repeated pairing of objects, places or events with the administration of cocaine and its subsequent euphoric effects. This clinical observation is supported by a number of laboratory studies that have demonstrated that cocaine's behavioural effects can readily become classically conditioned with specific environmental stimuli (Chapter III and IV; Barr *et al.*, 1983; Stewart *et al.*, 1984; Tatum and Seevers, 1929). Given the prevalence of these conditioned responses and their clinical significance, a better understanding of the neurobiology of this phenomenon may assist in the development of more rational treatments for cocaine abuse.

Although there is a large body of evidence implicating the mesolimbic DA system in the acute rewarding properties of cocaine (Di Chiara and Imperato, 1988a; Fibiger and Phillips, 1987; Roberts *et al.*, 1977, 1989; Ritz *et al.*, 1987), the conditioned responses to cocaine and other psychomotor stimulants is apparently DA-independent (Beninger and Hahn, 1983; Beninger and Herz, 1986; Weiss *et al.*, 1989; Carey, 1992). In Chapter III it was demonstrated that there was no evidence for a conditional increase in DA release in the nucleus accumbens in response to the presentation of a drug paired environment. This is consistent with an absence of evidence for conditioned dopaminergic activity following conditioning with a variety of agents such as cocaine (Barr *et al.*, 1983), fentanyl (Finlay *et al.*, 1988), morphine (Walter and Kuschinsky, 1989) and apomorphine (Möller *et al.*, 1987).

Although the findings of these studies indicated an absence of conditional DA release, all found significant conditioned behavioural changes.

In Chapter IV it was reported that exposure to a cocaine-paired environment produces a conditional increase in *c-fos* expression in various forebrain limbic regions, such as the cingulate cortex, claustrum, lateral septal nucleus and the amygdala. In agreement with our previous neurochemical findings, no conditional activation was observed in the nucleus accumbens. Taken as a whole these data suggested that cocaine-induced conditioned locomotion is associated with increased neuronal activation within various forebrain limbic structures known to be involved in emotion and learning (Powell *et al.*, 1990; Thomas *et al.*, 1991; Pascoe and Kapp, 1985; Lopez da Silva *et al.*, 1990; Davis, 1992). Consequently, it is possible that this specific form of classical conditioning involves similar neural circuits as other forms of learning (Mishkin and Aggleton, 1981).

Of the areas exhibiting an increase in Fos-positive neurons, the amygdala may be of particular importance. A large body of evidence suggests that amygdaloid nuclei are involved in stimulus-reward associations (Weiskrantz, 1956; Jones and Mishkin, 1972; Mishkin and Aggleton, 1981; Gaffan and Harrison, 1987; Cador *et al.*, 1989; Everitt *et al.*, 1991; Hiroi and White, 1991a; Kentridge *et al.*, 1991). Furthermore, Post *et al.* (1988) have reported that although lesions of the amygdala (electrolytic and 6-OHDA) do not affect cocaine-induced hyperactivity, they greatly attenuate environment-specific cocaine sensitization. Given these findings, the present study investigated the role of the amygdala in cocaine-induced conditional locomotion and cocaine-induced CPP. As previous studies have demonstrated that damage to fibres of passage within the amygdaloid complex produces behavioural effects that are unrelated to destruction of the amygdala (Riolobos and García, 1987; Dunn and Everitt, 1988), lesions were made using the fibre-sparing excitotoxin quinolinic acid.

(B) Materials and Methods

Subjects and Drugs

Subjects were 66 male Long Evans rats (Charles River, Quebec), weighing 270 - 310 g at the beginning of the experiments. The rats were group housed (3 per cage), on a 12-hr light/12-hr dark cycle (lights on 08:00), with food and water available *ad libitum*. All subjects were handled periodically for one week prior to surgery. All experimental procedures were conducted at approximately the same time each day, during the animals' light phase.

Both cocaine hydrochloride (10 mg/kg, dissolved in isotonic saline , BDH) and 0.9 % saline were injected *i.p.* in a volume of 1 ml/kg. The dose of cocaine is expressed as the weight of the salt.

Surgical Procedure

Subjects were anaesthetized with sodium pentobarbital (50 mg/kg, *i.p.*; BDH) and xylazine (5 mg/kg, *i.p.*; Haver) and mounted in a stereotaxic frame (incisor bar: + 5.0 mm; David Kopf Instruments). Bilateral lesions of the amygdala were produced by the infusion of 0.5 μl of quinolinic acid (0.12 M, in phosphate buffer, pH 7.1 - 7.4; RBI) at each of four injection sites (AP: +0.2 and -0.8 mm; ML: ±4.7 mm; DV: -7.9 from dura; relative to bregma; Pellegrino *et al.*, 1979). Control subjects received 0.5 μl infusions of phosphate buffer (0.12 M, pH 7.4). Infusions were made through 30-gauge stainless steel cannulae attached to pump driven (Harvard Apparatus) 5 μl syringes (Hamilton) by PE-10 tubing (Clay Adams). All infusions were made at a rate of 0.1 μl/min, and cannulae remained at the injection site for an additional 5 min following the injection to allow for the diffusion of the excitotoxin. Solutions of quinolinic acid were prepared immediately prior to each infusion to ensure the potency of the excitotoxin. Following the removal of the cannulae, topical antibiotic (Rifocin, Lepetit) was applied to the wound and the incision was sutured

with 4-0 silk. To reduce potential post-operative hypophagia and hypodipsia, the liquid diet Sustacal (Mead Johnson) was made available to all subjects for 7 - 10 days following surgery. During the 3 - 4 week post-operative recovery period subjects were handled daily. Subjects that exhibited spontaneous seizures or failed to resume normal feeding were sacrificed with sodium pentobarbital (100 mg/kg, *i.p.*).

Apparatus

Six circular (61 cm diameter) activity cages (BRS/LVE), as described in Chapter III, were used to measure locomotor activity during the training and testing of subjects in the cocaine-induced conditioned locomotion experiment.

Place preference conditioning was conducted in the same four identical shuttle boxes as utilized in the experiments in Chapter II.

Procedure

The effect of excitotoxic lesions of the amygdala on the classical conditioning of the locomotor stimulant effects of cocaine to a specific environment was examined in the first experiment. Both lesion and control rats were randomly assigned to 1 of 3 groups: conditioned, pseudoconditioned or control. Subjects were conditioned according to the same procedure as described in Chapter II, except that on the test day (48 hr after the final training session), subjects were placed in the locomotor cages and activity was monitored for 30 min.

Following completion of the conditioned locomotion experiment, both the lesioned and non-lesioned control (i.e. drug naive) subjects were tested for cocaine-induced CPP. The procedure for this experiment was identical to that utilized in Chapter II, except that the dose of cocaine utilized was 10 mg/kg, *i.p.*

Histology

Following the completion of the second experiment, the subjects were deeply anaesthetized with sodium pentobarbital (100 mg/kg, *i.p.*) and transcardially perfused with isotonic saline followed by 4% paraformaldehyde in phosphate-buffered (0.1 M) saline. Following the perfusion, brains remained in 4% paraformaldehyde for 24 - 48 hr before being transferred to a solution of 10% dimethylsulfoxide and 0.02% Na-azide in Na phosphate buffer (0.1 M) for 48 - 72 hr. Fifty μm coronal sections were cut on a freezing microtome and every fourth section was mounted on chrome-alum coated slides and stained with cresyl violet.

