INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

UMI

A Bell & Howell Information Company
300 North Zeeb Road, Ann Arbor MI 48106-1346 USA
313/761-4700 800/521-0600

A Study of Relapse to Cocaine Seeking in the Rat

Suzanne Erb

A Thesis

in

The Department

of

Psychology

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

September, 1996

Abstract

A Study of Relapse to Cocaine Seeking in the Rat

Suzanne Erb

The primary objective of this study was to determine whether brief exposure to a stressor would provoke relapse to cocaine-trained behaviour and, if so, whether such an effect could be blocked pharmacologically. Rats were initially trained to self-administer cocaine HCl (1.0 mg/kg/infusion, IV; one 3-hour session/day; 9-12 days). Subsequently, extinction conditions were introduced in which lever-pressing resulted in IV infusions of saline rather than of cocaine. Extinction conditions were maintained until animals made 15 or fewer responses in the 3-hour session, after which they received saline infusions at the start of each session until they made 10 or fewer responses in 3 hours. Subsequently, animals were tested for reinstatement of responding for saline infusions following a non-contingent injection of cocaine (2.0 mg/kg, IV) and exposure to intermittent footshock (10 min, 0.5 mA, 0.5 sec on, mean off period of 40 sec). In Experiment 1, footshock stress induced reinstatement of cocaine-trained behaviour after prolonged extinction and after a 4- to 6-week drug-free period; an effect comparable to that induced by a priming injection of cocaine. In Experiment 2, animals were given saline, cocaine, and footshock tests for reinstatement 20 min after pretreatment with saline and diazepam (0.75 or 1.5 mg/kg, IP). In this experiment, diazepam attenuated footshock- but not cocaine-induced reinstatement of cocaine-trained behaviour. These findings suggest that the neurochemical events mediating footshock- and cocaine-induced reinstatement of cocaine-trained behaviour are pharmacologically dissociable.

Acknowledgments

I would like to thank my supervisor Dr. Jane Stewart whose wisdom, constant support and encouragement, and willingness, at any time, to discuss problems and ideas I value highly. My sincerest thanks also to Yavin Shaham for providing me with the training and skills necessary to conduct the experiments and for continued willingness to answer questions and provide helpful criticism. My appreciation to the members of my committee, including Drs. Roy Wise, Barbara Woodside, and David Roberts. Thank you also to Demetra Rodaros for assisting with the surgeries. And finally, my thanks to Doug Funk for excellent talks, helpful suggestions, and good humour.

Table of Contents

List of Figures

A Study of Relapse to Cocaine Seeking in the Rat

Cocaine is among the most powerful reinforcers known and is considered to be one of the major drugs of abuse. Cocaine abuse is viewed as a pervasive public health problem and as such, it has become a highly-prioritized area of research, both clinically and preclinically.

Substance abuse or drug addiction, in general, can be defined as the habitual and compulsive self-administration of a drug (Jaffe, 1985). Although this definition is of limited utility in that it *describes* but does not *explain* drug addiction, it does address an essential aspect of addictive behaviour. Specifically, adjectives such as habitual and compulsive imply that individuals who abuse drugs are exposed repeatedly and chronically to them, and it is well-known that the effects of drugs can and do change with repeated exposure (Holman, 1994). The idea that drug effects change with repeated exposure is particularly relevant to the study of cocaine abuse. The transition from initial to chronic cocaine use is associated with the emergence of a number of characteristic pharmacological, psychological and behavioural effects; these long-term effects are important not only when considering the processes involved in the maintenance of the addictive behaviour, but also when considering the processes involved in the relapse to cocaine seeking after a period of abstinence.

Initial Cocaine Exposure: Acute effects

The retrospective self-reports of cocaine addicts, as well as information obtained from laboratory studies conducted with non-addicted cocaine users,

indicate that initial exposure to cocaine is associated with an elevated subjective sense of well-being (Gawin, 1991; Gawin & Ellinwood, 1988; Kumor, Sherer, Gomez, Cone & Jaffe, 1989; Van Dyke, Ungerer, Jatlow, Barash, & Byck, 1982). Users experience a non-distorted enhancement in the intensity of all normal pleasures, including heightened sexual feelings, increased interest in interpersonal relations, and correspondingly lowered social inhibitions. Initial cocaine exposure is also associated with increases in alertness and self-confidence and decreases in anxiety.

In the laboratory, monkeys and rats will rapidly learn to press a lever for intravenous (IV) infusions of cocaine; under a continuous schedule of reinforcement, in which each lever press results in an infusion of cocaine, animals will readily adopt a pattern of continuous and steady-state drug-taking behaviour (Roberts, 1992; see also Wise & Bozarth, 1987). Additionally, rats have been shown to exhibit a preference for a place paired with cocaine (5 .0 and 15.0 mg/kg) over a place paired with saline after just two conditioning trials (Gong, Neill, & Justice, 1996).

Cocaine is a psychostimulant drug that acts principally at norepinephrine (NA), serotonin (5-HT) and dopamine (DA) neuronal synapses. The drug inhibits neurotransmitter reuptake into the terminal, thereby interfering in the critical processes responsible for neurotransmitter inactivation (Roberts, 1992).

Although cocaine affects a number of neurotransmitter systems, the reinforcing effects of the drug have been attributed primarily to its effects on DA neurotransmission of the mesocorticolimbic system; in particular, the pathway originating in the ventral tegmental area (VTA) and terminating in the nucleus accumbens (NAcc) has been implicated in the reinforcing effects of cocaine

(Wise, 1982; Wise, 1984; Wise, 1989; Wise, Newton, Leeb, Burnette, Pocock, & Justice, 1995; Wise & Rompre, 1989; Wise, Spindler, de Wit, & Gerber, 1978). More recently, it has been shown that cocaine binds to the DA transporter. This binding results in a blockade of DA uptake and a subsequent potentiation of DA neurotransmission, particularly in mesocorticolimbic pathways (Kuhar, Ritz, & Boja, 1991; Ritz, Lamb, Goldberg, & Kuhar, 1987). The end result of these pharmacological actions is an increase in the extracellular concentration of DA in terminal limbic regions, such as the NAcc, that is available for post-synaptic binding and excitation.

Long-term Cocaine Exposure: Chronic effects

The transition from initial to chronic cocaine use is associated with a variety of psychological and behavioural changes (see Anthony, Tien, & Petronis, 1989; Gawin, 1991; Gawin & Ellinwood, 1988). As a cocaine user administers the drug with increasing frequency and regularity, a transition from occasional intermittent use to high-dose bingeing occurs. Each binge can last from 4 to 24 hours, with the number of binges per week ranging from one to seven. Users learn that high doses of the drug intensify the euphoria it produces; therefore, in the absence of self-imposed or external resource restrictions, the user will generally consume higher and higher doses of the drug. During binges, all thoughts tend to be focused on the euphoria induced by the drug; the user often withdraws socially, and the emergent negative effects of the drug, such as loss of sleep, lack of nourishment, anxiety, and withdrawal from family and friends, go unrecognized.

In animal studies of cocaine abuse, rats and monkeys have been reported to exhibit continuous cocaine self-administration (Gerber & Wise, 1989; Roberts,

1992). When given unlimited access to cocaine, rats will self-administer the drug to the point of death. Under these conditions of free access to the drug, rats appear to die not directly from an overdose, but rather from sleep deprivation and weight loss (Bozarth & Wise, 1985).

Animal studies of cocaine abuse have been extremely valuable for identifying neurochemical and behavioural changes that occur with repeated and chronic cocaine administration. Preclinical investigations have provided a means for evaluating pharmacological effects and behavioural correlates of chronic cocaine exposure in the absence of the complex cognitive, affective, and social interactions that must be considered in the case of human addiction. An outcome of these animal studies has been the identification of two major effects related to repeated cocaine exposure; the development of sensitization to the behavioural-activating effects of the drug and the emergence of anxiogenic drug effects.

Cocaine Sensitization

Sensitization refers to an enhancement in the magnitude of drug-induced behaviours and neurochemical events that occurs with repeated and intermittent exposure to a drug (Robinson & Becker, 1986). Sensitization of the psychomotor and stereotypic effects of a variety of psychostimulant drugs, including the amphetamines and cocaine, has been reported (see Robinson, 1993; Robinson & Becker, 1986). Kalivas and Duffy (1993a), for example, demonstrated a sensitized psychomotor response to an acute injection of cocaine (15 mg/kg) given up to 15 days after a five-day cocaine pretreatment (15 or 30 mg/kg/day); they also showed enhanced cocaine-induced DA efflux in the ventral striatum 11 to 15 days after the last cocaine pretreatment injection.

Likewise, cocaine-induced increases in psychomotor response and corresponding changes in DA efflux in the NAcc has been reported after repeated and intermittent exposure to cocaine (e.g., Kalivas, Sorg, & Hooks, 1993; Pettit, Pan, Parsons, & Justice, 1990; see also Kalivas & Duffy, 1993b)

Since similar neural pathways are implicated in the locomotor and reinforcing effects of cocaine, it would seem reasonable to speculate that the reinforcing effects of cocaine may also sensitize with repeated exposure to the drug (Robinson, 1993; Wise & Bozarth, 1987). Clinical observations of enhanced experiences of pleasure and euphoria with repeated cocaine exposure support the possibility that sensitization underlies cocaine addiction (Holman, 1994). At a preclinical level, there is also some evidence to suggest that sensitization to the reinforcing effects of cocaine occurs. For example, it has been shown that rats that have received previous experience with cocaine will learn to self-administer, what are for drug-naive rats, low non-reinforcing doses of cocaine (see Holman, 1994). Additionally, Shippenberg and Heidbreder (1995) reported that animals pretreated with cocaine showed a subsequent enhanced sensitivity to its reinforcing effects as demonstrated by a reduction in the number of conditioning trials and in the threshold dose required to establish a preference for a place paired with cocaine relative to a place paired with saline.

Cocaine-Induced Anxiogenic Effects

Cocaine addicts, while intoxicated, tend to become focused almost exclusively on the euphoria and pleasure sensations induced by the drug and, as a result, any emergent aversive drug effects generally go unrecognized (Gawin, 1991). Reports, however, of cocaine-induced panic attacks in human

addicts (Anthony et al., 1989) suggest that anxiogenic as well as sensitized reinforcing effects are associated with chronic cocaine use.

A number of animal studies have been conducted to investigate the anxiogenic effects of repeated cocaine exposure. In rats, for example, cocaine induces increases in defensive-withdrawal behaviour (a well-validated measure of anxiety); rats that are injected daily with cocaine (20 mg/kg/day), 20 minutes before being placed in an enclosed chamber within an open field, spend a greater amount of time inside the chamber when tested on days 6 and 14 than when tested on day 1 (Yang, Gorman, Dunn, & Goeders, 1992). In another study, Ettenberg and Geist (1991) reported that in rats trained to traverse an alleyway to obtain IV infusions of cocaine, successive daily trials were associated with a progressive increase in the number of retreats made from the goal box before actually entering it (see also Ettenberg & Geist, 1992). The authors argued "that the retreat behavior arose from learned associations between the stimuli of the goal box and the combined positive and negative consequences of cocaine administration" (Ettenberg & Geist, 1991, p. 459). As evidence that the retreat behaviour did in fact reflect the emergent anxiogenic effects of cocaine, they showed that pretreating the same animals with the benzodiazepine-receptor agonist diazepam before a trial significantly and markedly reduced the number of retreats exhibited. The ability of benzodiazepine agonists to attenuate the anxiety-inducing effects of cocaine after chronic exposure has also been found using defensive-withdrawal (e.g., Yang et al., 1992) and conditioned fear (e.g., Fontana & Commissaris, 1989) procedures.

In summary, preclinical studies of the effects of chronic exposure to cocaine suggest that the reinforcing effects of cocaine sensitize with experience

and that aversive drug effects related to anxiety emerge over time. Rats maintain a steady rate of cocaine self-administration over time (e.g., Gerber & Wise, 1989; Roberts, 1992), an observation that suggests that the drug has continued reinforcing effects with repeated exposure; however, cocaine-experienced rats also demonstrate increased defensive withdrawal behaviour (Yang et al., 1992) and enhanced conditioned fear responses (Fontana & Commissaris, 1989), suggesting that chronic cocaine exposure also produces anxiety. Animal studies, therefore, are in agreement with clinical observations that both reinforcing and anxiogenic effects occur in response to cocaine after chronic exposure.

The Relapse to Drug Taking: Abstinence from and reinitiation of cocaine seeking

Addictive behaviour, in reference to compulsive drug use, can be thought of as a highly recurrent pattern of drug taking, drug abstinence, and relapse to drug taking. Although the frequency with which this pattern is repeated may vary from addict to addict and from drug to drug, all addictive behaviours are characterized by alternating periods of drug taking and drug abstinence. Each transition from a period in which drug is not consumed to a period of drug consumption represents an instance of *relapse* .

The conceptualization of addiction as recurrent relapse to drug taking is particularly appropriate for describing the pattern of drug consumption typically exhibited by cocaine addicts. As was discussed previously, cocaine addicts tend to consume drug in binges. Following a binge, addicts often abstain from further drug use for several days before initiating another binge (Gawin, 1991;

Gawin & Ellinwood, 1988). In the case of cocaine addiction, then, instances of relapse occur frequently.

Although the concept of relapse is clearly important for understanding the mechanisms that maintain drug-taking behaviour over days, it is the relapse to drug taking that occurs after prolonged periods of abstinence, when drug in the body cannot directly influence desire or motivation for more drug, that is one of the major problems facing drug addicts and those attempting to treat drug addiction. Although progress has been made over the past several decades in understanding the mechanisms underlying the long-term relapse to drug taking (e.g., Stewart & de Wit, 1987), relapse remains a prevalent problem among drug addicts (Stitzer & Cox, 1996) and is a particularly prevalent problem among cocaine addicts (McKay, Rutherford, Alterman, Casciola, & Kaplan,1995).

Much of the thinking about drug addiction and relapse over the past 20 to 30 years has focused on the related roles of tolerance, physical dependence, and withdrawal in addictive behaviour. This focus can be attributed, at least in part, to the attention given to the long-term consequences of opioid use. Physical dependence develops after long-term exposure to opioids as assessed by the symptoms of withdrawal that occur upon discontinuation of drug use. As a result, investigators have hypothesized that it is the avoidance of the aversive physiological and affective consequences of withdrawal (for example, metabolic changes, irritability, and depression) that maintains drug-taking behaviour and that motivates relapse to drug taking following a period of abstinence (Siegel, 1979; Siegel, Hinson, Krank, & McCully, 1982; Solomon, 1977; Solomon & Corbit, 1974; Wikler & Pescor, 1967).

Although a number of *withdrawal-avoidance* hypotheses of relapse have been advanced, attempts to find a direct and important role for withdrawal in the relapse to drug taking have been unsuccessful (see Stewart, 1992; Stewart , de Wit, & Eikelboom, 1984). Even in the case of opioid abuse, a case in which the development of physical dependence clearly occurs, a link between withdrawal and relapse has not been established. For example, the concordance between withdrawal symptoms and subjective reports of craving in opiate addicts is not reliable (Childress, McLellen, Ehrman, & O'Brien, 1986; Tiffany, 1990). In animal studies as well, attempts to show a relationship between physical symptoms of withdrawal and drug-seeking or drug-taking of opioids have been unsuccessful. In rats trained to self-administer heroin, for example, a non-contingent injection of the training drug after a period of abstinence induces the reinstatement of drug-taking behaviour (e.g., Shaham & Stewart, 1995b); however, an injection of naltrexone or naloxone, opioid-receptor antagonists that induce acute withdrawal symptoms, is not effective in reinstating heroin-taking behaviour, either when given alone (Shaham & Stewart, 1996; Stewart & Wise, 1992) or when given after heroin (Shaham, Rajabi, & Stewart, 1996) or morphine (Shaham & Stewart, 1995b) exposure. Not only have withdrawal-avoidance hypotheses failed to show that the physical symptoms of opioid withdrawal provoke relapse to the abuse of such drugs, but withdrawal symptoms associated with other drugs of abuse, such as cocaine, also have not demonstrated a role for withdrawal in relapse.

Cocaine withdrawal is associated with elevated intracranial self-stimulation (ICSS) thresholds in rats, a finding believed to reflect cocaine withdrawal-induced anhedonia resulting from desensitization of the reward pathways mediating ICSS (Markou & Koob, 1991). As well, during withdrawal,

rats spend less time in the open arms of an elevated plus maze than when not in withdrawal, a behaviour believed to demonstrate withdrawal-induced anxiety (Sarnyai et al., 1995). In humans, cocaine withdrawal is associated with symptoms of anxiety, limited ability to experience pleasure (anhedonia), lack of energy (anergia), and boredom (Gawin, 1991; Gawin & Ellinwood, 1988; Weddington et al., 1990).

During withdrawal, cocaine addicts are generally cognizant of the adverse consequences of continued cocaine use and can, therefore, usually withstand the anhedonic dysphoria associated with withdrawal until they are confronted by reminders, or conditioned cues, of cocaine euphoria (Gawin, 1991). Even in cases where cocaine addicts attribute a relapse episode to the dysphoria induced by withdrawal, they often report that it is the memory of cocaine euphoria, rather than the distress of dysphoria, that is the primary motivation to seek out drug (Anthony et al., 1989; McKay et al., 1995). Such reports imply that the reminder of a "drug-like" state (cocaine-induced euphoria), rather than the attempt to avoid a "drug-opposite" state (withdrawal-induced dysphoria), is in many cases what induces craving for more drug and ultimately provokes relapse to drug taking (Stewart, 1992; Stewart & de Wit, 1987).

Over the past several decades, numerous studies have been conducted to test the effectiveness of various motivationally-significant stimuli in inducing, or *priming* , the reinstatement of drug-taking behaviour. Priming studies conducted with animals and humans have provided strong evidence in support of the hypothesis that the induction of a "drug-like" motivational state underlies the relapse to drug taking.

The Priming Model: Some background

It is well-known that the presentation of a reinforcing stimulus can *prime* motivation for continued reinforcement. At the beginning of the twentieth century, Pavlov (1919) wrote about how the ingestion of small amounts of food whets the appetite for more food:

> All the condiments and all the appetizers used before a substantial repast are obviously designed to provoke curiosity, interest and a greater desire for food. It is a well-known fact that a person who at first displays indifference to his customary meal afterwards begins to eat with gusto as if his taste has been stimulated by something piquant. (Pavlov, 1919, p.108).

It has been found experimentally that natural reinforcers such as food, water, brain stimulation, or other motivationally-significant stimuli all represent effective conditions for priming an organism to seek continued reinforcement (de Wit, 1996; Stewart & de Wit, 1987). For example, in rats trained to traverse a runway for food reinforcement, presentation of a small amount of food or water before a trial decreases latency to run and increases speed of running (Morgan & Fields, 1938). Similarly, presentation of nest-building materials to hamsters, in a home cage where such materials are already freely available, increases the likelihood that the hamster will engage in nest-building (Shettleworth, 1978).

Like the reinforcers just described, reinforcing drugs also produce a priming effect. In fact, one of the basic tenets of the organization Alcoholics Anonymous, that consumption of a single alcoholic beverage will greatly

increase the probability of a full relapse (Alcoholics Anonymous, 1955), supports the idea that a small amount of drug can prime desire for more drug.

In animal studies, the reinstatement of drug-taking behaviour can be defined, operationally, as an increase in the number of occurrences of the previously reinforced behaviour (e.g., number of lever presses on the previously-reinforced lever) in response to the presentation of a drug or drug-related stimulus after a period of extinction. Stretch and Gerber (1973) and Gerber and Stretch (1975) provided some of the earliest evidence that a non-contingent priming injection of a drug is able to reinstate previously-reinforced drug-taking behaviour in animals. These investigators trained squirrel monkeys to self-administer amphetamine. Once drug self-administration had stabilized, the animals were given several days of extinction sessions in which responding for drug resulted in infusions of saline rather than of drug. After several days of extinction, the monkeys were given non-contingent IV priming injections of amphetamine before the start of subsequent self-administration sessions; throughout these self-administration test sessions, responding continued to result in saline infusions. In both studies, it was found that a priming injection of the training drug reinstated drug-taking behaviour.

Since these initial reports of relapse to drug-taking behaviour in monkeys, this field of investigation has developed considerably. In rats, reinstatement of responding for drug by a priming injection of the same drug has been demonstrated in animals trained to self-administer morphine (Davis & Smith, 1976), heroin (de Wit & Stewart, 1983; Shaham et al., 1996; Shaham, Rodaros, & Stewart, 1994; Shaham & Stewart, 1995b; Shaham & Stewart, 1996), and cocaine (Comer, Lac, Curtis, & Caroll, 1993; de Wit & Stewart, 1981; Wise, Murray, & Bozarth, 1990; Worley, Valadez, & Schenk, 1994). Furthermore,

cross-over priming effects have been demonstrated in which drugs with similar pharmacological effects to the self-administered drug serve as effective stimuli for the reinstatement of drug-taking behaviour (for examples see de Wit & Stewart, 1981; Gerber & Stretch, 1975; Slikker, Brocco, & Keith, 1984; Stewart & Vezina, 1988; Wise et al., 1990). In general, drugs from the same pharmacological class as the self-administered drug, or those affecting similar neurotransmitter systems, appear to function most effectively in priming drug taking (Carroll & Comer, 1996; de Wit & Stewart, 1981).

The validity of drug-induced reinstatement of drug taking in animals as a model for relapse is supported by parallel studies conducted with human subjects. Priming injections of an abused drug are associated with increases on measures such as desire for drug, craving for drug, and willingness to work for more drug in alcoholics, (Bigelow, Griffiths, & Liebson, 1977; Hodgson, Rankin and Stockwell, 1979; Ludwig & Wikler, 1974) heroin addicts (Meyer & Mirin, 1979), nicotine addicts (Chornock, Stitzer, Gross, & Leischow, 1992), and cocaine addicts (Jaffe, Cascella, Kumor, & Shere, 1989; Preston, Sullivan, Strain, & Bigelow, 1992).

Priming Effects in Experienced Cocaine Users

As discussed above, it is well-established experimentally that a priming injection of cocaine after a drug-free period reinstates cocaine-trained behaviour in monkeys (Gerber & Stretch, 1975; Slikker et al., 1984) and rats (Comer et al., 1993; de Wit & Stewart, 1981; Stewart, 1984; Wise et al., 1990; Worley et al., 1994) with a history of cocaine self-administration. In a prototypic study, de Wit and Stewart (1981) used a *within-sessions* reinstatement procedure in which, following a period of self-administration training, rats were

exposed to drug self-administration, extinction (responding resulted in infusions of saline rather than drug), and reinstatement testing conditions within a single session. A range of cocaine priming doses (0.125 to 4.0 mg/kg, IV) were tested for effectiveness in restoring cocaine-trained behaviour. All but the lowest dose (0.125 mg/kg) were effective in reinstating responding for cocaine.

Drugs other than cocaine, thought to share some pharmacological actions in common with cocaine, have also been found to reinstate cocaine-trained behaviour. For example, in monkeys trained to self-administer cocaine, priming injections of amphetamine (Gerber & Stretch, 1975; Slikker et al. ,1984), morphine (Slikker et al., 1984), and codeine (Slikker et al., 1984) have all been shown to be effective in reinstating cocaine-trained behaviour. Numerous other reports of such *cross-over* priming effects have been reported in studies carried out in rats. Examples include the reinstatement of cocaine-trained behaviour by the DA-receptor agonist bromocriptine (Wise et al., 1990), by injection of morphine into the VTA (Stewart, 1984), by systemic injections of morphine, apomorphine, and amphetamine (de Wit & Stewart, 1981) and by caffeine (Worley et al., 1994).

Based on animal studies of cocaine priming effects, it may be predicted that in humans exposure to cocaine after a drug-free period should induce desire and craving for more drug. Indeed, clinical studies conducted with cocaine addicts confirm this prediction. Jaffe et al. (1989), for example, reported that 15 minutes after receiving an IV injection of cocaine, as compared to a placebo, subjects demonstrated a state of *needing* the drug as indicated by increased ratings in response to the question, "how much craving for the drug do you have right now" and *desire* for the drug as demonstrated by increased ratings in response to the question, "what is the most you would pay for the

drug"; over a subsequent two-hour period, subjects' ratings in response to these same questions declined to a pre-injection level. In another study, Preston et al. (1989) also reported increases in cocaine-craving following a priming injection of the drug and, in addition, they reported increases in ratings of *liking* the drug.

In summary, cocaine has been shown reliably to induce the reinstatement of cocaine-trained behaviour in animals and to induce desire and craving for cocaine in humans. Additionally, in animal studies, drugs sharing pharmacological effects in common with cocaine have also been shown to serve as effective stimuli for reinstating cocaine-trained behaviour. Taken together, these findings suggest that the induction of an appetitive-motivational state related to cocaine reinforcement may underlie the processes mediating relapse to cocaine seeking (Stewart, 1992; Stewart & de Wit, 1987).

Stress and Relapse: A possible role for stress in the relapse to cocaine seeking

Clearly, events other than re-exposure to drugs can provoke relapse to drug taking. In human laboratory studies, it has been shown that exposure to environmental stimuli associated with drug taking can elicit strong craving for drug in abstinent drug users (see Childress, Ehrman, Robins, & O'Brien, 1992); in particular, this has been shown to be the case in abstinent cocaine users (e.g., Childress, Ehrman, McLellan & O'Brien, 1987; Childress et al., 1986; see also Gawin, 1991). Likewise, exposure to drug-related conditioned stimuli has been shown to reinstate drug taking in rats trained to self-administer cocaine (de Wit & Stewart, 1981) and morphine (Davis & Smith, 1976), albeit not as effectively as re-exposure to drug itself. Studies such as these raise the possibility that exposure to environmental events may contribute to relapse.

One such event that has been implicated in the relapse to drug taking in humans (e.g., Ludwig & Stark, 1984; McKay et al., 1995), and that has been the subject of considerable interest in animal studies of drug abuse (see Kalivas & Stewart, 1991), is exposure to stress.

The concept of stress can be defined, in a general way, as an event that produces a profound physical or psychological change in an organism by disrupting the organism's normal steady state (Akil & Morano, 1995). These changes are manifested physiologically in a variety of central and autonomic responses (see Sapolsky, 1992). Acute physiological stress responses include NA release, which is associated with increased heart rate, blood pressure, and respiration, and activation of the hypothalamic-pituitary-adrenal (HPA) axis, resulting in the release, and subsequent elevation in plasma blood levels, of glucocorticoids. Chronic exposure to stress can induce the suppression of immune responses and can interfere in reproductive systems and the hormonal regulation of growth and development. Physiological changes, both acute and chronic, can be expressed psychologically in the form of, for example, depression, anxiety, and attention deficits. Other factors such as genetic constitution and behavioural 'coping' strategies may influence the interaction between exposure to stress and stress responses.

In recent years, the effects of acute stress (e.g., footshock, tail pinch, immobilization, social competition, exposure to novelty) on drug-seeking and drug-taking behaviours in animals has represented an area of growing interest in the field of drug addiction research. Substantial evidence exists to suggest that sensitization occurs in response to acute stress in much the same way as it occurs in response to psychostimulant drugs such as amphetamine and cocaine; changes in mesocorticolimbic DA transmission appear to underlie both

stress- and psychostimulant-induced sensitization (see reviews by Kalivas & Stewart, 1991; Sorg & Kalivas, 1993).

Since some of the effects of acute stress and psychostimulant drugs appear to be mediated by common neural pathways, it has been of interest to determine whether stressors can modify drug-seeking and drug-consuming behaviours. One approach to this area of study has been to determine whether an organism's response to stress might predict its vulnerability to the reinforcing effects of psychostimulant drugs. Piazza, Deminiere, Le Moal, and Simon (1989), for example, showed that a rat's level of locomotor activity in response to the stress of a novel environment predicted the likelihood that rat would initiate self-administration of low doses of amphetamine; rats that demonstrated a low level of activity in response to novelty (LR) did not acquire low dose amphetamine self-administration while rats that showed a high level of novelty-induced activity (HR) readily acquired the behaviour. These results raise the possibility that intrinsic neurochemical differences in mesocorticolimbic DA transmission exist between LR and HR rats; indeed, subsequent studies have shown that LR rats have a lower basal level of DA activity in the NAcc and exhibit less overflow of DA in NAcc in response to novelty-induced stress relative to HR rats (see Piazza & Le Moal, 1996). Although novelty-induced locomotor activity has been shown *not* to predict strength of amphetamine- (Erb & Parker, 1994) or cocaine-induced (Gong et al., 1996) place conditioning, other findings, in addition to those already mentioned, do suggest a correlation between an organism's initial response to stress and its vulnerability to the reinforcing effects of low doses of psychostimulant drugs (see Piazza and Le Moal, 1996).

As further evidence of a link between exposure to acute stress and sensitivity to the reinforcing effects of psychostimulant drugs, it has been shown that pre-exposure to acute stress sensitizes subsequent responses to psychostimulant drugs. Piazza, Deminiere, Le Moal, & Simon (1990), for example, demonstrated that in rats that received repeated tail pinch stress, a sensitized psychomotor response was demonstrated to a subsequent amphetamine challenge; as well, animals showed an increased likelihood to self-administer a low dose of amphetamine after receiving pre-exposure to tail pinch stress. Similar findings of stress-induced potentiation of self-administration have been reported with cocaine (see introduction, Experiment 1). The ability of stress to potentiate the initiation of psychostimulant self-administration is consistent with reports that pre-exposure to the drug itself can potentiate self-administration (Piazza et al., 1990), suggesting a cross-sensitization between the effects of acute stressors and psychostimulant drugs.

To date, the relationship between stressors and psychostimulants in drug-consuming behaviour has been investigated exclusively during the initiation and maintenance phases. However, in recent studies by Shaham, Stewart, and colleagues (e.g., Shaham et al., 1996; Shaham & Stewart, 1995b; Shaham & Stewart, 1996), exposure to acute footshock stress was shown to reinstate responding for the opioid, heroin; in fact, in these studies, footshock stress was found to be at least as effective as a priming injection of heroin for reinstating heroin-taking behaviour.

Opioid drugs, such as heroin, have known stimulant actions that, as is the case for the psychostimulants, are believed to be mediated by mesocorticolimbic DA transmission; cross-sensitization between morphine and psychostimulant drugs such as amphetamine (Stewart & Vezina, 1987; see also

Robinson, 1993) support this argument. It has also been found that footshock stress increases the breakpoint value for animals self-administering heroin on a progressive ratio schedule of reinforcement (Shaham & Stewart, 1994); this finding suggests that footshock stress enhances the reinforcing efficacy of heroin and supports the possibility that a common mechanism of action may underlie the stimulant effects of opioids and acute stress .

Since cocaine, like heroin, is readily self-administered by rats and since the psychostimulant effects of both drugs are mediated, at least in part, by their actions on mesocorticolimbic DA transmission, it may be predicted that exposure to footshock stress should reinstate cocaine-trained behaviour, as it does heroin-taking behaviour. The footshock-induced reinstatement of cocaine-trained behaviour is further predicted by the well-established finding that cross-sensitization occurs between the effects of acute stressors and psychostimulant drugs (Kalivas & Stewart, 1991; Piazza & Le Moal, 1996; Robinson, 1993; Sorg & Kalivas, 1993).

Purpose of Present Experiments

The primary objective of the present experiments was to determine whether brief exposure to stress would serve as an effective condition for provoking relapse to cocaine-trained behaviour. This was tested in Experiment 1 by comparing reinstatement by footshock stress to reinstatement after a priming injection of cocaine. Experiment 2 was done to test whether the reinstatement observed after stress and a cocaine priming injection could be blocked by administration of the anxiolytic drug, diazepam.

General Methods

Subjects

A total of 64 male Long Evans rats (32 supplied by Charles River, Canada and 32 supplied by Harlan Sprague-Dawley, United States) were used in the experiments. An additional 24 animals were used in pilot studies (8 supplied by Charles River and 16 by Harlan Sprague Dawley). All animals were drug-naive at the beginning of experimentation. Rats were housed in a humidity- and temperature-controlled colony room under a reversed light-dark schedule for two to three weeks before and for at least five days after surgery. Subsequently, rats were housed permanently in the self-administration boxes, unless otherwise specified (see experiment 1), and were maintained on a reversed light-dark schedule (lights on 1900 to 0900h). Food and water were continuously available to the animals.

Surgery

Rats were anesthetized with sodium pentobarbital (65 mg/kg, IP) and were injected with atropine sulfate (0.6 mg/ml; 0.3 ml/animal) just before surgery and with penicillin (Ayercillin 300 000 IU; 0.2 ml/animal) just after surgery. Intravenous silastic catheters (Dow Corning, inner diameter 0.02 in, outer diameter 0.037 in) were implanted into the right jugular vein. Silk sutures were used to secure the catheter to the vein. The catheter was then passed subcutaneously to the top of the skull where it exited into a connector (a modified 22 gauge cannula) mounted to the skull with jeweler's screws and dental cement. Animals were allowed at least 5 days to recover from surgery. A plastic cap was placed over the open end of the cannula during this period.

The catheters were flushed every 2 to 3 days during recovery with 0.1 ml of a saline-heparin solution (30 U/ml in experiment 1; 15 U/ml in experiment 2).

Apparatus

The self-administration boxes in which the animals were housed were constructed of plexiglas and were each one of three dimensions (4 boxes were 27 cm high by 27 cm wide by 27cm deep; 4 boxes were 26 cm by 30 cm by 27 cm; 8 boxes were 30 cm by 33 cm by 27 cm). The chambers were equipped with one retractable "active" lever (Med Associates, Lafayette, IN) and a second non-retractable "dummy" lever. Both levers were located 9 cm above the floor. Only responses on the active lever activated the infusion pump (Razel Scientific Instruments, Stamford, CT). Responses on the other lever were recorded but did not result in activation of the pump. Drug solution or vehicle was administered over a 20 second period at a volume of 0.13 ml. For the duration of the infusion, a white light located just above the active lever was lit. Lever presses during the 20-second infusion period were recorded but did not lead to additional infusions. All responses were collected automatically by computer.