The extent of the quinolinic acid lesions was examined microscopically. Subjects that were found to possess incomplete or misplaced lesions were excluded (n=5).

Statistical Analysis

Differences in locomotor counts during the conditioning trials were evaluated using a three-way (group x lesion x trial) analysis of variance with repeated measures (Huynh-Feldt adjustment of degrees of freedom). Separate univariate analyses were also performed to evaluate potential group differences between and within lesioned and non-lesioned subjects. Reverse Helmert contrasts were performed to further assess potential group differences. The conditioned locomotion results were evaluated using a two-way analysis (group x lesion). Additional univariate analyses were performed on the lesioned and non-lesioned subjects to evaluate potential group differences. These analyses also included Reverse Helmert group contrasts to further assess these data. Post-hoc comparisons were made using Scheffe's procedure. Paired t-tests were used to assess the time spent in the drug paired environment before and after place preference conditioning. These within-subject comparisons are justified, given that the preconditioning values for the time spent on the non-start side did not differ between the two groups [$t(21) = 0.99$, n.s.]. All statistical analyses were performed using SPSS:X version.3 software (SPSS:X User's Guide, 1988).

(C) Results

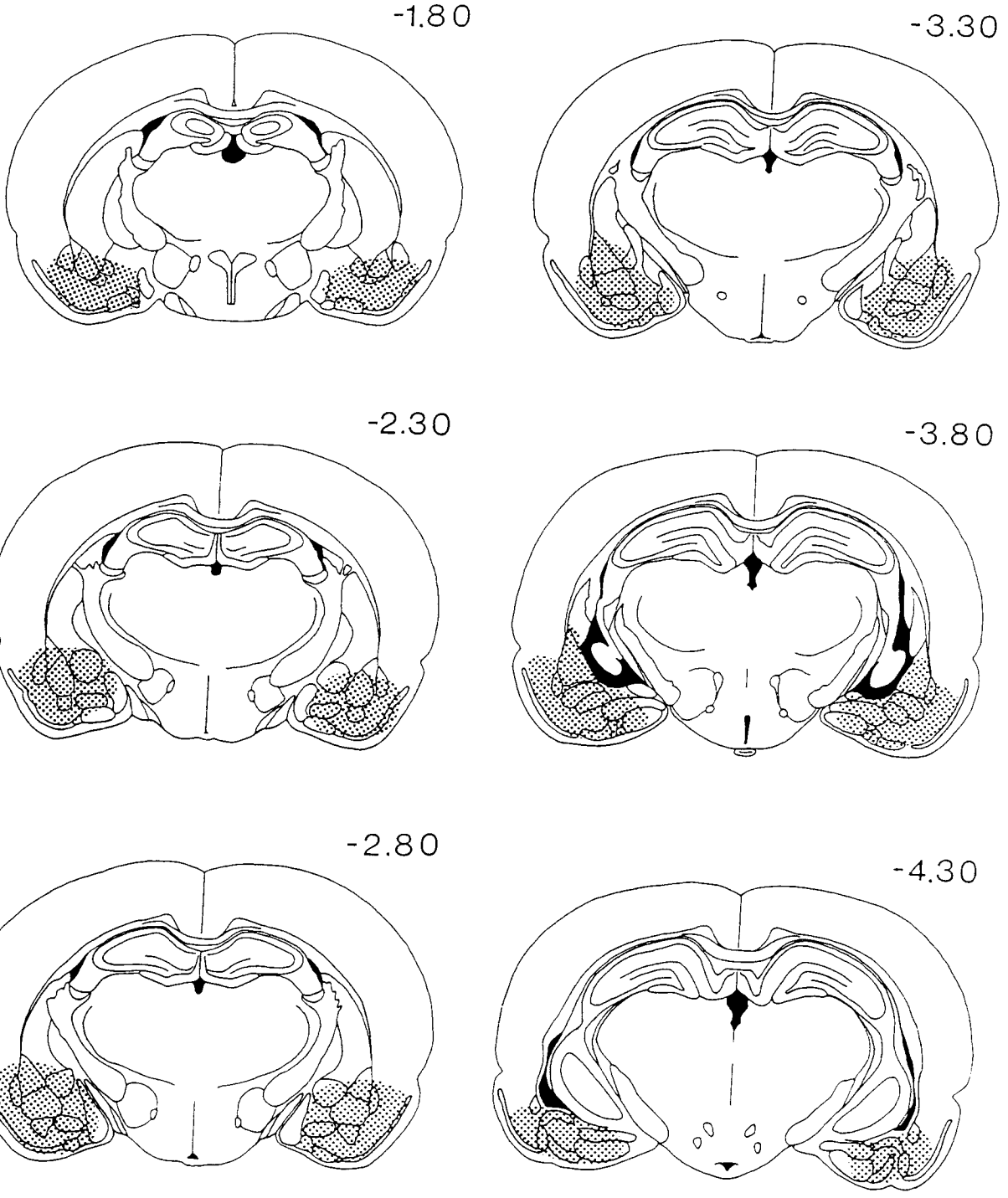
Histology

Quinolinic acid lesions produced extensive damage to the amygdaloid complex (Figure 22). A schematic representation of coronal sections from a representative lesioned subject are shown in Figure 23. Only subjects that exhibited extensive bilateral amygdala lesions were included in this study. The most rostral portion of the lesion generally included the anterior amygdaloid area, with some subjects exhibiting damage to the substriatal region and the ventral endopiriform nucleus. Generally, the central, lateral, basolateral and basomedial nuclei were almost completely destroyed in all subjects. However, the medial and cortical nuclei were partially spared in some subjects. As a result of the magnitude of these lesions, most subjects exhibited a degree of extra-amygdalar damage. Due to their close proximity to the amygdala, the dorsal and ventral endopiriform nuclei were damaged in almost all rats. A small number of subjects exhibited damage to the ventral portion of the caudate putamen. Many lesioned subjects also displayed damage to the piriform cortex. No subject included in the study appeared to possess damage to the hippocampus.

In addition to the extensive gliosis produced by the quinolinic acid lesions, many subjects exhibited enlargement of the inferior horn of the lateral ventricle. This result may have been due to liquifactive necrosis as a consequence of neuronal destruction. In a preliminary study, these ventricular enlargements were not observed in lesioned subjects that were perfused 2 - 3 weeks after surgery. This finding suggests that the 2 month interval between surgery and histology may have contributed to this result. In agreement with other investigators (Bermudez-Ratoni and McGaugh, 1991; Cahill and McGaugh, 1990; Jellestad and Cabrera, 1986), limited lesion-induced cavities were present in some subjects. This finding may also be a consequence of the long interval between surgery and histology, as subjects that were perfused 2 - 3 weeks after the infusion of quinolinic acid did not exhibit

Figure 22. Photomicrogragh of the amygdaloid region of a representative subject following infusions of quinolinic acid. This 50 μm section is approximately 2.8 mm posterior to bregma, according to the atlas of Paxinos and Watson (1986). Scale bar = 1 mm.

Figure 23. Schematic of a representative bilateral lesion of the amygdala following infusions of quinolinic acid. The stipled area represents the area of neuronal loss. The values to the upper right of each coronal section indicate the anterior/posterior distance from bregma, according to the altas of Paxinos and Watson (1986).