Each operant chamber was fitted to deliver constant-current, intermittent, inescapable, electric footshock through a scrambler to the grid floor (Grason-Stadler Generator #E1064GS). The foot shock was delivered according to a variable time schedule at a mean interval of 40 seconds (10-70 second range). Each shock (0.5 mA) was 0.5 seconds in duration.

Drugs

Cocaine HCL was obtained from BDH Chemicals in Toronto, Ontario, Canada and was dissolved in physiological saline. Diazepam was purchased

in injectable form from Hoffman-La Roche in Mississauga, Ontario and was injected IP at a dose of 0.75 or 1.5 mg/kg. The doses of diazepam were chosen on the basis of previous studies (e.g., Ettenberg & Geist, 1991) and are, reportedly, subthreshold doses for producing motor deficits in rats (e.g., Fanselow, & Helmstetter, 1988; Hottsenpiller & Williams, 1996; Rex, Stevens, & Fink, 1996).

General Procedure

The basic reinstatement procedure used in Experiments 1 and 2 is presented schematically in Appendix I. The procedure consisted of three phases: self-administration training (initiation and maintenance), extinction, and tests for reinstatement. During all phases of the procedure, animals were allowed to self-administer during one three-hour session each day (7 days/week), four to five hours after lights off. At 0830 each day, just before lights off, rats were weighed and their catheters were flushed with heparin (0.1 ml). The daily self-administration sessions began between 1400 and 1500. Rats, therefore, self-administered during the dark cycle and at a time that, presumably, did not interfere significantly with their daily feeding.

Self-administration training

Rats were trained to self-administer cocaine HCL (1.0 mg/kg/infusion, IV) on a fixed-ratio-1 schedule of reinforcement. At the beginning of each session, a red house light was illuminated, the active lever was introduced into the cage, and a white light just above the active lever was lit for the initial 30 seconds after presentation of the lever. The house light remained illuminated throughout the three-hour session. As indicated previously, responses on the active lever resulted in activation of the infusion pump and illumination of the light above the

lever for the 20 seconds of drug delivery. Additional lever presses during the drug-delivery period did not result in reactivation of the pump.

In early training sessions (1-4), if an animal did not initiate responding on the active lever within 30 minutes of the start of the session, a drop of water was placed on the lever in an attempt to direct the animal's attention to the lever; if the animal still did not respond, the pump was activated by placing the animal on the lever. Training sessions continued until each animal had achieved a minimum of eight consecutive and stable cocaine self-administration sessions. Stable responding was defined, roughly, as an average of six infusions per hour in any given session. Animals were generally self-administering cocaine at a stable rate within four training sessions; however, of the 64 animals trained to take cocaine, 10 did not achieve stable self-administration.

Extinction

Once animals demonstrated stable self-administration, extinction conditions were introduced. During extinction, responses on the active lever resulted in IV infusions of saline rather than cocaine. Otherwise, the conditions present during training remained the same throughout extinction.

Extinction conditions were continued for each animal until response rates had fallen to less than 15 responses in the three-hour period. Subsequently, these extinction conditions were continued with the difference that, in order to habituate the animals to injections and handling, sessions began with IV injections of saline delivered manually through a hand-held syringe and tubing. When an animal reached a criterion level of responding in response to a saline injection (2-6 sessions), defined as 10 or fewer responses on the active lever during a given three-hour session, tests for reinstatement began for that animal.

Tests for reinstatement

Throughout testing for reinstatement, responses on the active lever continued to result in IV infusions of saline. The following tests for reinstatement were given in both experiments. On the first day of formal testing for reinstatement, animals were given a non-contingent IV priming injection of saline five minutes before the session. The number of responses made in this saline test session served as the baseline measure. In subsequent sessions, a non-contingent priming injection of cocaine (2.0 mg/kg, IV; delivered manually as described for saline) five minutes before the beginning of the self-administration session, and a 10-minute exposure to intermittent footshock stress (0.5 mA) immediately preceding the self administration session were tested for effectiveness in restoring cocaine-trained behaviour. The priming dose of cocaine was chosen on the basis of a pilot experiment and is the one that induced a maximal response in a previous study (de Wit and Stewart, 1981). The footshock parameters were based on pilot studies with cocaine self-administration and on previously-published studies of reinstatement of heroin self-administration (Shaham & Stewart, 1995a; Shaham & Stewart, 1995b). Rats received two cocaine tests and two footshock tests. The tests were given daily; the cocaine and footshock tests were given on alternate days. Half of the animals received the cocaine test first and half of the animals were exposed to footshock stress first. In an attempt to equate the cocaine and footshock test conditions, an IV infusion of saline was given immediately before the start of footshock sessions.

Statistical Analysis

The main dependent measures for the tests for reinstatement were number of responses on the active lever and number of responses on the inactive lever. Separate analyses were conducted for responding on each lever. In both Experiments 1 and 2, Test Condition (Saline, Cocaine, Footshock) was a within-subjects factor and, in Experiment 2, Dose of the pretreatment drug was a between-subjects factor. When Test Condition was found to be a significant variable, an additional ANOVA was done using difference scores (number of responses on the active lever minus number of responses on the inactive lever).

In some instances, unequal numbers of subjects were tested under the cocaine and footshock test conditions (this typically occurred when subjects could not receive cocaine tests due to catheter failure); when this occurred, separate analyses were conducted for the cocaine test condition and for the footshock test condition, using the saline test condition as the control. When appropriate, differences between the various experimental conditions were analyzed using Fisher's LSD post-hoc comparisons, $p<.05$.

Experiment 1

Stressors such as footshock (Goeders & Guerin, 1994), observing another animal being exposed to footshock (Ramsey & Van Ree, 1993), and defeat stress (Haney, Maccari, Le Moal, Simon, & Piazza, 1995), have been found to potentiate the initiation of low dose cocaine self-administration. In addition, Miczek and Mutschler (1996) have demonstrated that defeat stress increases the rate of responding on a fixed-ratio schedule for IV cocaine self-administration during the maintenance phase.

Recently, Shaham, Stewart, and colleagues (e.g., Shaham et al., 1996; Shaham & Stewart, 1995b; Shaham & Stewart, 1996) have focused on the role that stress plays in the relapse to drug taking; they have reported that exposure to acute intermittent footshock stress is effective in reinstating heroin self-administration. Although numerous studies in rats and monkeys have shown that non-contingent injections of a self-administered drug effectively reinstate responding for that drug (see Introduction for references), Shaham and associates are the first to report on stress-induced relapse to drug taking.

Although exposure to acute stress has been shown to modify the initiation and maintenance of self-administration of cocaine, to date no data exists to indicate whether an acute stressor, such as footshock, might also induce relapse in animals experienced in the self-administration of cocaine. Therefore, using a between-sessions reinstatement procedure (see Shaham et al., 1994), the present experiment was conducted to determine whether exposure to footshock stress would induce relapse in animals experienced in cocaine self-administration after long-term extinction and after an additional 4- to 6-week drug-free period. Since non-contingent injections of cocaine have been shown

previously to reinstate cocaine-trained behaviour in rats (e.g., de Wit & Stewart, 1981) and to elicit cocaine craving in humans (Jaffe et al., 1989; Preston et al., 1989), footshock-induced responding was compared to that induced by a priming injection of cocaine.

Method

Subjects

Sixteen male Long Evans rats (supplied by Charles River Canada) were prepared with IV catheters. The animals were maintained as described in the general methods with the exception that they were housed in the colony room for a four-week period after the first set of reinstatement tests.

Procedure

Self-administration training, extinction, and testing for reinstatement was conducted as described in the general methods. In this experiment, however, two sets of tests for reinstatement were given. After the first set of tests was completed, all animals were removed from the testing environment for four weeks. Subsequently, they were returned to the self-administration boxes where extinction conditions were reintroduced and, as was the case during the first extinction phase, maintained until animals made 15 or fewer responses in a three-hour session. Again, saline injections were given before the start of subsequent daily self-administration sessions until animals reached the criterion level of responding of 10 or fewer responses in a three-hour session. Following extinction, animals were given one test for reinstatement under each of the following conditions: saline injections, IP or IV, cocaine injections, IV; and footshock stress. Saline injections were given IP rather than IV to animals that

no longer had patent catheters; these animals did not receive cocaine tests for reinstatement.

Results

Self-administration Training and Extinction Phases

During the maintenance period of the training phase, animals were self-administering approximately seven to eight infusions per hour of 1.0 mg/kg/infusion, cocaine HCl. Animals achieved a stable rate of self-administration within one to three sessions. The mean (±SEM) number of infusions made on the last two days of training was 21.46 (±2.20) and 24.15 (±1.80) infusions in each three-hour session. As expected, animals showed an increase in responding on the active lever on the first day of extinction, mean (±SEM) number of infusions of 59.67 (±11.52); responding extinguished over the following 4 to 11 days.

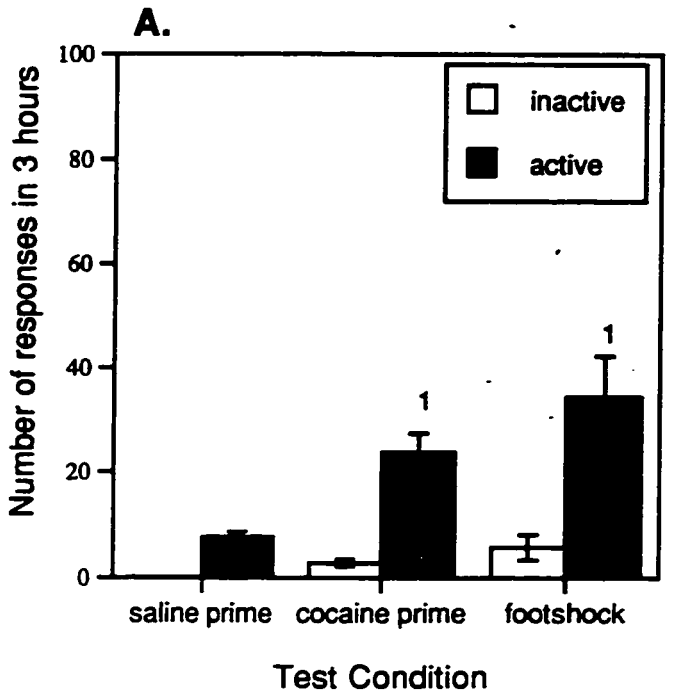
Reinstatement Test Phase 1

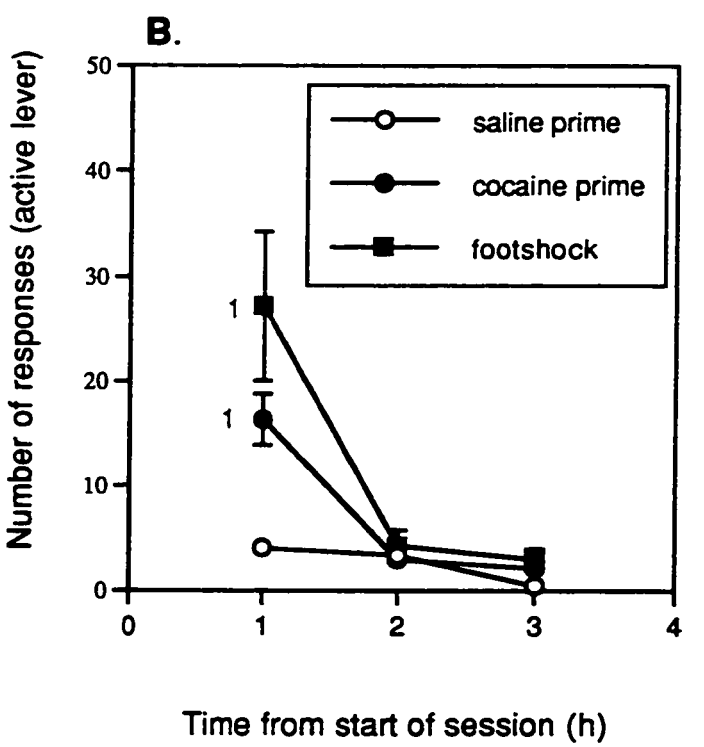
Of the 16 animals initially trained to self-administer cocaine, 12 were used in the first set of reinstatement tests. Two animals were excluded from the study due to catheter failure early in training and another two were excluded because they failed to acquire and maintain a stable level of self-administration over the training period.

Animals were tested twice after priming injections of cocaine and twice after exposure to footshock. Because no differences were found between the two tests in each condition, and because there was no evidence for an order effect for Test Condition, mean scores for each of the Test Conditions were used in the ANOVAs. Figure 1A shows the mean (±SEM) number of

Figure 1. Reinstatement test phase 1: **A**: Mean (±SEM) number of responses on the previously inactive and active levers in the 3-hour test for reinstatement after a non-contingent IV injection of saline, a non-contingent IV priming injection of cocaine (2.0 mg/kg), and intermittent footshock stress (10 minutes, 0.5 mA, 0.5 seconds on, mean off period of 40 seconds). **B**: Mean (±SEM) number of responses on the previously active lever during each hour following the saline, cocaine, and footshock primes. 1 Different from the Saline test condition, $p<.05$.

after 4 to 11 days of extinction





responses on the active and inactive levers under the various Test Conditions. A repeated-measures ANOVA on number of responses on the active lever for Test Condition (Saline, Cocaine, Footshock) was significant ($F[2,22]=9.22$, $p<.001$). A similar analysis on number of responses on the inactive lever also revealed a significant effect for Test Condition, ($F[2,22]=4.59$, $p<.03$). Therefore, difference scores (number of responses on the active lever minus number of responses on the inactive lever) were calculated for responding under each of the Test Conditions and these scores were entered into a repeated measures ANOVA. This analysis also revealed a significant effect of Test Condition, ($F[2,22]=6.27$, $p<.01$). Post-hoc comparisons revealed reliable differences between the Saline and Cocaine test conditions and between Saline and Footshock test conditions, but no differences between Cocaine and Footshock test conditions; the same comparisons were significant whether number of responses on the active lever was entered as the dependent measure (differences indicated in Figure 1) or difference scores were entered as the dependent measure.

Figure 1B presents the mean (±SEM) number of responses on the active lever during each Hour of Testing under the various Test Conditions. A repeated-measures ANOVA for Hour of Testing (1,2,3) and Test Condition (Saline, Cocaine, Footshock) revealed significant main effects for Hour ($F[2,22]=24.84$, $p<.001$) and for Test Condition ($F[2,22]=9.22$, $p<.001$), and a significant Hour X Test Condition interaction ($F[4,44]= 6.5$, $p<.001$). Subsequent simple effects analyses for the factor of Test Condition at each Hour revealed a significant effect for the first hour, ($F[2,22]=7.72$, $p<.01$). Comparisons between the various Test Conditions during the first hour of testing revealed significant differences between Saline- and Cocaine-induced

levels of responding and between Saline- and Footshock-induced levels of responding; in both cases, responding was higher under Footshock and Cocaine test conditions than under Saline conditions. Significant differences are indicated in Figure 1B.

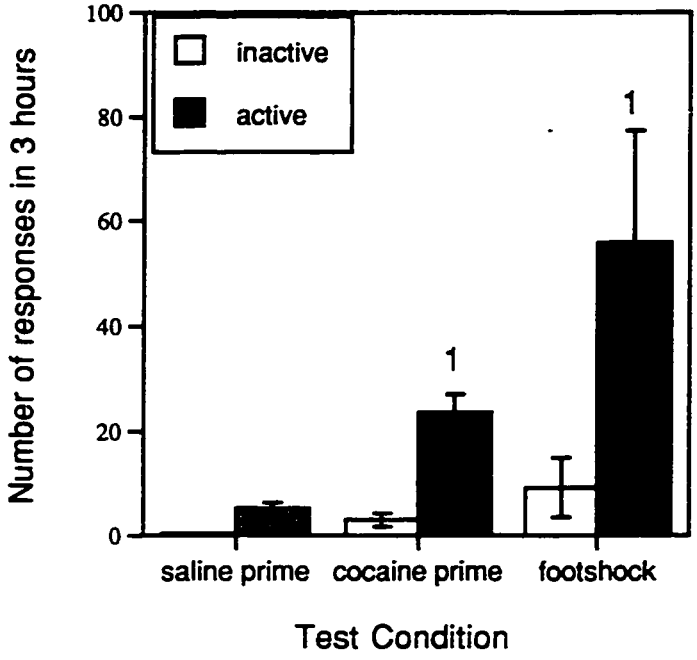
Reinstatement Test Phase 2

Eleven animals were returned to the self-administration boxes after four weeks in the animal colony. Extinction conditions were in place for several days until the number of responses on the active lever had reached the baseline criterion of 10 responses or less in three hours. Response rates were quite high on the first two days of extinction when animals were first re-exposed to the stimuli normally presented at the beginning of the daily session. The mean (±SEM) number of responses made by all animals was 23.91 (±3.17) and 22.55 (±3.28) during the first two three-hour daily sessions. As in the tests for reinstatement, most of the responses occurred in the first hour of the session.