-1.80
-3.30
-2.30
-3.80
-2.80
-4.30

this histological feature. An additional factor worth noting is that quinolinic acid was prepared fresh before each infusion. Preliminary findings suggested that this procedure produced greater neurotoxicity than when toxin was prepared prior to surgery.

Conditioned Locomotion

Amygdala lesions did not alter basal or cocaine-induced locomotion as no significant difference in locomotor counts was observed between lesioned and non-lesioned subjects for any of the experimental groups [$F(1,60)=0.09$, n.s.] (Figure 24). Moreover, individual univariate analyses demonstrated that locomotor counts did not differ between lesioned and non-lesioned subjects that received saline, such as the control [$F(1,21)=0.75$, n.s.] or pseudoconditioned [$F(1,19)=0.00$, n.s.] groups, or the conditioned subjects that received cocaine [$F(1,20)=0.01$, n.s.]. However, a significant group effect was observed [$F(2,60)=90.34$, $p < 0.001$]. This group effect was due to the cocaine-induced locomotor counts of the conditioned subjects, as a Reverse Helmert analysis of this group effect revealed that there was no significant difference between the control and pseudoconditioned groups [$F(1,60)=0.01$, n.s.], although a highly significant difference between these control groups and the conditioned group [$F(1,60)=180.67$, $p < 0.001$] was present. A nonsignificant group x lesion interaction [$F(2,60)=0.03$, n.s.] further illustrates that the amygdala lesions failed to reliably alter locomotor activity. Although no significant effect of trials was observed [$F(6.78, 407.01)=0.79$, n.s.], the group x trial interaction was significant [$F(13.57., 401.01)=67.79$, $p < 0.001$]. Separate univariate analyses for each of the three groups demonstrated that this effect was due to a significant decrease in locomotor counts over trials for the control [$F(6.37, 133.79)=17.48$, $p < 0.001$] and pseudoconditioned [$F(8.73, 165.91)=20.63$, $p < 0.001$] groups. The conditioned subjects just failed to exhibit a significant increase in locomotor counts over trials [$F(8.06, 161.18)=1.92$, $p < 0.06$].

The conditioning procedure produced a notable conditional behavioural effect, as indicated by a significant group effect [$F(2,60)=20.87$, $p < 0.001$] (Figure 25). A Reverse

Helmert contrast further indicated that there was no significant difference between the control and pseudoconditioned groups [$F(1,60)=0.46$, n.s.], but that the conditioned groups had significantly greater locomotor counts than other groups [$F(1,60)=41.29$, $p < 0.001$]. This behavioural effect was not affected by amygdala lesions as there was no significant effect of lesion [$F(1,60)= 0.12$, n.s.] or group x lesion interaction [$F(2,60)=0.60$, n.s.]. Individual analyses demonstrated that amygdala lesions did not affect locomotor counts for control [$F(1,21)=2.19$, n.s.], pseudoconditioned [$F(1,19)=0.02$, n.s.] or conditioned [$F(1,20)=0.07$, n.s.] subjects. Moreover, separate univariate analyses demonstrated that significant group effects were apparent for both non-lesioned [$F(2,27)=17.71$, $p < 0.001$] and lesioned [$F(2,33)=7.162$, $p < 0.005$] subjects. In addition, Reverse Helmert contrasts demonstrated that control and pseudoconditioned groups do not differ significantly from each other for either non-lesioned [$F(1,27)=1.27$, n.s.] or lesioned [$F(1,33)=0.04$, n.s.] subjects, while conditioned subjects exhibited significantly more locomotor counts than these controls for both non-lesioned [$F(1,27)=34.15$, $p < 0.001$] and lesioned [$F(1,33)=14.33$, $p < 0.001$] subjects. Post-hoc comparisons revealed that both non-lesioned and lesioned conditioned subjects exhibited significantly more locomotor counts than either control or pseudoconditioned subjects ($p < 0.05$; Figure 25).

Conditioned Place Preference

Non-lesioned subjects exhibited a robust cocaine-induced CPP, as illustrated by a significant increase in the time spent on the drug-paired side of the apparatus [$t(9)=6.20$, $p < 0.001$] (Figure 26). In contrast, lesioned subjects did not exhibit an increase in time spent on the drug-paired side of the apparatus [$t(12)=0.33$, n.s.] (Figure 26), suggesting that cocaine-induced CPP was blocked by the amygdala lesions.

Figure 24. Total locomotor counts during the 10 days of locomotor conditioning for non-lesioned control (△, n=10), pseudoconditioned (□, n=10) and conditioned (o, n=10) subjects, as well as lesioned control (▲, n=13), pseudoconditioned (■, n=11) and conditioned (●, n=12) subjects. Conditioned subjects received cocaine (10 mg/kg, *i.p.*) prior to being placed in the locomotor apparatus, while pseudoconditioned and control subjects were injected with saline. Values represent the group mean ± SEM.