Figure 2 presents the mean number of responses that animals made on the active and inactive levers in the tests for reinstatement. In this second set of reinstatement tests, 6 of the 11 animals were tested in the Cocaine test condition (those having patent catheters) and11 were tested in the Saline (IV or IP saline injections) and Footshock test conditions. Because of the unequal numbers of animals tested in each condition, comparisons were made between the Saline condition and each of the other test conditions, separately, using t-tests for related samples. Animals responded significantly more often after the priming injection of cocaine than after saline injection ($t[5]=3.36$, $p < .05$), and more often after exposure to footshock stress than after saline injection ($t[10]=3.1$, $p < .03$). Significant differences are indicated in Figure 3.

Figure 2. Reinstatement test phase 2: Mean (±SEM) number of responses on the previously inactive and active levers in the 3-hour session following a non-contingent IV or IP saline injection (n=11), a non-contingent IV priming injection of 2.0 mg/kg cocaine (n=6), and intermittent footshock stress (10 minutes, 0.5 mA, 0.5 seconds on, mean off period of 40 seconds), n=11. 1 Different from the Saline test condition, $p<.05$.

The reinstatement of cocaine-trained behaviour after a 4- to 6- week drug-free period



Discussion

The major finding from the present study is that acute exposure to intermittent footshock stress can serve as a powerful stimulus for relapse in animals trained to self-administer cocaine. The effect of footshock, at the parameters used here, was comparable to the reinstatement induced by a priming injection of cocaine and was demonstrated in rats after a one- to two-week extinction period and, again, after an additional four- to six-week drug-free period. These results are consistent with previous reports of reinstatement of heroin-taking behaviour induced by both priming injections of heroin and exposure to intermittent footshock after prolonged extinction and a drug-free period (Shaham & Stewart, 1995b). In fact, the magnitude of reinstatement induced by drug or footshock in cocaine-taking animals, in the present experiment, is strikingly similar to that induced by these events in heroin-taking animals.

Although the capacity of environmental stressors to modify psychostimulant self-administration has been the subject of considerable study (Goeders & Guerin, 1994; Miczek & Mutschler, 1996; Piazza et al., 1990), the present study would appear to be the first report of stress-induced relapse to cocaine-taking in laboratory animals. In this experiment, footshock stress induced the reinstatement of cocaine-taking at least as effectively as a non-contingent priming injection of the drug, itself.

In the present study it was also found that for cocaine self-administration, as was found for heroin self-administration (Shaham & Stewart, 1995b; Shaham et al., 1994), priming injections of the self-administered drug can induce relapse after a prolonged extinction period and an extended drug-free

period. Previous studies of drug-induced reinstatement of cocaine-trained behaviour have been done using a within-session procedure in which animals had been taking drug in the same session as testing occurred (e.g., Comer et al., 1993; de Wit & Stewart, 1981; Wise et al, 1990) or following only a few drug-free days (Gerber & Stretch, 1975; Slikker et al., 1984). The present findings, therefore, are important in the context of finding an animal model for the mechanisms underlying relapse to drug taking in humans, where relapse can occur after long periods of abstinence (Childress et al., 1986; O'Brien , Ehrman, & Ternes, 1986).

The procedures used in the present experiment may be useful for the study of the effects of drug-associated events and conditioned stimuli on relapse to drug taking in animals (de Wit and Stewart, 1981; Stewart, 1992). It was found here, not unexpectedly, that when animals were returned to the self-administration boxes after four weeks in the colony room, the stimuli previously associated with the beginning of cocaine self-administration sessions evoked levels of responding on the active lever that were as high as those seen after a priming injection of cocaine in the tests for reinstatement. These observations lend support to the argument that particularly strong classical and operant conditioning occurs to cues associated with cocaine-taking (Gawin, 1991) and that such cues often trigger craving and relapse to cocaine-taking in humans (Childress et al., 1986).

Finally, although in the present experiment exposure to brief footshock stress proved to be as effective a stimulus for relapse to cocaine-trained behaviour as a priming injection of the drug, it cannot be determined on the basis of these findings whether common mechanisms of action mediate the relapse induced by both events. Shaham and Stewart (1995b), for example,

reported a comparable magnitude of reinstatement by footshock and by a priming injection of heroin; however, in a subsequent study (Shaham & Stewart, 1996), they showed that the mechanisms underlying the two reinstatement events are pharmacologically dissociable. Since heroin- and footshock-induced reinstatement of heroin-taking behaviour do not appear to be mediated by identical processes, even though they share certain effects and actions in common, it is conceivable that different processes may also be important for the footshock- and cocaine-induced reinstatement of cocaine-trained behaviour. This possibility was addressed in Experiment 2.

Experiment 2

Chronic cocaine use is associated with the emergence of drug-related anxiogenic effects. In humans, cocaine abuse has been known to precipitate panic attacks (Anthony et al., 1989). Likewise in animals, chronic cocaine administration results in increased defensive-withdrawal behaviour (Yang et al., 1992) and enhanced fear-potentiated acoustic startle responses (Willick & Kokkinidis, 1995); both of these procedures are well-validated measures of anxiety. It is interesting to note that cocaine withdrawal has also been associated with anxiety in humans (Weddington et al., 1990) and in animals (Sarnyai et al., 1995). Such findings suggest a role for anxiety in the maintenance of cocaine self-administration and subsequent cocaine abuse.

The fact that anxiety occurs as a consequence of cocaine use and withdrawal raises the possibility that it may also play a role in relapse to cocaine use. Because cocaine and footshock stress have some anxiogenic effects in common (see, for example, Yang et al., 1992; Ettenberg & Geist, 1991; Hottenspiller & Williams, 1996; Quintero, Henney, Lawson, Mellanby, & Gray,

1985) and because both events provoke relapse to cocaine-taking (Experiment 1), it would seem possible that the elicitation of these anxiogenic effects might play a role in relapse. Manipulation of the anxiogenic effects of a cocaine prime and footshock stress may, therefore, provide a way of determining if, or to what degree, anxiety is related to their ability to provoke relapse.

Benzodiazepines have been used frequently in the treatment of a variety of anxiety-related neuropsychiatric disorders. The primary pharmacological action of the benzodiazepines is to inhibit neurotransmission in a variety of cortical, cerebellar, and limbic regions of the brain by binding to the $GABA_A$ receptor complex. This binding facilitates the subsequent binding of the inhibitory neurotransmitter GABA, itself, which causes the opening of the $GABA_A$ chloride channel. Consequently, Cl^- enters the cell causing hyperpolarization and inhibition of further cell firing (see Cooper, Bloom & Roth, 1996; Kandel, 1991; Redmond & Huang, 1979). It is considered that one of the effects of these compounds is the reduction of transmission of the NA neurons originating in the locus coeruleus and projecting diffusely to the hypothalamus and cortex, whose high activity is associated with fear and anxiety (Kandel, 1991; Redmond & Huang, 1979). In primates, for example, diazepam (1.0 mg/kg) has been shown to reverse a variety of anxiety-related physiological responses, including increases in heart rate, blood pressure, and plasma levels of NA induced by the anxiogenic benzodiazepine-receptor antagonist beta-carboline-3-carboxylic acid ethyl ester (Crawley et al., 1985). Similarly, in rats, observing another animal being exposed to footshock stress was found to induce release of NA in the hypothalamus, amygdala, and locus coeruleus, an effect that was reversed by pretreatment with 5.0 mg/kg diazepam (Tanaka et al., 1991).

As described in the Introduction, administration of benzodiazepine-receptor agonists, such as diazepam and chlordiazepoxide (CDP), effectively reduces (e.g., Yang et al., 1992) or reverses (Ettenberg & Geist, 1991) the anxiogenic effects of cocaine. In a similar way, benzodiazepines have been shown to effectively attenuate stress-induced anxiety. For example, CDP reduces the suppressive effects of punished responding when given to animals that have had electric shock paired with lever-pressing for sucrose (Quintero et al., 1985; Rawlins, Feldon, Salmon, Gray, & Garrud, 1980) or food (Izenwasser, Blake, Goeders, & Dworkin,1989). Similarly, CDP effectively reduces behaviours associated with frustrative nonreward; in rats trained to traverse an alleyway for food reward, on a partial or continuous schedule of reinforcement, administration of CDP during extinction sessions, in which no trials are rewarded, results in an increased resistance to extinction (Bucklan, Mellanby & Gray, 1986; Feldon & Gray, 1981; Salmon & Gray, 1985). Finally, diazepam has been shown to reduce anxiety-induced feeding behaviours, as demonstrated by decreased food-hoarding behaviour (McNamara & Whishaw, 1990) and increased likelihood of approaching the centre of an open field to obtain food (Rex et al., 1996).

The primary purpose of the present study was to determine whether the anxiogenic effects of stress play an important role in the footshock-induced reinstatement of cocaine-trained behaviour found in Experiment 1. Since similar anxiogenic effects are associated with chronic cocaine use and with the effects of acute stressors, it is conceivable that the anxiogenic effects of footshock stress are important for its effectiveness in reinstating cocaine-trained behaviour. If the anxiogenic effects are an important factor in stress-induced relapse, then the anxiolytic actions of a benzodiazepine should prevent

footshock-induced relapse. In Experiment 2, this hypothesis was tested by comparing, in rats, the footshock-induced reinstatement of responding after saline pretreatment with footshock-induced responding after diazepam pretreatment. The effect of diazepam on relapse induced by a priming injection of cocaine was also tested. The same footshock and cocaine test parameters that were used in Experiment 1 were used in this experiment.

Method

Subjects

Forty-eight male Long Evans rats (16 supplied by Charles River and 48 supplied by Harlan Sprague-Dawley) were used in the present experiment. The animals were maintained as described in the general methods.

Procedure

Self-administration training and extinction phases were conducted as described in the general methods. A diagram showing the experimental design used for the tests for reinstatement can be found in Appendix II. As was the case in Experiment 1, rats received two cocaine tests for reinstatement and two footshock tests for reinstatement; however, in this experiment, a second saline test was given between the first and second sets of cocaine and footshock tests. Animals, therefore, received a total of six tests for reinstatement. Before each of the first 3 tests, all animals were pretreated with saline (1ml/kg, IP, No Drug); before the second 3 tests , animals were pretreated with diazepam (0.75 or 1.5 mg/kg, IP, Drug). (For an explanation of this design see Appendix III.) Therefore, as is depicted in Appendix II, each animal received two Pretreatment Conditions (No Drug, Drug) and, within each Pretreatment Condition, they

received three Test Conditions for reinstatement (Saline, Cocaine, and Footshock). Pretreatment injections were given 15 minutes before a saline infusion or a priming injection of cocaine and 10 minutes before the start of the 10 minute footshock stress session, thus ensuring that all pretreatment injections were given 20 minutes before the start of the self-administration test sessions.

Not all animals had patent catheters at the time of testing. For those that did have patent catheters, IV infusions of saline were give five minutes before the start of saline tests and immediately before the start of footshock sessions, as described in the general methods; animals that did not have functional catheters at the time of testing (n=8) were handled in the same manner as those receiving saline infusions (n=17), but were not injected before saline tests. As described in the general methods, half of the animals received the cocaine priming injection first and half were exposed to footshock stress first; the order in which each rat received the test conditions for the first set of three reinstatement tests was maintained for the second set of three tests.

Results

Training and Extinction Phases

During the maintenance period of the training phase, animals self-administered approximately seven infusions per hour, 1.0 mg/kg/infusion cocaine HCL. This rate of infusions per hour is similar to that reported in Experiment 1. The rate of self-administration stabilized within one to four sessions. The mean (±SEM) number of infusions made in each three-hour session on the last two days of training was, respectively, 21.48 (±1.81) and 22.52 (±2.24). On the first day of extinction, a characteristic increase in the

number of responses made on the active lever was observed, 75.16 (±12.72) responses in three hours; responding extinguished over a subsequent 5 to 13 day period.

Reinstatement Test Phase

Of the 48 animals initially trained to self-administer cocaine, 25 were available for testing for reinstatement. Five animals were excluded from the study due to catheter failure early on in training, 8 were excluded because they failed to acquire a stable rate of self-administration over the training period, and 10 died during the course of the experiment or were sacrificed before or during testing due to poor health.

Of the 25 animals that were used in the tests for reinstatement, 8 did not have patent catheters at the time of testing; these animals received only Saline and Footshock tests for reinstatement, whereas animals with patent catheters received the three Test Conditions: Saline, Cocaine, and Footshock. Additionally, the footshock test data for two animals that responded at an unusually high level in the No Drug pretreatment condition (354 and 581 responses, respectively) were omitted from the analyses on the grounds that their scores exaggerated unfairly the differences between the No Drug and Drug pretreatment conditions. The data obtained from the cocaine tests for two animals were omitted from analysis because these animals convulsed after receiving the IV priming injection of cocaine in the No Drug pretreatment condition and were unresponsive for the duration of the subsequent test session. Because of the unequal numbers of animals tested in each condition, separate analyses were conducted between the Cocaine and Saline test conditions and between the Footshock and Saline test conditions.

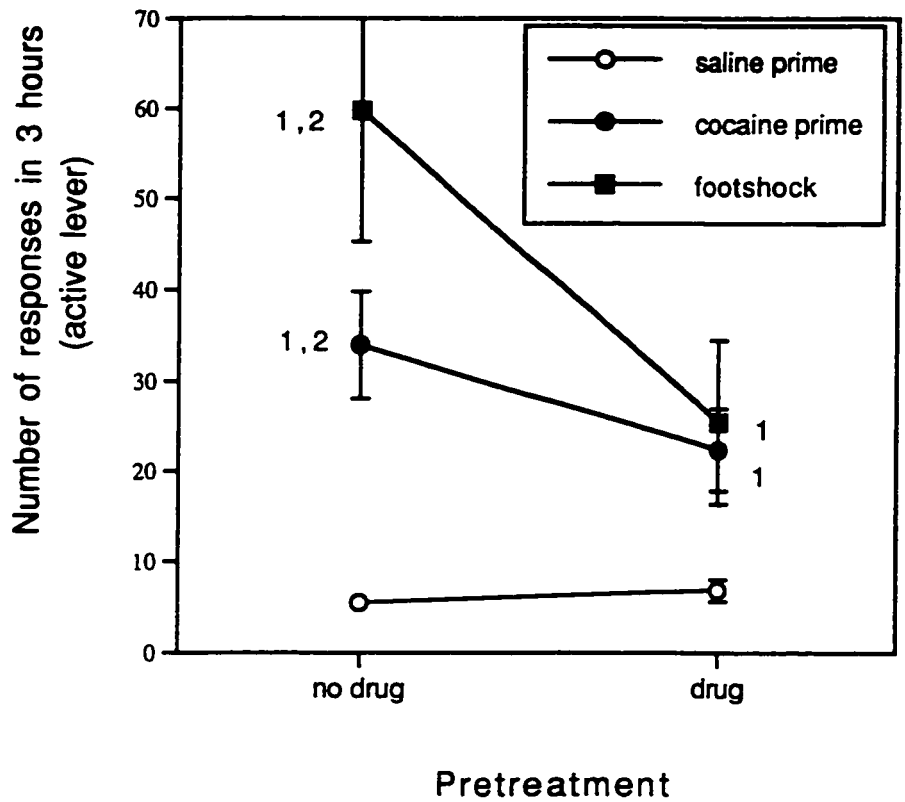
Responses on the active lever in 3 hours

Initial mixed-factor ANOVAs on number of responses on the active lever were conducted for the within-subjects factors of Pretreatment (No Drug, Drug) and Test Condition (Saline, Cocaine; Saline, Footshock) and for the between-subjects factor of Diazepam Dose (0.75 mg/kg, 1.5 mg/kg). The ANOVA conducted with Footshock as one of the test conditions revealed significant main effects for Pretreatment ($F[1,21]=4.53$, $p<.05$) and Test Condition ($F[1,21]=12.37$, $p<.01$) and for the interaction between Pretreatment X Test Condition ($F[1,21]=6.10$, $p<.03$). The ANOVA conducted with Cocaine as a test condition also revealed significant main effects for Pretreatment ($F[1,12)=7.72$, $p<.03$) and Test Condition ($F[1,12]=25.9$, $p<.001$) and for the interaction between Pretreatment X Test Condition ($F[1, 12]=9.26$, $p<.01$). In neither of these analyses was the effect of Diazepam Dose significant. Therefore, the data were combined for the two groups of animals (2 doses) in subsequent analyses.