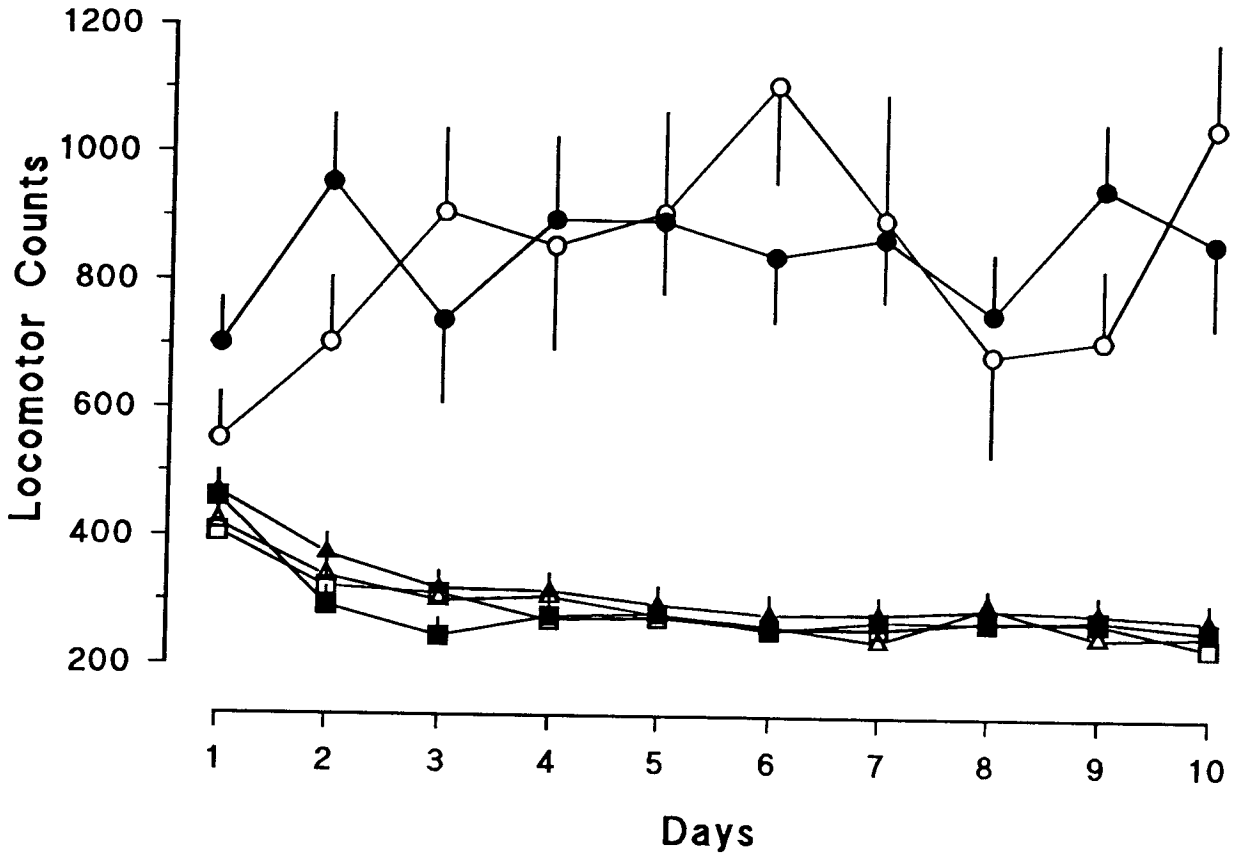

1200
1000
800
600
400
200
Locomotor Counts
1
2
3
4
5
6
7
8
9
10
Days

Figure 25. Total locomotor counts for non-lesioned (□) control (n=10), pseudoconditioned (n=10) and conditioned (n=10) subjects, as well as lesioned (■) control (n=13), pseudoconditioned (n=11) and conditioned (n=12) subjects following 10 days of conditioning with cocaine (10 mg/kg, *i.p.*). Testing occurred 48 hr after the last training session, and was conducted in the same apparatus as used for the training procedure. Values represent the group mean + SEM. * $p < 0.05$ compared to control or pseudoconditioned subjects.

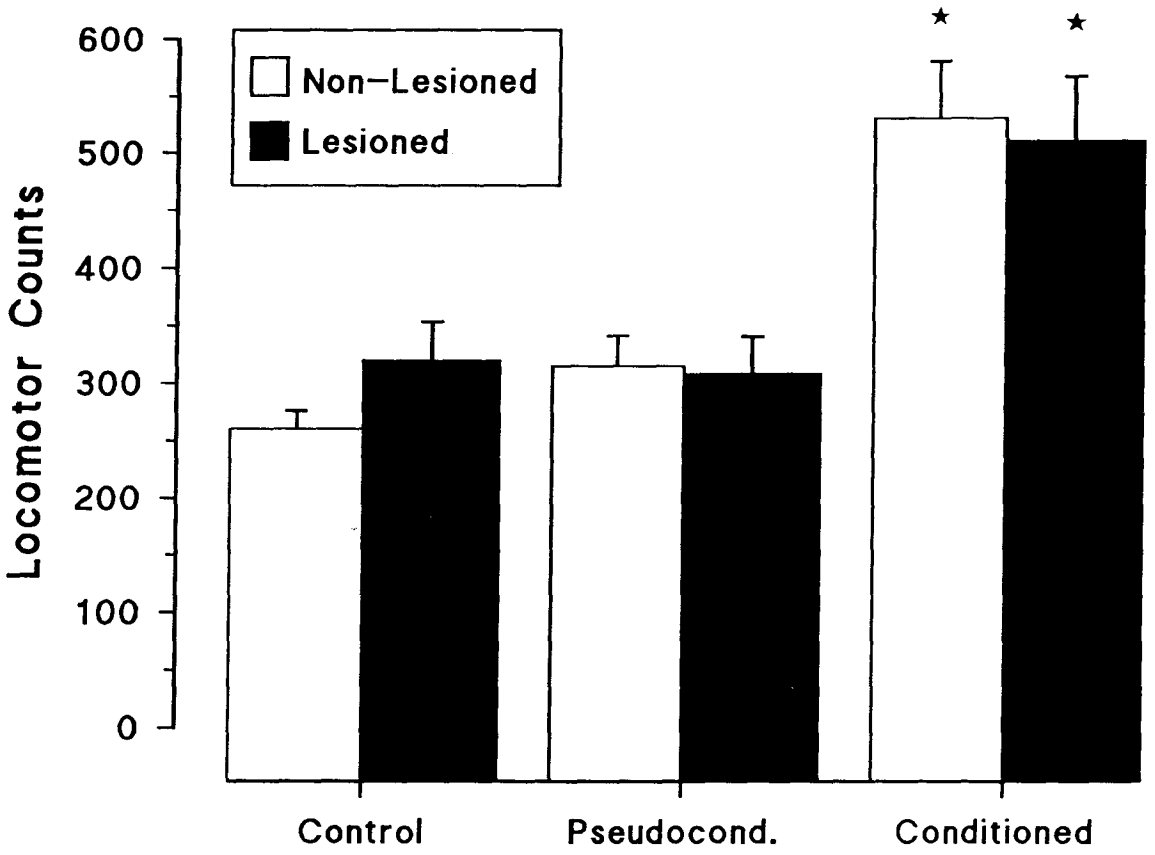
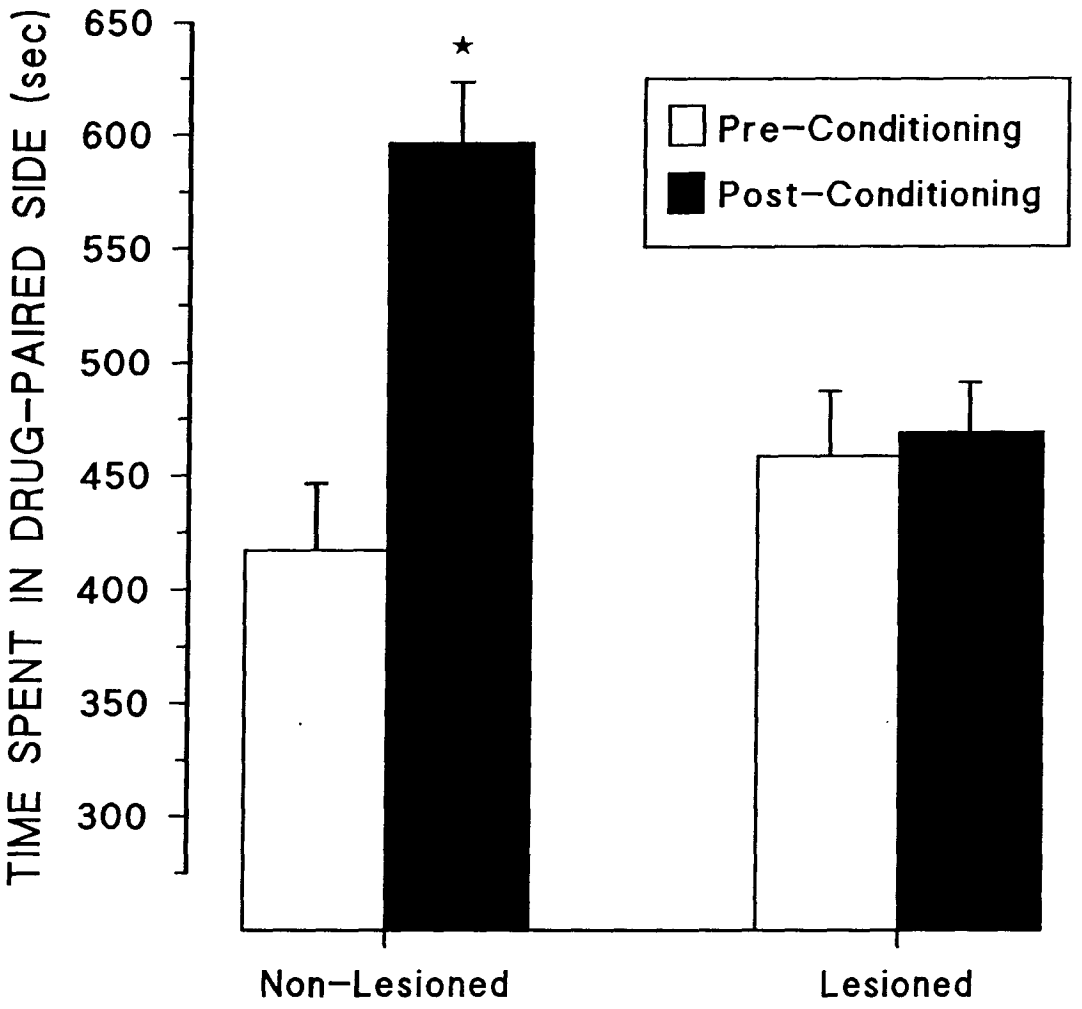