Figure 3 presents the mean number of responses on the active lever (all animals) for each Test Condition under each Pretreatment Condition. It can be seen that animals responded more often following the Cocaine and Footshock tests, regardless of Pretreatment, but that the effects of footshock were markedly reduced after Drug pretreatment. The ANOVA for Footshock versus Saline (all animals) revealed significant main effects for Pretreatment ($F[1,22]=5.21$, $p<.05$) and Test Condition ($F[1,22]=13.22$, $p<.01$) and for the Pretreatment X Test Condition interaction ($F[1,22]=6.85$, $p<.03$); the same analysis conducted with Cocaine as a test condition revealed only a significant main effect for Test Condition ($F[1,13]=25.3$,

Figure 3. Mean (±SEM) number of responses on the previously active lever in the 3-hour test for reinstatement after an IV saline injection (n=24), IV injection of 2.0 mg/kg cocaine (n=16), and intermittent footshock stress (10 minutes, 0.5 mA, 0.5 seconds on, mean off period of 40 seconds), n=23 for No Drug and Drug pretreatment conditions. 1 Different from the Saline test condition (same pretreatment); 2 Different from Drug pretreatment, $p<.05$.

Effect of No Drug and Drug pretreatment on the reinstatement of cocaine-trained behaviour after 5 to 13 days of extinction



p<.001) and for the Pretreatment X Test Condition interaction (F[1,13]=5.66, p<.05). Significant post-hoc comparisons are indicated in Figure 3.

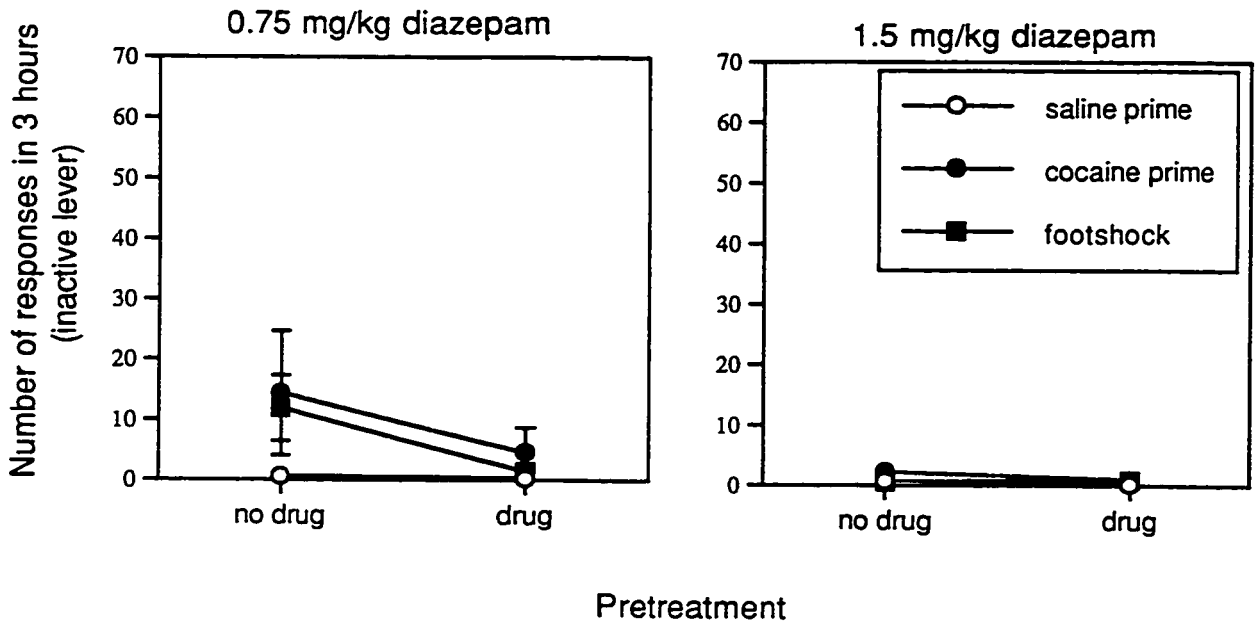
Responses on the inactive lever in 3 hours (analyses for non-specific motoric effects)

Similar analyses to those conducted for responding on the active lever were conducted for responding on the inactive lever. Responding on the inactive lever in self-administration studies is typically taken as a measure of and control for non-specific effects on motor activity. Diazepam has been reported to produce motor deficits in rats only at doses higher than the ones used in this experiment (Fanselow & Helmstetter, 1988; Hottenspitter & Williams, 1996; Rex et al., 1996). It was important, however, to determine whether non-specific responding was affected by the treatments used here.

Figure 4 presents the mean number of responses on the inactive lever for animals in each dose group under the two Pretreatment and three Test Conditions. A mixed-factor ANOVA for the factors of Pretreatment, Test Condition, and Diazepam Dose, with Footshock as a test condition, revealed main effects for Dose (F[1,21]=5.87, p<.03), Pretreatment (F[1,21]=5.59, p<.03) and Test Condition (F[1,21]=6.66, p<.03) and several interactions (see Appendix IV for source tables), including a three-way interaction between Pretreatment X Test Condition X Dose (F[1,21]=5.67, p<.03). No significant effects were found, however, when subsequent repeated-measures analyses for the factors of Pretreatment and Test Condition at each Dose were conducted. Visual inspection of Figure 4 suggests that the interactions obtained in the initial analysis (the analysis in which Dose was included as a factor) can be attributed to an unusually high level of footshock-induced responding in the No Drug pretreatment

Figure 4. Mean (±SEM) number of responses on the inactive lever in the 3-hour tests for reinstatement (Saline, Cocaine, Footshock) for No Drug and Drug, 0.75 or 1.5 mg/kg diazepam, pretreatment conditions, $p<.05$.

Effect of No Drug and Drug pretreatment on responding on the inactive lever



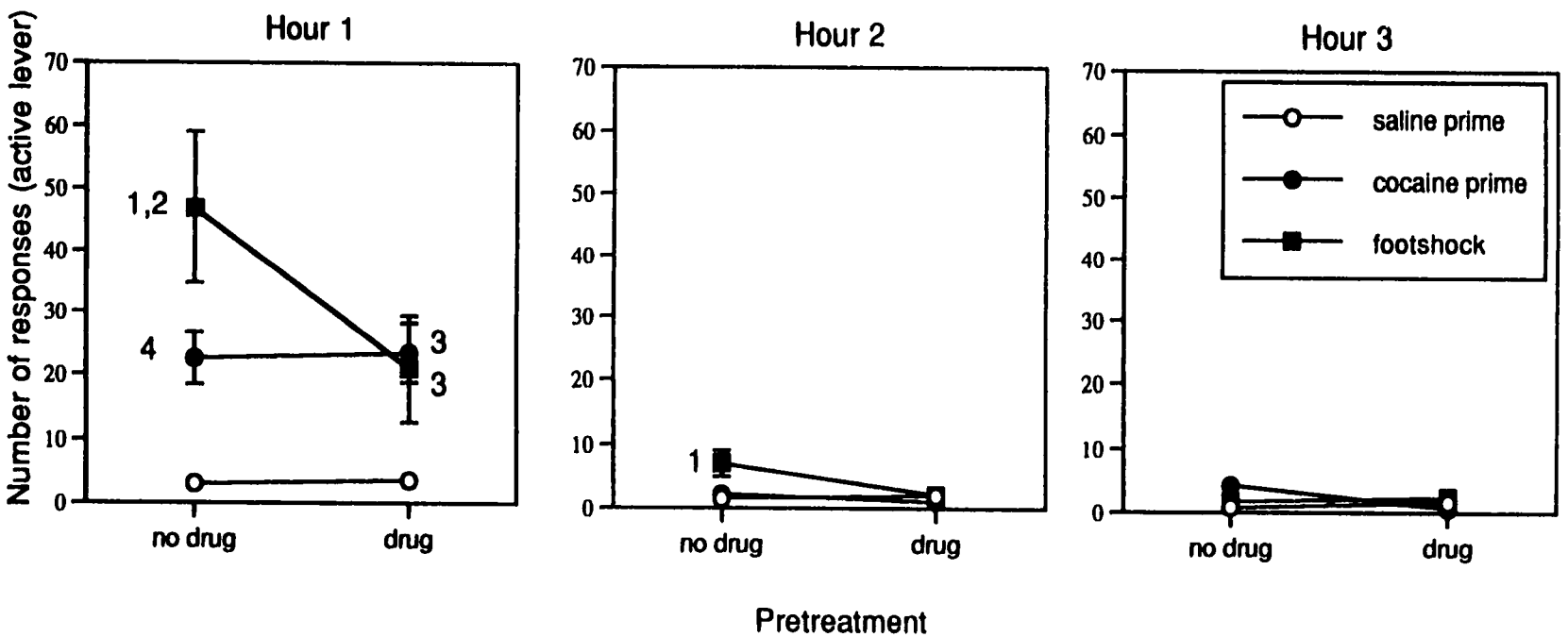
condition by animals assigned to the low but not high dose of diazepam. This apparent discrepancy in footshock-induced level of responding on the inactive lever between the two dose groups is puzzling since there were no differences in handling between the groups. A similar explanation would appear to account for the significant effect for Pretreatment ($F[1,12]=5.61$, $p<.05$) and for the Pretreatment X Test Condition interaction ($F[1,12]=5.29$, $p<.05$) when Cocaine was a test condition. Overall, therefore, the analyses conducted for responses on the inactive lever do not provide evidence of diazepam-induced suppression of activity. However, as a final precaution, an additional analysis was carried out using difference scores (number of responses on the active lever minus number of responses on the inactive lever). These scores were entered into mixed-factor ANOVAs for the factors of Pretreatment, Test Condition, and Diazepam Dose. With one exception, these analyses and the subsequent post-hoc tests revealed the same pattern of results that was found when the analyses were done using only the responses on the active lever (see figure 3). The difference occurred in the Cocaine test condition where the interaction between Pretreatment X Test Condition was no longer significant ($F(1,12)= 1.89$, $p=.20$). This finding suggests that when the anomalous differences in responding on the inactive lever were factored out, cocaine-induced reinstatement of responding was similar in both the No Drug and Drug pretreatment conditions and, if anything, there was a slight *increase* in cocaine-induced responding after diazepam pretreatment.

Responses on the active lever in each hour of testing

Figure 5 presents the mean number of responses on the active lever during each hour of testing under the various Pretreatment and Test Conditions for the two dose groups combined. It can be seen that most of

Figure 5. Mean (±SEM) number of responses on the previously active lever during each hour of testing after the Saline (IV), Cocaine (2.0 mg/kg, IV) and footshock (10 minutes, 0.5 mA, 0.5 seconds on, mean off period of 40 seconds) primes for No Drug and Drug pretreatment conditions. 1 Different from Saline test (No Drug pretreatment); 2 Different from Footshock test (Drug pretreatment); 3 Different from Saline test (Drug pretreatment); 4 Cocaine tests (No Drug, Drug pretreatment) different from Saline tests (No Drug, Drug pretreatment), $p<.05$.

Effect of No Drug and Drug pretreatment on the reinstatement of cocaine-trained behaviour at each hour of testing



the responding occurred in the first hour of testing under all conditions. In Hour 1, footshock-induced responding was lower after the injection of diazepam (Drug) than it was after saline (No Drug); it can be seen that diazepam did not change the response to the priming injection of cocaine. Mixed-factor ANOVAs were conducted initially for the within-subjects factors of Pretreatment (No Drug, Drug), Test Condition (Saline, Cocaine; Saline, Footshock) and Hour of Testing (1,2,3) and the between-subjects factor of Diazepam Dose (0.75 mg/kg, 1.5 mg/kg). These analyses revealed no significant effects for Dose or for any of the interactions between Dose and any one or more of the other factors. The data from both dose groups were, therefore, combined and were entered into three-way repeated-measures ANOVAs (see Appendix V for source tables). In summary, both analyses revealed significant effects for Pretreatment and Hour (Test Condition was significant only for the cocaine analysis) plus a significant three-way interaction.

To further analyze both three-way interactions, repeated-measures analyses were conducted for the factors of Pretreatment and Test Condition at each Hour (see Appendix VI for source tables). In summary, significant effects were found during the first hour of testing for both analyses and also during the second hour of testing for the footshock analysis only. In both analyses there was a significant effect for Test Condition in Hour 1; in the footshock analysis only, the main effect for Pretreatment and a Pretreatment X Test Condition interaction were also significant. The analysis with footshock during the second hour of testing revealed a significant main effect for Test Condition and a Pretreatment X Test Condition interaction.

Significant post-hoc comparisons are indicated in Figure 5. These comparisons showed that, for the footshock analysis, the Pretreatment X Test

Condition interaction in hour 1 and hour 2 can be attributed to a higher level of responding after Footshock in the No Drug than Drug pretreatment condition and no difference in the Saline test condition between pretreatments; furthermore, in hour 1, responding after Footshock was higher than after Saline, regardless of Pretreatment Condition. Thus, diazepam attenuated, but did not block, the footshock-induced reinstatement of cocaine-trained behaviour observed after No Drug pretreatment. For the cocaine analysis, comparisons conducted in the first hour of testing for the main effect of Test Condition showed that animals responded more under Cocaine than Saline test conditions, regardless of pretreatment.

Discussion

The principle finding in Experiment 2 is that, under the conditions of the experiment, administration of the anxiolytic drug diazepam attenuates footshock-induced reinstatement of cocaine-trained behaviour in rats, but not reinstatement induced by cocaine. This study would appear to be the first suggesting that anxiety could play a role in *relapse* to cocaine-taking. There are two reasons, however, why it cannot be said that anxiety is a necessary condition for provoking relapse to cocaine-taking: First, diazepam did not block stress-induced relapse, and second, diazepam did not affect relapse induced by a priming injection of cocaine. Similar doses of diazepam to those used in the present study have been shown to effectively block behaviour patterns considered to reflect stress-induced (e.g., McNamara & Whishaw, 1990; Rex et al., 1996) and cocaine-induced (Ettenberg & Geist,1991) anxiety. It is not likely, therefore, that the doses of diazepam used in the present study were simply subthreshold. Since cocaine and footshock stress share some but not all neurochemical effects in common, and since in the present experiment

diazepam attenuated the effect of one event but not the other, the mechanisms mediating cocaine- and footshock-induced reinstatement of cocaine-taking appear to be pharmacologically dissociable. Similarities and differences in the stimulant effects of cocaine and footshock and possible ways in which such similarities and differences may account for the results of the present experiment will be considered in the General Discussion.

Also requiring consideration in Experiment 2 is the finding that diazepam itself did not induce the reinstatement of cocaine-trained behaviour. The fact that diazepam was without effect on the reinstatement of cocaine-trained behaviour is consistent with previous reports that diazepam (0.25 to 1.0 mg/kg, IM) does not reinstate cocaine-trained behaviour in monkeys (Slikker et al., 1984). The ineffectiveness of diazepam in inducing relapse to cocaine-taking, in this and a previous study (i.e., Slikker et al., 1984), may be attributable to differences between diazepam and cocaine in their pharmacological actions (see Carroll & Comer, 1996). The administration of reinforcing doses of a variety of drugs from different drug families do not reinstate responding for cocaine; examples include heroin, clonidine, methohexital, nicotine, ethanol, and pentobarbitol (de Wit & Stewart, 1981; Gerber & Stretch, 1975; Slikker et al., 1984; Wise et al., 1990).

Finally, on a number of grounds, it can be argued that diazepam did not produce a suppression in general motor activity in the present study. First, although locomotor activity was not monitored in this study, the doses of diazepam used here are, according to previous reports (e.g., Fanselow & Helmstetter, 1988; Hottsenpillar & Williams, 1996; Rex et al., 1996), subthreshold for producing deficits in locomotor activity. Second, analyses conducted for responding on the inactive lever did not reveal systematic

differences between Pretreatment Conditions, suggesting that diazepam did not produce global deficits in activity. Third, differences were not found between the No Drug and Drug pretreatment conditions under Saline test conditions, suggesting that diazepam did not alter baseline levels of responding. Finally, when pretreated with diazepam, animals showed footshock- and cocaine-induced reinstatement of responding that was higher than responding under Saline test conditions, further evidence that diazepam did not produce general motor deficits.

In summary, the same doses of diazepam that attenuated footshock-induced reinstatement of cocaine-trained behaviour were not effective in altering reinstatement induced by a priming injection of cocaine. Furthermore, diazepam itself did not modify reinstatement of responding for cocaine.

General Discussion

The present experiments provide the first evidence for reinstatement of cocaine-trained behaviour by brief exposure to an acute stressor. In both Experiments 1 and 2, footshock stress was shown to be at least as effective as a priming injection of cocaine in reinstating cocaine-trained behaviour. In Experiment 1, the reinstatement of cocaine-taking by footshock stress and by a priming injection of cocaine was found not only after 4 to 11 days of extinction but also after an additional four- to six-week drug-free period. In Experiment 2, it was found that pretreatment with the anxiolytic drug diazepam attenuated footshock- but not cocaine-induced reinstatement of cocaine-trained behaviour.