600
500
400
300
200
100
0
Locomotor Counts
Non-Lesioned
Lesioned
★
★
Control
Pseudocond.
Conditioned

Figure 26. Effect of cocaine (10 mg/kg, *i.p.*) on the time spent in the drug-paired (nonstart) compartment before (□) and after (■) conditioning for non-lesioned (n=10) and lesioned (n=13) subjects. Values represent the group mean + SEM. * indicates a significant within-group difference ($p < 0.05$) of pre- vs. post-conditioning scores.


TIME SPENT IN DRUG-PAIRED SIDE (sec)
650
600
550
500
450
400
350
300
★
Pre-Conditioning
Post-Conditioning
Non-Lesioned
Lesioned

(D) Discussion

In agreement with previous reports (Post *et al.*, 1988; Cador *et al.*, 1989), excitotoxic lesions of the amygdala did not affect basal or cocaine-induced locomotor activity. This finding indicates that the amygdaloid complex is not a substrate for the unconditioned psychomotor stimulant effects of cocaine. Lesions of the amygdaloid complex also failed to affect cocaine-induced conditional locomotion, a somewhat unexpected result given previous data implicating these nuclei in this form of classical conditioning (Chapter IV; Post *et al.*, 1988). Despite an absence of effect of these excitotoxic lesions on the unconditioned locomotor effects of cocaine, as well as cocaine-induced conditional locomotor activity, destruction of the amygdaloid complex blocked cocaine-induced CPP.

Conditioned Locomotion

The failure of amygdala lesions to alter cocaine-induced conditional locomotion was unanticipated given that electrolytic and 6-OHDA lesions of the amygdala have been reported to greatly attenuate environment-specific cocaine-sensitization (Post *et al.*, 1988) and that exposure to a cocaine-paired environment produces an increase in *c-fos* expression in the amygdala (Chapter IV). Moreover, a large body of evidence strongly implicates various nuclei of the amygdala in stimulus-reward learning (Weiskrantz, 1956; Jones and Mishkin, 1972; Mishkin and Aggleton, 1981; Gaffan and Harrison, 1987; Cador *et al.*, 1989; Everitt *et al.*, 1991; Hiroi and White, 1991a; Kentridge *et al.*, 1991). The second experiment demonstrated that cocaine-induced CPP was blocked by excitotoxic lesions of the amygdala, indicating that destruction of the amygdala can affect certain forms of cocaine-induced conditioning.

One explanation for the failure of amygdala lesions to affect cocaine-induced conditional locomotion relates to the learning demands of this paradigm. Although lesions of the amygdaloid complex clearly can produce impairments in learning of stimulus-reward

associations (Weiskrantz, 1956; Jones and Mishkin, 1972; Gaffan and Harrison, 1987; Cador *et al.*, 1989; Kesner *et al.*, 1989; Cahill and McGaugh, 1990; Kentridge *et al.*, 1991), the deficits are evident only in certain situations. For example, both Weiskrantz (1956) and Jones and Mishkin (1972) have noted that amygdala lesions do not completely or irreversibly block the learning of all stimulus-reward associations. Specifically, Jones and Mishkin (1972) suggest that "... only when associative learning demands are high, as in discrimination reversal, for example, will the animal show a severe and prolonged impairment." There is considerable experimental support for this proposal as many investigators have reported that amygdala lesions fail to produce substantial deficits in certain tasks that are based on the formation of associations between specific stimuli and biologically relevant events (Schwartzbaum, 1965; Pelligrino, 1968; Shuckman *et al.*, 1969; Slotnick, 1985; Cahill and McGaugh, 1990; Kentridge *et al.*, 1991). It is noteworthy that the conditioned locomotion procedure utilized in the present study involved multiple cocaine-environment pairings, and required a rudimentary discrimination involving multimodal stimuli (homecage versus testcage), and a contextual conditioned stimulus for cocaine administration. Although no single critical factor appears to predict if amygdala lesions will produce deficits in learning, the design of the present conditioned locomotion experiment was not biased in favor of observing a lesion effect, given the long post-operative recovery period before conditioning, the relative simplicity of the task and the number of conditioning trials. Similar factors may also contribute to the discrepancy between the present findings and those of Post *et al.* (1988), who reported that amygdala lesions attenuate environment-specific cocaine sensitization. These investigators used a one-trial learning paradigm, which may have biased their result in favor of observing a lesion effect. It is also important to note that this interpretation of the present results does not preclude the possibility that the amygdala is involved in this form of conditioning in non-lesioned subjects, given that recovery of function via other structures and pathways is possible (Jones and Mishkin, 1972).

Conditioned Place Preference

Although lesions of the amygdaloid complex failed to attenuate cocaine-induced conditional locomotion, the destruction of this region blocked cocaine-induced CPP. This result is in agreement with previous reports that destruction of specific amygdaloid nuclei attenuate amphetamine- (Hiroi and White, 1991a) and food- (Everitt *et al.*, 1991) induced CPP. Although the findings of Hiroi and White (1991a) and Everitt *et al.* (1991) implicate different amygdaloid nuclei as being critically involved in CPP, the extensive pathways between amygdaloid nuclei (Krettek and Price, 1978; Nitecka *et al.*, 1981; Ottersen, 1982; Aggleton, 1985; Smith and Millhouse, 1985) suggest that several nuclei may be involved in the learning of associations between specific stimuli and biologically relevant events. For example, in addition to the well documented role of the central nucleus in conditioned fear (Applegate *et al.*, 1982; Pascoe and Kapp, 1985; Hitchcock and Davis, 1986, 1987; Davis, 1992), it has recently been demonstrated that the lateral and/or basolateral nuclei of the amygdala play a role in fear-potentiated startle responses (Sananes and Davis, 1992). The importance of a given nucleus in stimulus-reward associations may depend on the modality of the incoming sensory information, the specific demands of the associations and the characteristics of the response. Although the present findings demonstrate that lesions of the amygdala abolish cocaine-induced CPP, it cannot be determined from the present results if this effect is due to the blockade of the aquisition or expression of conditioning. Hiroi and White (1991a) have obtained data that are consistent with a role for the amygdala in the expression of amphetamine-induced CPP, as well as a potential role in the acquisition of conditioning. This is consistent with other data showing a role of the amygdala in both the acquistion and expression of stimulus-reward associations (Jones and Mishkin, 1972; Mishkin and Aggleton, 1981; Murray, 1991; Davis, 1992; Helmstetter, 1992). The present CPP data support the proposal that nuclei within the amygdaloid complex can play an essential role in the association of environmental stimuli with reward.

The Role of the Amygdala in Cocaine-induced Conditioning

Although both cocaine-induced conditional locomotion and CPP involve the formation and expression of environmental stimulus-drug associations, only cocaine-induced CPP was affected by amygdala lesions. This differential effect may reflect the distinct learning demands of the two paradigms. For example, subjects in the CPP experiment were required to discriminate between two highly similar environments, while subjects in the conditioned locomotion experiment were only required to discriminate a novel environment from their homecage. However, these results may also reflect the fact that stimulant-induced conditional locomotion and CPP are behaviourally distinct phenomena, and hence could be subserved by different neural circuits. Stimulant-induced conditional locomotion is clearly a form of classical conditioning, with the conditioned response resembling the unconditioned response. The conditioned response of CPP, however, appears to be a form of approach/orienting behaviour and does not resemble the unconditioned response to cocaine, and hence cannot be explained in terms of traditional Pavlovian conditioning (Wise, 1989). It is noteworthy that a recent study has demonstrated that lesions of the central nucleus of the amygdala can differentially affect different classes of appetitively conditioned behaviours (Gallagher *et al.*, 1990). Specifically, destruction of the central nucleus impaired the acquisition of conditioned orienting responses, but produced no deficit in the conditioning of behaviours originally evoked by the unconditioned stimulus. The differential effect of amygdala lesions on conditional locomotion and CPP is remarkably similar to these findings, and may provide support for the proposal that the acquisition of these two classes of appetitively conditioned responses are subserved by distinct neural mechanisms (Holland, 1984). Taken as a whole, the data from these experiments support the proposal that nuclei within the amygdaloid complex can play a role in the association of environmental stimuli with reward. However, the importance of this structure appears to be dependent on the demands of the learning paradigm and/or the responses examined.

VI. General Discussion

The preceding chapters have presented the findings of experiments that have assessed various aspects of the neurobiology of cocaine's behavioural actions in relation to its abuse potential. The first series of experiments (Chapter II) evaluated the behavioural and neurochemical interactions between buprenorphine and cocaine. The resulting data suggested that buprenorphine, like other opioids, can increase the rewarding effects of cocaine. Moreover, the ability of buprenorphine to potentiate the cocaine-induced increases in interstitial DA in the nucleus accumbens provides a potential explanation for this behavioural effect.

Given the large body of evidence that implicates the mesolimbic dopaminergic system in the unconditioned behavioural effects of cocaine, the second series of experiments (Chapter III) examined whether stimuli previously paired with cocaine administration can elicit increases in interstitial DA in the nucleus accumbens that are similar to the unconditioned effects of this drug. When administered acutely, cocaine produced a potent unconditioned increase in interstitial DA concentrations in the nucleus accumbens. Although repeated pairing of cocaine with a specific environment produced conditioned locomotion upon subsequent presentation of that environment, there was no concomitant conditional increase in DA release. These data do not support the hypothesis that stimuli paired with cocaine produce their behavioural effects by eliciting similar neurochemical effects as cocaine.

To further elucidate the neurobiology of cocaine-induced environment-specific conditioning, expression of Fos, a putative marker of neuronal activity, was examined in the forebrain of rats exposed to an cocaine-paired environment (Chapter IV). Consistent with its stimulant actions, cocaine produced an increase in locomotion that was accompanied by an increase in Fos expression within specific limbic regions (cingulate cortex, claustrum, piriform cortex, lateral septal nucleus, paraventricular nucleus of the thalamus, lateral habenula, and amygdala), as well as the basal ganglia (dorsomedial striatum and nucleus

accumbens). In agreement with our previous results, exposure of rats to the cocaine-paired environment produced a conditional increase in locomotion. In addition to this behavioural effect, conditioned subjects exhibited a significant increase in the number of Fos-positive neurons within the cingulate cortex, claustrum, lateral septal nucleus, paraventricular nucleus of the thalamus, lateral habenula and the amygdala, suggesting increased neuronal activity within these regions. In contrast to the dramatic effects observed in these structures, no conditional activation was observed within the piriform cortex, nucleus accumbens, or dorsal striatum, suggesting that these brain regions are not involved in the conditioned locomotor response. These findings suggest that specific limbic regions exhibit increased neuronal activation during the presentation of cocaine-paired cues and may be involved in the formation of associations between cocaine's stimulant actions and the environment in which the drug administration occurred. Although the nucleus accumbens is necessary for the reinforcing and locomotor effects of cocaine, it did not exhibit a conditional Fos response, further suggesting that different neural circuits are involved in the unconditioned and conditioned locomotor effects of cocaine.

The final series of experiments (Chapter V) evaluated the role of the amygdaloid complex in cocaine-induced conditional locomotion and CPP. Quinolinic acid lesions of the amygdaloid complex did not affect basal or cocaine-induced locomotion, suggesting that the amygdala does not mediate the unconditioned psychomotor stimulant effects of this drug. Preconditioning lesions also failed to affect cocaine-induced conditional locomotion. This lack of effect was contrasted by a complete blockade of cocaine-induced CPP by the amygdaloid lesions. These data demonstrate that cocaine-induced stimulus-reward conditioning can be differentially affected by lesions of the amygdala.

The impetus for the first series of experiments (Chapter II) was a report that buprenorphine suppresses cocaine self-administration by rhesus monkeys, and therefore might be useful in the pharmacotherapy of cocaine abuse (Mello *et al.*, 1989). However, the present findings, as well as the results of two recent double-blind, controlled studies

(Johnson *et al.*, 1992; Kosten *et al.*, 1992), suggest that the buprenorphine will not be an effective pharmacotherapy for cocaine abuse. In addition to the aforementioned studies that have examined the efficacy of buprenorphine in the treatment of cocaine abuse, numerous clinical and preclinical studies have evaluated a variety of drugs as potential pharmacotherapies for cocaine abuse. Although it is possible that this approach may result in the discovery of a "magic bullet" for treating cocaine abuse, present understanding of the neurobiology of cocaine's behavioural actions suggests that this goal is unlikely to be realized, particularly given the theoretical orientation of the majority of recent investigations, as will be discussed.

As discussed previously, a large body of evidence indicates that the mesolimbic DA system plays a fundamental role in the reinforcing properties of cocaine (Di Chiara and Imperato, 1988; Fibiger *et al.*, 1992; Fibiger and Phillips, 1987; Johanson and Fischman, 1989; Roberts *et al.*, 1977, 1989). Accordingly, the majority of preclinical and clinical studies of potential pharmacotherapeutic treatments for cocaine abuse have been directed at altering dopaminergic transmission. However, the normal biological function of the mesolimbic dopaminergic projection appears to be intimately involved in the rewarding and/or incentive motivational effects of natural stimuli such as food and sex (Fibiger, in press; Fibiger and Phillips, 1987; Nomikos *et al.*, in preparation; Pfaus *et al.*, 1990; Wise and Rompre, 1989). Therefore, drug therapies that block or attenuate dopaminergic transmission, although highly effective in reducing the reinforcing effects of cocaine in both rats and non-human primates (Bergman *et al.*, 1990; Roberts *et al.*, 1989), also decrease the rewarding or hedonic value of natural stimuli (Fibiger, in press; Fibiger and Phillips, 1987; Willner *et al.*, 1991). This hypothesis is supported by the findings that neuroleptics produce dysphoria in normal subjects (Belmaker and Wald, 1977; Heninger *et al.*, 1965; Simonsone, 1964; Willner, 1983) and that these DA receptor antagonists are considered unacceptable by patients seeking treatment for cocaine abuse (Gawin, 1986; Gawin and Kleber, 1986; Sherer *et al.*, 1989). As the mesolimbic DA system plays a critical role in the reinforcing effects of

both cocaine and natural stimuli, such as sex and food, it is improbable that a drug can be developed that will decrease the actions of cocaine, without also attenuating the hedonic and/or incentive motivational properties of natural reinforcers.