Since in Experiments 1 and 2 footshock stress and a priming injection of cocaine were both effective in reinstating cocaine-trained behaviour, superficially there would appear to be no difference between reinstatement of

cocaine-taking by footshock or by cocaine. This observation, in combination with evidence that acute stressors and psychostimulant drugs such as cocaine share a number of common neurochemical effects, suggests that footshock stress may reinstate cocaine-trained behaviour by mimicking the actions of a priming injection of cocaine. This same hypothesis was initially proposed by Shaham & Stewart (1995b) to account for their finding of a comparable magnitude of reinstatement by footshock stress and a priming injection of heroin in heroin-experienced animals; in subsequent studies, however, they showed pharmacologically that the processes mediating these two effects are in fact not identical. For example, Shaham and Stewart (1996) showed that the selective D1-like receptor antagonist SCH 23390, the selective D2-like receptor antagonist raclopride, and the opioid antagonist naltrexone each blocked heroin-induced reinstatement, but were without effect on footshock-induced reinstatement. Additionally, in rats experienced in heroin self-administration, heroin had a greater effect on DA overflow in the NAcc and on locomotor activity than did footshock stress. Finally, Shaham et al. (1996) showed that a "maintenance" dose of heroin (delivered continuously throughout extinction and testing via an Alzet osmotic minipump) attenuated heroin-induced reinstatement of heroin-taking behaviour but was without effect on reinstatement induced by footshock stress. These studies provide convincing evidence that the neurochemical events underlying heroin- and footshock-induced reinstatement of heroin-taking behaviour are not identical. Cocaine and footshock stress, like heroin and footshock stress, have some neurochemical actions in common; the neurochemical dissociations found between the effects of the drug and the stressor in inducing the reinstatement of heroin-taking behaviour, however, raise the possibility that the neurochemical events mediating cocaine- and

footshock-induced reinstatement of cocaine-trained behaviour may also be dissociable.

Possible Mechanisms Mediating the Reinstatement of Cocaine-Trained behaviour by Footshock Stress and a Priming Injection of Cocaine

A number of neurochemical and neurophysiological similarities and differences between the effects of an acute injection of cocaine and exposure to footshock stress need to be considered when speculating about possible mechanisms mediating the reinstatement of cocaine-trained behaviour by each event. First, however, it is important to recall that although initial use of cocaine is associated with a positive affect in humans (Gawin, 1991; Gawin & Ellinwood, 1988; Kumor et al., 1989; Van Dyke et al., 1982) and is readily self-administered by monkeys and rats (Roberts, 1992; Wise & Bozarth, 1987), with continued use of the drug anxiogenic effects associated with, for example, increased heart rate, blood pressure, and respiration, also emerge. As was discussed in the Introduction, Ettenberg and Geist (1991; 1993) demonstrated the concurrent positive and negative effects associated with repeated cocaine use; they showed that, in rats trained to traverse a runway for IV infusions of cocaine, successive trials were associated, increasingly, with a conflict approach-avoidance behaviour as the animal arrived at the goal box.

The mesocorticolimbic DA system is considered the primary system mediating the appetitive-reinforcing effects of chronic cocaine use (e.g., Wise, 1984), whereas activity within the ascending NA systems (Redmond & Huang, 1979) and the HPA (hypothalamic-pituitary-adrenal) axis (e.g., File, Zangrossi, Sanders, & Mabbutt, 1994) is considered to be related to the anxiogenic effects

of the drug. Since both an acute injection of cocaine and footshock stress increase extracellular DA and NA, and since both also activate the HPA axis, it is conceivable that any one or more of these actions underlie reinstatement induced by them. In the next sections, several hypotheses that focus on similar and dissociable effects of cocaine and footshock stress, and that may account for the results obtained in Experiments 1 and 2, will be considered.

Hypothesis I: The mesocorticolimbic DA system mediates footshock- and cocaine-induced reinstatement of cocaine-trained behaviour.

Consider first the possibility that both cocaine and footshock stress reinstate cocaine-trained behaviour through their actions on mesocorticolimbic DA transmission. Both increase extracellular DA in the terminal regions of the neurons but through different mechanisms.

In Experiment 2, diazepam, a drug that among its other actions reduces the release of DA in the NAcc (Invernizzi, Pozzi & Samanin, 1991), attenuated footshock but not cocaine-induced reinstatement of cocaine-trained behaviour. Rather than implying a difference in the neurochemical events underlying reinstatement by footshock and by cocaine, this dissociation might be explained in terms of differences in the effects of diazepam on footshock- and cocaine-induced changes in mesocorticolimbic DA transmission. As was discussed in the Introduction, cocaine acts to block DA reuptake and, as a consequence, increases the concentration of DA in the terminal region available for postsynaptic activation; footshock stress, on the other hand, increases the concentration of DA at the terminal through DA cell firing (Maeda & Mogenson, 1982). Since cocaine acts to block DA reuptake, any DA released after an acute injection of the drug should have the opportunity to bind postsynaptically.

In addition, there is evidence that with repeated exposure to cocaine higher levels of extracellular DA per unit dose of cocaine are available in terminal regions (e.g., Kalivas & Duffy, 1993a; Kalivas et al., 1993; Pettit et al., 1990). Thus, it is possible that, even in the presence of diazepam's potential inhibitory effect on neurotransmission, following an injection of cocaine, enough DA will enter the synapse to effectively activate postsynaptic neurons. Footshock stress, on the other hand, causes increases in NAcc DA (e.g., Shaham & Stewart, 1996) by increasing cell firing; clearly, diazepam would reduce the availability of DA in the synapse. Furthermore, whatever DA was able to enter the terminal region could, and likely would through reuptake, be taken back into the cell.

The explanation just offered for the differential effects of diazepam on footshock- and cocaine-induced reinstatement of cocaine-trained behaviour is largely speculative. Indirect evidence for the proposed effect of diazepam on footshock stress comes from reports that 5.0 mg/kg diazepam reverses stress-induced increases in NAcc levels of DOPAC in response to acute tail pinch stress (D'Angio, Serrano, Rivy, & Scatton, 1987). Similarly, 2.0 mg/kg diazepam was shown to block immobilization stress-induced increases in extracellular DOPAC in the NAcc (Serrano, D'Angio, & Scatton, 1989). Additionally, 5.0 mg/kg but not 1.0 mg/kg diazepam has been found to significantly reduce extracellular levels of DA, DOPAC, and HVA in the NAcc (Invernizzi et al., 1991). In Experiment 2, diazepam (0.75 and 1.5 mg/kg) partially antagonized the effect of footshock on reinstatement of responding at lower doses than those that have been shown previously to block stress-induced increases in DOPAC in the NAcc (D'Angio et al., 1987; Serrano et al., 1989). This, in combination with the report that, in itself, 1.0 mg/kg diazepam

has no effect on extracellular levels of DA in the NAcc (Invernizzi et al., 1991), suggests that the doses of diazepam used in Experiment 2 may have been too low to reduce stress-induced increases in DA activity to the degree necessary for the footshock effect to be blocked.

Diazepam, at the doses used in Experiment 2, did not attenuate reinstatement of responding by a priming injection of cocaine; however, since diazepam inhibits DA neurotransmission, and since cocaine reinforcement is believed to be mediated primarily by mesocorticolimbic DA (e.g., Wise, 1984), diazepam might be expected to reduce the reinforcing efficacy of cocaine. Evidence that benzodiazepines alter the reinforcing efficacy of cocaine has been found in self-administration studies with rats. Goeders, McNulty, Mirkis, & McAllister (1989) found that a range of doses of CDP (0.3 to 1.0 mg/kg) increased drug intake in animals self-administering 0.5 mg/kg/infusion of cocaine, whereas a higher dose of CDP (10 mg/kg) decreased drug-intake; in animals that self-administered 1.0 mg/kg/infusion cocaine, on the other hand, low doses of CDP did not affect drug-intake while the high dose significantly reduced drug-intake (see also Goeders, 1992). These findings suggest that CDP reduced the reinforcing efficacy of cocaine by opposing the pharmacological effects of the drug; since the reinforcing effects of cocaine are considered to be mediated primarily by mesocorticolimbic DA (e.g., Wise, 1984), it would seem reasonable to suggest that diazepam interfered with cocaine reinforcement through its action on DA neurotransmission.

Although benzodiazepines appear to reduce the reinforcing efficacy of cocaine self-administration (Goeders, 1992; Goeders et al., 1989), it is not surprising that diazepam did not modify cocaine-induced reinstatement in Experiment 2. According to the hypothesis that mesocorticolimbic DA mediates

footshock and cocaine-induced reinstatement, diazepam does not modify cocaine-induced reinstatement, under the conditions of Experiment 2, because enough DA is able to reach the synapse to, in a sensitized mesocorticolimbic DA system, cause sufficient postsynaptic activation to provoke relapse. de Wit and Stewart (1981) showed that a range of IV cocaine priming doses (0.25 to 4.0mg/kg) effectively reinstated cocaine-trained behaviour. In the present experiment, a priming dose falling well within this range, 2.0 mg/kg, was used; therefore, even if diazepam did alter the reinforcing efficacy of the priming injection, as the results of Goeders and colleagues (1989) would suggest, it does not appear that it did so to the degree necessary for the priming injection to be pharmacologically ineffective. It is conceivable that if either a higher dose of diazepam and/or a lower priming dose of cocaine was used, diazepam may also interfere in the effectiveness of a priming injection of cocaine to induce relapse. Therefore, it is possible that at certain doses of diazepam and cocaine, diazepam may interfere in the ability of cocaine to provoke relapse by interfering in the neurotransmission of pathways mediating the reinforcing effects of cocaine.

Clearly, the degree to which mesocorticolimbic DA mediates footshock- and cocaine-induced reinstatement of cocaine-trained behaviour requires direct empirical testing. A number of possibilities for testing the hypothesis will be considered shortly.

Hypothesis II: NA and DA underlie footshock- and cocaine-induced reinstatement of cocaine-trained behaviour

Chronic cocaine-taking is associated not only with effects related to its actions on the mesocorticolimbic DA system, but also, it is associated with

physiological symptoms of anxiety, such as increased heart rate, blood pressure, and respiration. These autonomic responses occur, at least in part, as the result of the actions of NA systems (Redmond & Huang, 1979). An alternative hypothesis to Hypothesis I, therefore, is that NA as well as DA underlies the reinstatement of cocaine-trained behaviour. Both footshock stress and cocaine act on DA and NA systems, suggesting that the physiological and psychological effects associated with the actions of both DA and NA could be important for inducing a "drug-like" motivational state facilitating the reinitiation of cocaine-trained behaviour.

As was mentioned in Experiment 2, release of NA by neurons originating in the locus coeruleus, is associated with fear and anxiety (Kendel et al., 1991; Redmond & Huang, 1979). Furthermore, administration of benzodiazepine-receptor agonists, such as diazepam, reportedly inhibits footshock-induced increases in cortical NA neurotransmission (Rossetti, Portas, Pani, Carboni , & Gessa, 1990). It is conceivable, therefore, that in Experiment 2 diazepam attenuated footshock-induced reinstatement of cocaine-taking by altering stress-induced NA release.

In general, the effects of footshock, cocaine, and diazepam on NA systems are similar to those described above for the DA systems; footshock increases NA neurotransmission, cocaine blocks NA reuptake, and diazepam inhibits NA neurotransmission. Therefore, if NA did play a role in reinstatement by both cocaine and footshock, the same explanation offered in Hypothesis I concerning DA might apply to NA; that is, the differential effect of diazepam on footshock and cocaine might be due to the differences in the way in which the manipulation alters NA availability. This argument, however, relies on the assumption that NA systems, like the mesocorticolimbic DA system, become

sensitized with repeated exposure to cocaine; that is, a sensitized NA system would help to explain how enough NA is available in the synapse to act effectively postsynaptically in the presence of diazepam-induced inhibition of neurotransmission. To date, psychostimulant-induced sensitization of NA systems has not been demonstrated; however, there is some evidence that animals repeatedly exposed to chronic cold stress exhibit enhanced NA efflux in the medial prefrontal cortex (Gresch, Sved, Zigmond, & Findlay, 1994) and enhanced hippocampal release of NA (Nisenbaum, Zigmond, Sved, & Abercrombie, 1991) in response to subsequent tail pinch stress (see also Anisman & Zacharko, 1986). These findings suggest that under conditions of chronic stress, sensitization of NA systems occurs; it is possible, therefore, that psychostimulant-induced sensitization of these systems may also occur.

It is also possible that NA mediates reinstatement of cocaine-trained behaviour, at least in part, through its actions on mesocorticolimbic DA transmission. Stimulation of the locus coeruleus, for example, has been shown to elicit increases in mesocorticolimbic DA cell firing (Grenhoff, Nissell, Ferre, Aston-Jones, & Svensson, 1993), while toxin-induced depletion of NA reportedly suppresses mesocorticolimbic DA release (Lategan, Marien, & Colpaert, 1992). It is possible that the modulatory effect of NA on mesocorticolimbic DA is important in the case of footshock-induced reinstatement; that is, in Experiment 2 the inhibition of NA-induced DA cell firing by diazepam may have attenuated the ability of footshock to provoke relapse to cocaine-trained behaviour. Diazepam, therefore, may attenuate footshock-induced reinstatement of cocaine-trained behaviour indirectly, by NA-induced inhibition of mesocorticolimbic DA cell firing and directly by inhibition of NA (see above) and DA (see Hypothesis I) cell firing. In the case of cocaine-induced

reinstatement, it may be argued, as before, that even in the presence of diazepam enough DA and NA will enter the synapse to effectively activate postsynaptic neurons.

Hypothesis III: CRF mediates the effects of footshock and cocaine on the reinstatement of cocaine-trained behaviour.

Corticotropin releasing factor (CRF) is a peptide of the hypothalamus that acts directly in the brain, as well as being involved in the pituitary release of ACTH. Increased CRF release, like increased NA release, is associated with physiological symptoms of anxiety such as increased heart rate and blood pressure (e.g., Fisher et al., 1982). In fact, CRF and NA have been shown to interact in a number of ways. For example, administration of a CRF antagonist attenuates immobilization stress-induced release of NA in the medial prefrontal cortex (Shimizu, Nakane, Hori, & Hayashi, 1994) and, likewise, ICV administration of CRF, itself, has been found to enhance NA release in the hypothalamus (Emoto, Yokoo, Yoshida, & Tanaka, 1993). Recently, several behavioral studies have been conducted to consider the possible role of CRF in stress-induced anxiety. For example, rats that are exposed to social defeat stress and are subsequently placed in a plus maze spend less time in the open arms of the maze relative to non-stressed rats; the anxiogenic response exhibited by socially-defeated rats is blocked by pretreatment with a CRF antagonist (Manzaghi et al., 1994). Likewise, intracerebroventricular (ICV) injections of CRF suppress reinforced responding in a conflict test (Britton, Morgan, Rivier, Vale & Koob, 1985) and potentiate acoustic startle responses (Swerdlow, Geyer, Vale, & Koob, 1986); in both cases, however, pretreatment with CDP attenuates the CRF-induced anxiogenic responses. It is possible, therefore, that in the present experiments, diazepam attenuated footshock-

induced reinstatement of cocaine-trained behaviour by inhibiting CRF release. In support of this hypothesis, it has been found recently that CRF delivered into the lateral ventricles reinstates heroin-taking behaviour in rats (Shaham et al., in preparation). Clearly, the role of CRF in relapse warrants further investigation.

Consideration of a general activation of behaviour hypothesis of footshock-induced relapse

One question that arises concerning the ability of stress to reinstate drug-taking behaviour is whether it does so through a general arousal and activation of behaviour. For example, it may be argued that in Experiments 1 and 2, footshock stress resulted in a general behavioural activation facilitating lever pressing and that, in Experiment 2, pretreatment with diazepam interfered in this activation by making animals more relaxed, in general. In studies of the footshock-induced reinstatement of heroin-taking behaviour, this general-activation hypothesis of footshock-induced relapse has been considered in a number of ways. First, Shaham & Stewart (1996) showed that the magnitude of the reinstatement effect was not correlated with the magnitude of the effect on either locomotor activity or on levels of extracellular DA in the NAcc. A dose of heroin that was more effective than footshock in stimulating locomotor activity and DA in the NAcc tended to be less effective than footshock in reinstating heroin-taking behaviour; furthermore, pharmacological studies have indicated that the neurochemical mechanisms underlying the two effects are not identical (Shaham et al., 1996; Shaham & Stewart, 1996). Additionally, Shaham et al. (in-press) showed that exposure to other highly arousing events, such as presentation of a sexually-receptive female without physical access, and copulation with the receptive female, do not induce reinstatement of heroin-

taking in the heroin-trained male rat. Finally, precipitated opioid withdrawal, a condition known to cause hyperactivity (see Bhargava, 1994), does not reinstate heroin-taking behaviour in rats (Shaham & Stewart, 1995b; Shaham & Stewart, 1996).

On the basis of the findings just discussed, it does not appear that footshock stress reinstates heroin-taking behaviour through a general arousal and activation of behaviour. Since, in heroin-experienced animals, the magnitude of the reinstatement effect does not correlate with either locomotor activity or extracellular levels of DA in the NAcc, it is unlikely that such correlations would be found in cocaine-experienced animals.