As DA receptor antagonists appear to be unsuitable in the pharmacotherapy of cocaine abuse, researchers have also examined the potential use of both direct and indirect DA receptor agonists to treat cocaine abuse, hypothesizing that they could be utilized in a manner similar to methadone in the treatment of heroin abuse. One obvious problem with this approach is that many indirect DA agonists, such as amphetamine, methylphenidate, GBR 12909 and mazindol, also possess considerable abuse liability (Bergman *et al.*, 1989; Johanson and Fischman, 1989; Mansbach and Balster, 1993; Ritz *et al.*, 1987; Roberts, 1993; Sannerud and Griffiths, 1992). The use of these cocaine-like drugs is also problematic insofar as they may act as powerful cues that could lead to increased drug craving, and therefore a return to drug use (Jaffe *et al.*, 1989; O'Brien *et al.*, 1992; Stewart *et al.*, 1984).

In addition to the aforementioned practical difficulties in the use of dopaminergic agonists in the pharmacotherapy of cocaine abuse, the theoretical basis for this approach is highly questionable. The basic premise behind this hypothesis is that cocaine craving is the consequence of a decrease in dopaminergic transmission as a result of abstinence from cocaine (Dackis and Gold, 1985; Spealman, 1992). This hypothesis is based on a withdrawal model of drug abuse, similar to those that have been proposed to explain opioid abuse (Wise and Bozarth, 1987). The general inadequacies of these models is discussed extensively by Wise and Bozarth (1987), and therefore only the principal flaw of this model in relation to cocaine abuse will be reviewed.

A clear prediction of the DA depletion hypothesis of cocaine abuse (Dackis and Gold, 1985) and other withdrawal theories is that craving should be alleviated by taking cocaine. In contrast, clinical and laboratory results indicate that craving for cocaine is reported to be most intense following drug use, while blood levels of cocaine are still elevated (Gawin and Kleber, 1986; Jaffe *et al.*, 1989). It is noteworthy that similar results

have also been reported for heroin (Meyer and Mirin, 1979). These findings are in agreement with results that indicate that drug self-administration is stimulated by the presence of the drug, not its absence (De Wit and Stewart, 1981, 1983; Gerber and Stretch, 1975; Stewart *et al.*, 1984; Wise *et al.*, 1990). These data clearly fail to provide support for the use of DA receptor agonists to treat cocaine abuse. In summary, the aforementioned data suggest that because the mesolimbic DA system is intimately involved in the reinforcing and/or incentive motivational properties of natural rewarding stimuli, drugs that attenuate cocaine's primary rewarding effects are likely to interfere with the rewarding properties of natural reinforcers, and hence be unacceptable in the treatment of substance abuse. Also, there is virtually no support for the hypothesis that treatment with cocaine-like drugs will decrease craving for cocaine; on the contrary, these drugs appear to increase cocaine craving.

Although the DA projection to the nucleus accumbens is strongly implicated in the reinforcing properties of cocaine, the neurobiology of cocaine-induced conditioned responses is largely unknown. As discussed previously, this aspect of cocaine's action appears to be an important component in its abuse liability (Gawin, 1991; O'Brien *et al.*, 1992). In addition to its potential clinical importance, a better comprehension of the neurobiology of this phenomenon may also directly contribute to our understanding of the neural circuitry involved in the classical conditioning of natural rewards to environmental stimuli. Taken as a whole, the present findings (Chapters III, IV and V) suggest that the neural circuits underlying the conditioned response to the presentation of cocaine-paired stimuli differ somewhat from those responsible for the unconditioned response to cocaine. Moreover, these data suggest that limbic structures involved in the stimulus-reward conditioning of natural reinforcers may also be involved in cocaine-induced conditioned responses.

The apparent DA-independent nature of the conditioned locomotor response to cocaine-paired stimuli (Chapter III and IV) has potential implications for the treatment for cocaine abuse; however, the generalizability of these findings to other abused substances and

natural reinforcers is not clear. Although there is strong support for a dopaminergic role in the acquisition of various stimulus-reward associations (Beninger and Hahn, 1983; Beninger and Herz, 1986; Beninger and Phillips, 1980; Hiroi and White, 1989; Horvitz and Ettenberg, 1989; Spyraki *et al.*, 1982b; Weiss *et al.*, 1989), the role of DA in the expression of these associations remains uncertain. In support of a non-dopaminergic mechanism for the expression of these conditioned behaviors, the presentation of stimuli that signal the availability of food have not been found to increase interstitial DA concentrations in the nucleus accumbens, as measured by *in vivo* microdialysis, despite increases in locomotor activity or lever pressing (Hernandez and Hoebel, 1988; Nomikos *et al.*, in preparation; Radhakishun *et al.*, 1988). In agreement with these data, the DA receptor antagonist pimozide fails to block the conditioned locomotor response to the presentation of food-paired stimuli (Horvitz and Ettenberg, 1991). Moreover, responding for conditioned rewarding stimuli is unaffected by 6-OHDA lesions of the nucleus accumbens, leading the authors to suggest that "the information about the conditioned reinforcer is not directly dependent upon activity in the ventral striatal DA system" (Robbins *et al.*, 1989). The results from these conditioning studies employing non-drug reward are in agreement with the present results (Chapter III and IV), as well as previous studies that have failed to observe conditional dopaminergic activity following conditioning with a variety of drugs (Barr *et al.*, 1983; Finlay *et al.*, 1988; Möller *et al.*, 1987; Walter and Kuschinsky, 1989).

In contrast to those studies that suggest that conditioned stimuli can activate behavior through non-dopaminergic pathways, Blackburn *et al.* (1989b) found that presentation of discrete stimulus that predicted the availability of food produced an increase in the DOPAC/DA ratio, which suggests an increase in DA turnover. Moreover, responding to this conditioned stimulus was reduced following administration of DA receptor antagonists (Blackburn *et al.*, 1987, 1989a). The activity of DA neurons have also been demonstrated to increase in anticipation of a food reward; however, this apparent conditional response decreases with continued training (Ljungberg *et al.*, 1992). Based on the ability of intra-

accumbens α-flupenthixol to attenuate the expression of amphetamine-induced CPP, Hiroi and White (1990) suggest that "when animals encounter environmental cues which have previously been paired with a primary reward, dopamine is released in the nucleus accumbens". These authors have also reported that systemically administered D1 and D2 receptor antagonists block the expression of amphetamine-induced CPP (Hiroi and White, 1991b). Finally, the local administration of amphetamine or DA into the nucleus accumbens can increase responding for a stimulus previously paired with a natural reward, suggesting that DA can affect this conditioned response (Taylor and Robbins, 1984, 1986; Robbins *et al.*, 1989). In summary, these data suggest that dopaminergic transmission can affect the expression of responses to stimuli previously paired to primary rewards.