Summary of Hypothesized Neurochemical Mechanisms Mediating Cocaine- and Footshock-Induced Reinstatement of Cocaine Seeking

Three hypotheses have been proposed here to try to explain the results obtained in Experiments 1 and 2. Although it is convenient for the purposes of discussion to consider potential mechanisms of action separately, it is both possible and likely that all three of these mechanisms contribute to reinstatement of responding by footshock stress and cocaine. Not only is activation of DA, NA, and CRF systems common to both footshock stress and cocaine but, in addition, all three systems have been shown to interact with one another (see, for example, Emoto et al., 1993; Grenhoff et al., 1993; Lategan et al., 1992; Shimizu et al., 1994).

Future Directions

A number of pharmacological and neurochemical studies need to be carried out to determine the degree to which DA, NA, and CRF, as well as other

systems such as 5-HT systems, mediate reinstatement of cocaine-taking by footshock stress and by a priming injection of cocaine.

As was discussed previously, Shaham and Stewart (1996) investigated the role of DA in footshock- and heroin-induced reinstatement of heroin-taking behaviour by administering a variety of selective and non-selective DA antagonists before the various tests for reinstatement. A similar experiment could be carried out in rats experienced in cocaine self-administration to determine the role of DA in footshock- and cocaine-induced reinstatement of responding. Furthermore, the degree to which DA and NA underlie cocaine- and footshock-induced reinstatement of cocaine-trained behaviour could be investigated by considering the effects of not only DA antagonists, but also NA antagonists, on reinstatement by footshock stress and by cocaine. In conjunction with pharmacological studies aimed at determining the degree to which DA and NA mediate footshock and cocaine-induced reinstatement, it may be interesting to determine whether psychostimulant-induced sensitization in fact occurs within NA systems. Additionally, a number of parallel microdialysis and reinstatement studies could be carried out in an attempt to further determine whether diazepam attenuates footshock-induced reinstatement through its actions on DA and NA neurotransmission.

Experiments also need to be conducted to investigate the effects of CRF on the reinstatement of cocaine-trained behaviour. A first step would be to determine whether an ICV injection of CRF, itself, reinstates cocaine-trained behaviour as, it would appear, it does heroin-taking behaviour (Shaham et al., in preparation). Next, it could be considered whether a CRF antagonist or antibody would interfere in footshock- or cocaine-induced relapse; administration of such a compound before footshock and cocaine tests for

reinstatement would provide a viable way of determining to what degree CRF underlies the ability of each of these stimuli to provoke relapse.

Implications of the Present Findings for Relapse Prevention Treatment Strategies

The present findings have a number of implications in terms of considering future relapse prevention strategies for human cocaine addicts. The fact that stress-induced relapse was attenuated by administration of diazepam suggests that suppressing stress responses related to, for example, increased NA release and increased activity of the HPA axis during abstinence, may provide a viable way of preventing stress-induced relapse to drug-taking in human cocaine addicts.

Although pharmacological modification of physiological stress responses may represent a promising strategy for the prevention of relapse to cocaine-taking under certain conditions, the present studies suggest that if cocaine is taken, such interventions are not likely to be effective in preventing a full relapse; in Experiment 2, diazepam did not attenuate the reinstatement of cocaine-trained behaviour by cocaine, itself. Furthermore, one of the most common events reported to induce relapse to cocaine-taking is exposure to stimuli associated with the drug-taking environment (Childress et al., 1986; see also Gawin, 1991); it is well-known that such conditioned stimuli activate the appetitive systems facilitating motivation to seek drug, in particular the mesocorticolimbic DA system (Stewart, 1992). It would seem important, therefore, that any cocaine relapse prevention strategy also consider a probable role for DA in relapse to cocaine-taking.

Clearly, further preclinical characterization of the factors mediating relapse to cocaine-taking is required. The present findings, however, suggest that a complex of neurochemical systems is likely to be involved and that pharmacological interventions aimed at modifying NA and DA neurotransmission may, in conjunction with other therapies, provide a useful approach to treating cocaine addiction.

References

Akil, H.A., & Morano, M.I. (1995) Stress. In: *Psychopharmacology: The Fourth Generation of Progress*, F.E. Bloom & D.J. Kupfer, eds., Raven Press, New York, pp. 773-785.

Alcoholics Anonymous. (1955) Alcoholics Anonymous World Services Inc., New York.

Anisman, H., & Zacharko, R.M. (1986) Behavioral and neurochemical consequences associated with stressors. *Annals of the New York Academy of Science, 467*, 205-225.

Anthony, J.C., Tien, A.Y., & Petronis, K.R. (1989) Epidemiologic evidence on cocaine use and panic attacks. *American Journal of Epidemiology, 129*, 543-549.

Bhargava, H.N. (1994) Diversity of agents that modify opioid tolerance, physical dependence, abstinence syndrome and self-administrative behavior. *Pharmacological Review, 46*, 293-324.

Bigelow, G.E., Griffiths, R.R., & Liebson, I.A. (1977) Pharmacological influences upon human ethanol self-administration. In: *Alcohol Intoxication and Withdrawal*, M.M. Gross, ed., Plenum Press, New York, pp. 523-538.

Bozarth, M.A., & Wise, R.A. (1985) Toxicity associated with long-term intravenous heroin and cocaine self-administration in the rat. *Journal of the American Medical Association, 254*, 81-83.

Britton, K.T., Jr., Morgan, J., Rivier, W. Vale, & Koob, G.F. (1985) Chlordiazepoxide attenuates response suppression induced by corticotropin-releasing factor in the conflict test. *Psychopharmacology, 86,* 170-174.

Buckland, C., Mellanby, J., & Gray, J.A. (1986) The effects of compounds related to γ-aminobutyrate and benzodiazepine receptors on behavioural responses to anxiogenic stimuli in the rat: Extinction and successive discrimination. *Psychopharmacology, 88,* 285-295.

Carroll, M.E., & Comer, S.D. (1996) Animal models of relapse. *Experimental and Clinical Psychopharmacology, 4,* 11-18.

Chornock, W.M., Stitzer, M.L., Gross, J., & Leischow, S. (1992) Experimental model of smoking re-exposure: Effects on relapse. *Psychopharmacology, 108,* 495-500.

Childress, A.R., Ehrman, R., McLellen, A.T., & O'Brien, C. (1987) Conditioned craving and arousal in cocaine addiction: A preliminary report. *National Institute of Drug Addiction Monograph Series, 81,* 74-80.

Childress, A.R., Ehrman, R., Rohsenow, D.J., Robbins, S.J., O'Brien, C.P. (1992) Classically conditioned factors in drug dependence. In: *Substance abuse: A comprehensive textbook, 2nd ed.,* J.W. Lowinson, P. Luiz, R.B. Millman, & G. Langard, eds., Williams and Wilkins, Baltimore, pp. 56-69.

Childress, A.R., McLellen, A.T., Ehrman, R.N., & O'Brien, C.P. (1986) Extinction of conditioned responses in abstinent cocaine or opioid users. *National Institute of Drug Addiction Monograph Series, 76,* 189-195.

Cooper, J.R., Bloom, F.E., & Roth, R.H. (1996) *The Biochemical Basis of Neuropharmacology*, Oxford University Press, Oxford.

Comer, S.D., Lac, S.T., Curtis, L.K., & Carroll, M.E. (1993) Effects of buprenorphine and naltrexone on reinstatement of cocaine-reinforced responding in rats. *The Journal of Pharmacology and Experimental Therapeutics*, *267*, 1470-1477.

Crawley, J.N., Ninan, P.T., Pickar, D., Chrousos, G.P., Linnoila, M., Skolnick, & P., Paul, S.M. (1985) Neuropharmacological antagonism of the beta-carboline-induced "anxiety" response in rhesus monkeys. *The Journal of Neuroscience*, *5*, 477-485.

D'Angio, M., Serrano, A., Rivy, J.P., Scatton, B. (1987) Tail-pinch stress increases extracellular DOPAC levels (as measured by in vivo voltammetry) in the rat nucleus accumbens but not frontal cortex: Antagonism by diazepam and zolpidem. *Brain Research*, *409*, 169-174.

Davis, W.M., & Smith, S.G. (1976) Role of conditioned reinforcers in the initiation, maintenance and extinction of drug-seeking behavior. *Pavlovian Journal*, 11, 222-236.

de Wit, H. (1996) Priming effects with drugs and other reinforcers. *Experimental and Clinical Psychopharmacology*, *4*, 5-10.

de Wit, H., & Stewart, J. (1981) Reinstatement of cocaine-reinforced responding in the rat. *Psychopharmacology*, *75*, 134-143.

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

Emoto, H., Yokoo, H., Yoshida, M., & Tanaka, M. (1993) Corticotropin-releasing factor enhances noradrenaline release in the rat hypothalamus assessed by intracerebral microdialysis. *Brain Research, 601*, 286-288.

Erb, S.M., & Parker, L.A. (1994) Individual differences in novelty-induced activity do not predict strength of amphetamine-induced place conditioning. *Pharmacology, Biochemistry and Behavior, 48*, 589-596.

Ettenberg, A., & Geist, T.D. (1991) Animal model for investigating the anxiogenic effects of self-administered cocaine. *Psychopharmacology, 103*, 455-461.

Ettenberg, A., & Geist, T.D. (1992) Qualitative and quantitative differences in the operant runway behavior of rats working for cocaine and heroin reinforcement. *Pharmacology, Biochemistry and Behavior, 44*, 191-198.

Fanselow, M.S., & Helmstetter, F.J. (1988) Conditioned analgesia, defensive freezing, and benzodiazepines. *Behavioral Neuroscience, 102*, 233-243.

Feldon, J., & Gray, J.A. (1981) The partial reinforcement extinction effect after treatment with chlordiazepoxide. *Psychopharmacology, 73*, 269-275.

File, S.E., Zangrossi, H., Jr., Sanders, F.L., & Mabbutt, P.S. (1994) Raised corticosterone in the rat after exposure to the elevated plus-maze. *Psychopharmacology, 113*, 543-546.

Fisher, L.A., Rivier, J., Rivier, C., Spiess, J., Vale, W., & Brown, M.R. (1982) Corticotropin-releasing factor (CRF): central effects on mean arterial pressure and heart rate in rats. *Endocrinology, 110*, 2222-2224.

Fontana, D.J., & Commissaris, R.L. (1989) Effects of cocaine and conflict behavior in the rat. *Life Sciences, 45*, 819-827.

Gawin, F.H. (1991) Cocaine addiction: Psychology and neurophysiology. *Science, 251*, 1580-1586.

Gawin, F.H., & Ellinwood, E.H., Jr. (1988) Cocaine and other stimulants: Actions, abuse, and treatment. *The New England Journal of Medicine, 318*,1173-1182.

Gerber, G.J., & Stretch, R. (1975) Drug-induced reinstatement of extinguished self-administration behavior in monkeys. *Pharmacology, Biochemistry and Behavior, 3*, 1055-1061.

Gerber, G.J., & Wise, R.A. (1989) Pharmacological regulation of intravenous cocaine and heroin self-administration in rats: A variable dose paradigm. *Pharmacology, Biochemistry and Behavior, 32*, 527-531.

Goeders, N.E. (1992) Potential involvement of anxiety in the neurobiology of cocaine. *Annals of the New York Academy of Sciences, 654*, 357-367.

Goeders, N.E., & Guerin, G.F. (1994) Non-contingent electric footshock facilitates the acquisition of intravenous cocaine self-administration in rats. *Psychopharmacology, 114*, 63-70.

Goeders, N.E., McNulty, M.A., Mirkis, S., & McAllister, K.H. (1989) Chlordiazepoxide alters intravenous cocaine self-administration in rats. *Pharmacology, Biochemistry and Behavior, 33*, 859-866.

Gong, W., Neill, D.B., & Justice, J.B., Jr. (1996) Locomotor response to novelty does not predict cocaine place preference conditioning in rats. *Pharmacology, Biochemistry and Behavior, 53*, 191-196.

Grenhoff, J., Nissell, M., Ferre, S., Aston-Jones, G., & Svensson, T.H. (1993) Noradrenergic modulation of midbrain dopamine cell firing elicited by stimulation of the locus coeruleus in the rat. *Journal of Neural Transmission, 93*, 11-25.

Gresch, P.J., Sved, A.F., Zigmond, M.J., & Finlay, (1994) Stress-induced sensitization of dopamine and norepinephrine efflux in medial prefrontal cortex of the rat. *Journal of Neurochemistry, 63*, 575-583.

Haney, M., Maccari, S., Le Moal, M., Simon, H., Piazza, P.V. (1995) Social stress increases the acquisition of cocaine self-administration in male and female rats. *Brain Research, 698*, 46-52.

Hodgson, R., Rankin, H., & Stockwell, T. (1979) Alcohol dependence and the priming effect. *Behavior Research and Therapy, 17*, 379-387.

Holman, R.B. (1994) Biological effects of central nervous system stimulants. *Addiction, 89*, 1435-1441.

Hotsenpiller, G., & Williams, J.L. (1996) Conditioned fear and analgesia to conspecific odors: Benzodiazepine and 5-HT1A agonists. *Psychobiology, 24*, 118-126.

Invernizzi, R., Pozzi, L., & Samanin, R. (1991) Release of dopamine is reduced by diazepam more in the nucleus accumbens than in the caudate nucleus of conscious rats. *Neuropharmacology, 30*, 575-578.

Izenwasser, S., Blake, M.J., Goeders, N.E., & Dworkin, S.I. (1989) Punishment modifies the effects of chlordiazepoxide and benzodiazepine receptors. *Pharmacology, Biochemistry and Behavior, 32,* 743-748.

Jaffe, J.H. (1985) Drug addiction and drug abuse. In A.G. Gilman, L.S. Goodman, & A. Gilman, eds., *The Pharmacological Basis of Therapeutics,* Macmillan Press: New York, pp. 532-581.

Jaffe, J.H., Cascella, N.G., Kumor, K.M., & Sherer, M.A. (1989) Cocaine-induced cocaine craving. *Psychopharmacology, 97,* 59-64.

Kalivas, P.W., & Duffy, P. (1993a) Time course of extracellular dopamine and behavioral sensitization to cocaine. I. Dopamine axon terminals. *The Journal of Neuroscience, 13,* 266-275.

Kalivas, P.W., & Duffy, P. (1993b) Time course of extracellular dopamine and behavioral sensitization to cocaine. II. Dopamine Perikarya. *The Journal of Neuroscience, 13,* 276-284.

Kalivas, P.W., Sorg, B.A., & Hooks, M.S. (1993) The pharmacology and neural circuitry of sensitization to psychostimulants. *Behavioral Pharmacology, 4,* 315-334.

Kalivas, P.W., & Stewart, J. (1991) Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. *Brain Research Reviews, 16,* 223-244.

Kandel, E.R. (1991) Disorders of mood: Depression, mania, and anxiety disorders. In: *Principals of Neural Science, 3rd ed.*, E.R. Kandel, J.H. Schwartz, & T.M. Jessel, eds., Appleton & Lange, Connecticut.

Kuhar, M.J., Ritz, M.C. & Boja, J.W. (1991) The dopamine hypothesis of the reinforcing properties of cocaine. *Trends in Neuroscience, 14*, 299-302.

Kumor, K.M., Sherer, M.A., Gomez, J., Cone, E., & Jaffe, J.H. (1989) Subjective response during continuous infusion of cocaine. *Pharmacology , Biochemistry and Behavior, 33*, 443-452.

Lategan, A.J., Marien, M.R., & Colpaert, F.C. (1992) Suppression of nigrostriatal and mesolimbic dopamine release following noradrenaline depletion by DSP-4: A microdialysis study. *Life Sciences*, 50, 995-999.

Ludwig, A.M., & Stark, L.H. (1974) Alcohol craving: Subjective and situational aspects. *Quarterly Journal of Studies in Alcohol, 35*, 899-905.

Ludwig, A.M., & Wikler, A. (1974) "Craving" and relapse to drink. *Quarterly Journal of Studies on Alcohol, 35*, 108-130.

Maeda, H., & Mogenson, G.J. (1982) Effects of peripheral stimulation on the activity of neurons in the ventral tegmental area, substantia nigra, and midbrain reticular formation of rats. *Brain Research Bulletin, 8*, 7-14.

McKay, J.R., Rutherford, M.J., Alterman, A.I., Cacciola, J.S., & Kaplan, M.R. (1995) An examination of the cocaine relapse process. *Drug and Alcohol Dependence, 38*, 35-43.

Markou, A., & Koob, G.F. (1991) Postcocaine anhedonia: An animal model of cocaine withdrawal. *Neuropyshopharmacology, 4*, 17-26.

McNamara, R.K., & Whishaw, I.Q. (1990) Blockade of hoarding in rats by diazepam: An analysis of the anxiety and object value hypotheses of hoarding. *Psychopharmacology, 101*, 214-221.

Meyer, R.E., Mirin, S.M. (1979) *The Heroin Stimulus: Implications for a Theory of Addiction.* Plenum Medical Book Company, New York.

Menzaghi, F., Howard, R.L., Heinrichs, S.C., Vale, W., Rivier, J., Koob, G.F. (1994) Characterization of a novel and potent corticotropin-releasing factor antagonist in rats. *The Journal of Pharmacology and Experimental Therapeutics, 269*, 564-572.