Although there is support for the hypothesis that the expression of conditioned stimulus-reward associations is not dependent on an increase in dopaminergic transmission in the nucleus accumbens, other data appear to suggest that DA does play a critical role in the conditioned stimulus activation of these behaviors. A number of factors may account for these discrepancies, such as differences in the learning paradigms or responses measured (*e.g.* responding for a conditioned reinforcer vs. conditioned locomotion vs. CPP), the nature of the unconditioned reward (*e.g.* drug vs. non-drug) or the characteristics of the conditioned stimulus (*e.g.* explicit vs. contextual cues), and clearly future studies should attempt to evaluate the importance of these specific considerations. It is possible that increased DA release is a critical component for the expression of certain forms of stimulus-reward learning, while others are independent of an increase in dopaminergic transmission. However, it should also be noted that some of the data that have been proposed to indicate that enhanced DA release is essential for the expression of stimulus-reward associations do not provide direct support for this hypothesis. Specifically, the ability of DA receptor antagonists to reduce or block a behavioural response does not provide evidence that *enhanced* DA release is associated with the production of the behaviour; rather, these data simply suggest that there is a DA-dependent component to the expression of these

behaviours. The competitive blockade produced by DA receptor antagonists suggests that these drugs may produce their largest effects when dopaminergic transmission is reduced, not elevated. Therefore, the reduction of a conditioned response following the administration of a DA receptor antagonist may reflect the attenuation of necessary modulatory effects of basal DA release. Specifically, the blockade of dopaminergic transmission may alter the efficacy of transmission of neural signals from the amygdala or subiculum, for example, to the subpallidal area via the nucleus accumbens. This proposal is compatible with electrophysiological studies that provide evidence for a neuromodulatory role of DA in the nucleus accumbens (Yim and Mogenson, 1982, 1986, 1988). A similar hypothesis has been proposed to account for the finding that 6-OHDA lesions block the facilitation of responding for a conditioned reinforcer produced by intra-accubmens amphetamine, but fail to affect basal responding for the conditioned stimulus (Robbins *et al.*, 1989). Specifically, Robbins and colleagues (1989) have proposed that the information regarding the association between the primary reward and the conditioned stimulus is dependent on projections from the amygdala and other limbic structures, such as the subiculum and the prefrontal and entorhinal cortex, to the nucleus accumbens, whereas the mesolimbic dopaminergic projection plays a modulatory role, enhancing the performance of those behaviours resulting from presentation of the conditioned stimulus. Despite the fact that the ability of neuroleptics to affect the performance of conditioned response does not necessarily indicate enhanced dopaminergic transmission, the fact remains that additional results also provide evidence for conditional dopaminergic activity (Blackburn *et al., 1989*b; Ljungberg *et al.*, 1992). Future studies using *in vivo* techniques, such as microdialysis, should be utilized to resolve these discrepancies by directly examining if enhanced DA release is associated with the performance of specific conditioned behaviors.

Given the aforementioned findings that indicate that stimuli paired to either drug or natural rewards can apparently produce conditioned responses in the absence of increased DA release in the nucleus accumbens and perhaps through non-dopaminergic pathways, the

cocaine-induced conditional responses examined in the present studies (Chapters II, IV and V) may be mediated by the same neural mechanism involved in the classical conditioning of natural rewards to environmental stimuli (Robbins *et al.*, 1989). This proposal is further supported by the fact that the presentation of a cocaine-paired environment increased Fos expression in the amygdala and that lesions of the amygdala blocked cocaine-induced CPP, which are in agreement with a large body of evidence that suggests the amygdala is involved in stimulus-reward learning (Weiskrantz, 1956; Jones and Mishkin, 1972; Mishkin and Aggleton, 1981; Gaffan and Harrison, 1987; Cador *et al.*, 1989; Everitt *et al.*, 1991; Hiroi and White, 1991a; Kentridge *et al.*, 1991). To further evaluate this proposal the direct examination of single- or multi-unit activity in the amygdala and other regions implicated in stimulus-reward associations could be examined during the acquisition and expression of both cocaine- and natural reward-induced conditional behaviours. This examination during responding for a cocaine-paired stimulus would also be a highly worthwhile area of investigation. One distinct advantage of the use of this paradigm is that the data from previous studies that have used natural rewards, such as food, water and receptive sexual partners, could be directly compared to the results obtained using cocaine as the reward (Robbins *et al.*, 1989). Moreover, the use of a different paradigm would allow for the examination of the generalizability of the present findings to other forms of stimulus-reward conditioning.

Cocaine-related stimuli can elicit conditioned responses, including drug-like physiological changes and reports of craving, in individuals who abuse cocaine (Ehrman *et al.*, 1992; O'Brien *et al.*, 1992). Although craving is a subjective state, it appear to be highly correlated to verbal and physiological measures (Pickens and Johanson, 1992). Assuming that the neural mechanisms involved in the conditioned responses of cocaine abusing individuals and the subjects in the present studies are similar, the proposed neural mechanisms underlying the cocaine-induced conditioned responses provide a potential framework for understanding cocaine craving, as well as predictions regarding treatment.

The DA-independent nature of the conditioned response suggests that craving should not necessarily be associated with euphoria or positive feelings elicited by cocaine itself. This prediction is supported by the finding that less than half of the subjects who reported increases in craving in response to cocaine related cues indicated that this was associated with feelings of a "cocaine high" (Ehrman *et al.*, 1992). A related prediction is that cocaine craving should be relatively resistant to neuroleptic treatment. Although no published reports have directly examined this possibility, it remains a testable hypothesis for future investigation.

The other major conclusion of the present findings, as discussed previously (Chapters III, IV and V), is that cocaine-induced conditional responses are potentially subserved by the same neural mechanisms involved in the formation of associations between specific stimuli and other classes of biologically relevant events. This suggests that the established principles of classical conditioning should also be applicable to cue-induced cocaine craving. Although this has not been directly investigated, the importance of generalization and spontaneous recovery in extinction-based treatment of cocaine abusing individuals has been recognized (Hammersley, 1992; O'Brien *et al.*, 1992). However, the profound resistance to extinction exhibited by chronic cocaine users related to the presentation of cocaine related cues appears unusually protracted (O'Brien *et al.*, 1992). Although this pattern of extinction may simply reflect the large number of cocaine-stimulus pairings that have occurred prior to presentation for treatment, this uncharacteristic resistance to extinction may alternatively reflect the supraphysiological stimulation of the meso-accumbens pathway produced by cocaine during the acquisition of this stimulus-reward association. Future studies that evaluate the relationship between dopaminergic stimulation during the acquisition of stimulus-reward associations and its subsequent effect on rate of extinction and number of spontaneous recoveries will directly address this hypothesis. Finally, the present hypothesis concerning the neurobiology of cocaine-induced conditioned responses would suggest that the development of a pharmacotherapy to reduce cravings is unlikely. If cravings are the

result of the same neural processes involved in the expression of other stimulus-reward associations, it is highly improbable that a drug will be developed to "erase" the memory of cocaine.

VII. References

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