Miczek, K., & Mutschler, N. (1996) Activational effects of social stress on IV cocaine self-administration in rats. *Psychopharmacology*, in press.

Morgan, C.T., & Fields, P.E. (1938) The effects of variable preliminary feeding upon the rats speed of locomotion. *Journal of Comparative and Physiological Psychology, 26*, 331-348.

Neisenbaum, L.K., Zigmond, M.J., Sved, A.F., & Abercrombie, E.D. (1991) Prior exposure to chronic stress results in enhanced synthesis and release of hippocampal norepinephrine in response to a novel stressor. *The Journal of Neuroscience, 11*, 1478-1484.

O'Brien, C., Ehrman, R.N., & Ternes J.W. (1986) Classical conditioning in human opioid dependence. In: *Behavioral Analysis of Drug Dependence,* S.R. Goldberg, & I.P. Stolerman, eds., Academic Press, New York, pp. 329-356.

Pavlov, I.P. (1919) Lectures on the work of the principal digestive glands. In: *Experimental Psychology and Other Essays*, I.P. Pavlov, ed., Philosophical Library, New York, 108-109.

Pettit, H.O., Pan, H.-T., Parsons, L.H., & Justice, J.B. (1990) Extracellular concentraions of cocaine and dopamine are enhanced during chronic cocaine administration. *Journal of Neurochemistry, 55*, 755-760.

Piazza, P.V., & Le Moal, S. (1996) Pathophysiological basis of vulnerability to drug abuse: Interaction between stress, glucocorticoids and dopaminergic neurons. *Annual Review of Pharmacology and Toxicology 36*, 359-378.

Piazza, P.V., Deminiere, J. -M., Le Moal, M., & Simon, H. (1989) Factors that predict individual vulnerability to amphetamine self-administration. *Science, 245*, 1511-1513.

Piazza, P.V., Deminiere, J.-M., Le Moal, M., & Simon, H. (1990) Stress- and pharmacologically-induced behavioural sensitization increases vulnerability to acquisition of amphetamine self-administration. *Brain Research, 514*, 22-26.

Preston, K.L., Sullivan, J.T., Strain, E.C., & Bigelow, G.E. (1992) Effects of cocaine alone and in combination with bromocriptine in human cocaine abusers. *The Journal of Pharmacology and Experimental Therapeutics, 262*, 279-291.

Quintero, S., Henney, S., Lawson, P., Mellanby, J., & Gray, J.A. (1985) The effects of compounds related to γ-aminobutyrate and benzodizepine receptors on behavioural responses to anxiogenic stimuli in the rat: Punished barpressing. *Psychopharmacology, 85*, 244-251.

Ramsey, N.F., & Van Ree, J.M. (1993) Emotional but not physical stress enhances intravenous cocaine self-administration in drug-naive rats. *Brain Research, 608*, 216-222.

Rawlins, J.N.P., Feldon, J., Salmon, P., Gray, J.A., & Garrud, P. (1980) The effects of chlordiazepoxide HCl administration upon punishment and conditioned suppression in the rat. *Psychopharmacology, 70*, 317-322.

Redmond, D.E., Jr., & Huang, Y.H. (1979) Locus coeruleus and anxiety. *Life Sciences, 25*, 2149-2162.

Rex, A., Stephens, D.N., & Fink, H. (1996) "Anxiolytic" action of diazepam and abecarnil in a modified open field test. *Pharmacology, Biochemistry, and Behavior, 53*, 1005-1011.

Ritz, M.C., Lamb, R.J., Goldberg, S.R., & Kuhar, M.J. (1987) Cocaine receptors on dopamine transporters are related to self-administration of cocaine. *Science, 23*, 1219-1223.

Roberts, D.C.S. (1992) Neural substrates mediating cocaine reinforcement: The role of monoamine systems. In: *Cocaine: Pharmacology, Physiology, and Clinical Strategies*, J.M. Lakoski, M.P. Galloway, & F.J. White, eds., CRC Press, Boca Raton, Florida, pp. 73-90.

Robinson, T.E. (1993) Persistent sensitizing effects of drugs on brain dopamine systems and behavior: Implications for addiction and relapse. In: *The Biological Basis of Substance Abuse*, J. Barchas, & S. Korenman, eds., Oxford University Press, Oxford.

Robinson, T.E., & Becker (1986) Enduring changes in brain and behavior produced by chronic amphetamine administration: A review and evaluation of animal models of amphetamine psychosis. *Brain Research Reviews, 396*, 157-198.

Rossetti, Z.L., Portas, C., Pani, L., Carboni, S., & Gessa, G.L. (1990) Stress increases noradrenaline release in the rat frontal cortex: Prevention by diazepam. *European Journal of Pharmacology, 176*, 229-231.

Salmon, P., & Gray, J.A. (1985) Comparison between the effects of propranolol and chlordiazepoxide on timing behaviour in the rat. *Psychopharmacology, 87*, 219-224.

Sapolsky, R.M. (1992) Neuroendocrinology of the stress-response. In: *Behavioral Endocrinology*, J.B. Becker, S.M. Breedlove, & D. Crews, eds., Library of Congress, Massachusetts, pp. 287-324.

Sarnyai, Z., Biro, E., Gardi, J., Vecsernyes, M., Julesz, J., & Telegdy, G. (1995) Brain corticotropin-releasing factor mediates 'anxiety-like' behavior induced by cocaine withdrawal in rats. *Brain Research, 675*, 89-97.

Serrano, A., D'Angio, M., & Scatton, B. (1989) NMDA antagonists block restraint-induced increase in extracellular DOPAC in rat nucleus accumbens. *European Journal of Pharmacology,162*, 157-166.

Shaham, Y., Funk, D., Erb, S., Brown, T., Walker, C.-D., & Stewart, J. Corticosterone does not contribute to relapse to heroin-seeking in rats induced by footshock stress or priming injections of heroin, in preparation.

Shaham, Y., Puddicombe, J., & Stewart, J. (1996) Sexually arousing events do not induce relapse to heroin-seeking in sexually experienced male rats. *Physiology and Behavior*, in-press.

Shaham, Y., Rajabi, H., & Stewart, J. (1996) Relapse to heroin-seeking under opioid maintenance: The effects of opioid withdrawal, heroin priming and stress. *The Journal of Neuroscience, 16*, 1957-1963.

Shaham, Y., Rodaros, D., & Stewart, J. (1994). Exposure to mild stress enhances the reinforcing efficacy of intravenous heroin self-administration in rats. *Psychopharmacology, 114*, 523-527.

Shaham, Y., & Stewart, J. (1994) Exposure to mild stress enhances the reinforcing efficacy of intravenous heroin self-administration in rats. *Psychopharmacology, 114*, 523-537.

Shaham, Y., & Stewart, J. (1995a) Characterization of stress- and heroin-primed relapse to heroin-seeking behavior in rats. *Abstract for the Society for Neuroscience, 21*, 725.

Shaham, Y., & Stewart, J. (1995b) Stress reinstates heroin-seeking in drug-free animals: An effect mimicking heroin, not withdrawal. *Psychopharmacology, 119*, 334-341.

Shaham, Y., & Stewart, J. (1996) Effects of opioid and dopamine receptor antagonists on relapse induced by stress and re-exposure to heroin in rats. *Psychopharmacology, 125*, 385-391.

Shettleworth, S.J. (1978) Reinforcement and the organization of behavior in golden hamsters: Sunflower seed and nest paper reinforcers. Animal *Learning and Behavior, 6*, 352-362.

Shimizu, N., Nakane, H., Hori, T., & Hayashi, Y. (1994) CRF receptor antagonist attenuates stress-induced noradrenaline release in the medial prefrontal cortex of rats. *Brain Research, 654*, 145-148.

Shippenberg, T.S., & Heidbreder, Ch. (1995) Sensitization to the conditioned rewarding effects of cocaine: Pharmacological and temporal characteristics. *The Journal of Pharmacology and Experimental Therapeutics, 273*, 808-815.

Siegel, S. (1979) The role of conditioning in drug tolerance and addiction. In: *Psychopathology in Animals: Research and Clinical Implications*, J.D. Keehn, ed., Academic Press, New York, pp. 143-168.

Siegel, S., Hinson, R.E., Krank, M.D., & McCully, J. (1982) Heroin "overdose" death: Contribution of drug-associated environmental cues. *Science, 216*, 436-437.

Slikker, W., Jr., Brocco, M.J., & Keith, F.K., Jr. (1984) Reinstatement of responding maintained by cocaine or thiamylal. T*he Journal of Pharmacology and Experimental Therapeutics, 228*, 43-52.

Solomon, R.L. (1977) An opponent-process theory of motivation: IV. The affective dynamics of addiction. In: *Psychopathology: Experimental Models*, J.D. Maser & M.E.P. Seligman, eds., Freeman Press, San Francisco, pp.66-103.

Solomon, R.L., & Corbit, J.D. (1974) An opponent process theory of motivation: Temporal dynamics of affect. *Psychological Review, 81*, 119-145.

Sorg, B.A., & Kalivas, P.W. (1993) Behavioral sensitization to stress and psychostimulants: Role of dopamine and excitatory amino acids in the mesocorticolimbic system. *The Neurosciences, 5,* 343-350.

Stewart, J. (1984) Reinstatement of heroin and cocaine self-administration behavior in the rat by intracerebral application of morphine in the ventral tegmental area. *Pharmacology, Biochemistry & Behavior, 20*, 917-923.

Stewart, J. (1992) Neurobiology of conditioning to drugs of abuse. *Annals of the New York Academy of Sciences, 654*,335-345.

Stewart, J., & de Wit, H. (1987) Reinstaement of drug-taking behavior as a method of assessing incentive motivational properties of drugs. In: *Methods of Assessing the Reinforcing Properties of Abused Drugs*, M.A. Bozarth, ed., Springer-Verlag, New York, pp. 211-227.

Stewart, J., de Wit, H., & Eikelboom, R. (1984) Role of unconditioned and conditioned drug effects in the self-administration of opiates and stimulants. *Psychological Review, 91*, 251-268.

Stewart, J., & Vezina, P. (1987) Environment-specific enhancement of the hyperactivity induced by systemic or intra-VTA morphine injections in rats preexposed to amphetamine. *Psychobiology, 15*, 144-153.

Stewart, J., & Vezina, P. (1988) A comparison of the effects of intra-accumbens injections of amphetamine and morphine on the reinstatement of heroin intravenous self-administration behavior. *Brain Research, 457*, 287-294.

Stewart, J., & Wise, R.A. (1992) Reinstatement of heroin self-administration habits: Morphine prompts and naltrexone discourages renewed responding after extinction. *Psychopharmacology, 108*, 79-84.

Stitzer, M.L., & Cox, W.M. (1996) Introduction to special section. Relapse to substance abuse: Recent findings from basic and clinical research. *Experimental and Clinical Psychopharmacology, 4*, 3-4.

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

Swerdlow, N.R., Geyer, M.A., Vale, W.W., & Koob, G.F. (1986) Corticotropin-releasing factor potentiates acoustic startle in rats: Blockade by chlordiazepoxide. *Psychopharmacology, 88*, 147-152.

Tanaka, M., Tsuda, A., Yokoo, H., Yoshida, M., Mizoguchi, K., & Shimizu, T. (1991) Psychological stress-induced increases in noradrenaline release in rat brain regions are attenuated by diazepam, but not by morphine. *Pharmacology, Biochemistry, & Behavior, 39*, 191-195.

Tiffany, S.T. (1990) A cognitive model of drug urges and drug-use behavior: The role of automatic and nonautomatic processes. *Psychological Review, 97*, 147-168.

Van Dyke, C., Ungerer, J., Jatlow, P., Barash, P., & Byck, R. (1982) Intranasal cocaine: Dose relationships of psychological effects and plasma levels. *International Journal of Psychiatry in Medicine, 12*, 1-13.

Weddington, W.W., Brown, B.S., Haertzen, C.A., Cone, E.J., Dax, E.M., Herning, R.I., Michaelson, B.S. (1990) Changes in mood, craving, and sleep during short-term abstinence reported by male cocaine addicts. *Archives of General Psychiatry, 47*, 861-868.

Wikler, A., & Pescor, F.T. (1967) Classical conditioning of a morphine abstinence phenomenon, reinforcement of opioid-drinking behavior and "relapse" in morphine-addicted rats. *Psychopharmacologia, 10*, 255-284.

Willick, M.L., & Kokkinidis, L. (1995) Cocaine enhances the expression of fear-potentiated startle: Evaluation of state-dependent extinction and the shock-sensitization of acoustic startle. *Behavioral Neuroscience, 109*, 929-938.

Wise, R.A. (1982). Neuroleptics and operant behavior: The anhedonia hypothesis. *Behavioral and Brain Sciences, 5*, 38-87.

Wise, R.A. (1984) Neural mechanisms of the reinforcing action of cocaine. In: *Cocaine: Pharmacology, Effects, and Treatment of Abuse*, J. Grabowski, ed., National Institute of Drug Abuse, Washington, D.C., pp. 15-33.

Wise, R.A. (1989) The brain and reward. In: T*he Neuropharmacological Basis of Reward*, J.M. Liebman & S.J. Cooper, eds. Oxford University Press, Oxford, pp. 377-424.

Wise, R.A. (1993) In vivo estimates of extracellular dopamine and dopamine metabolite levels during intravenous cocaine and heroin self-administration. *The Neurosciences, 5*, 337-342.

Wise, R.A., & Bozarth, M.A. (1987) A psychomotor stimulant theory of addiction. *Psychological Review, 94*, 469-492.

Wise, R.A., Murray, A., & Bozarth, M.A. (1990) Bromocriptine self-administration and bromocriptine-reinstatement of cocaine-trained and heroin-trained l lever pressing in rats. *Psychopharmacology, 100*, 355-360.

Wise, R.A., Newton, P., Leeb, K., Burnette, B., Pocock, D., Justice, J.B., Jr. (1995) Fluctuations in nucleus accumbens dopamine concentration during intravenous cocaine self-administration. *Psychopharmacology, 120*, 10-20.

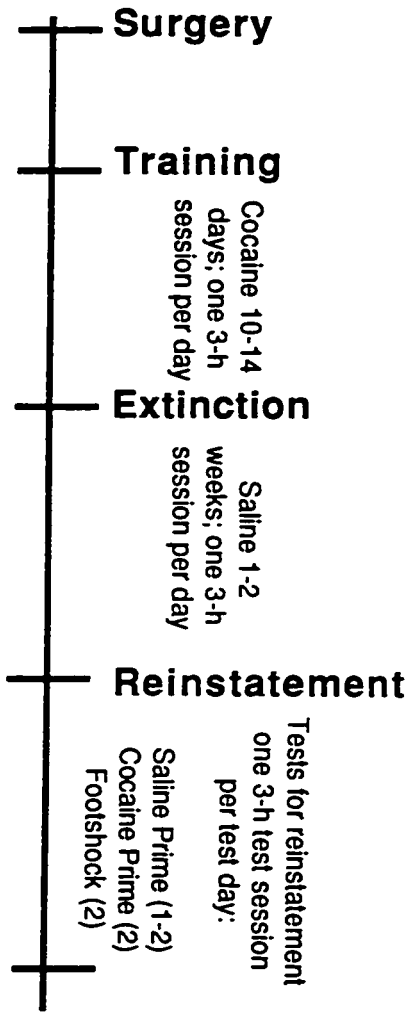
Wise, R.A., & Rompre, P.-P. (1989) Brain dopamine and reward. *Annual Review of Psychology, 40*, 191-225.

Wise, R.A., Spindler, J., de Wit, H., & Gerber, G.J. (1978) Neuroleptic-induced "anhedonia" in rats: Pimozide blocks the reward quality of food. *Science, 201*, 262-264.

Worley, C.M., Valadez, A., & Schenk, S. (1994) Reinstatement of extinguished cocaine-taking behavior by cocaine and caffeine. *Pharmacology, Biochemistry and Behavior, 48*, 217-221.

Yang, X.-M., Gorman, A.L., Dunn, A.J., & Goeders, N.E. (1992) Anxiogenic effects of acute and chronic administration: Neurochemical and behavioral studies. *Pharmacology, Biochemistry and Behavior, 41*, 643-650.

Appendices


Surgery
Training
Cocaine 10-14 days; one 3-h session per day
Extinction
Saline 1-2 weeks; one 3-h session per day
Reinstatement
Tests for reinstatement one 3-h test session per test day:
Saline Prime (1-2)
Cocaine Prime (2)
Footshock (2)

	Pretreatment			
Test Condition	0.75 mg/kg Diazepam		1.5 mg/kg Diazepam	
Saline	no drug	drug	no drug	drug
Cocaine	no drug	drug	no drug	drug
Footshock	no drug	drug	no drug	drug

Appendix III

In a pilot study, the order in which animals received the No Drug and Drug pretreatment conditions was counterbalanced. The results from this study, however, indicated that rats that received Drug (diazepam) pretreatments first were less likely to demonstrate footshock-induced reinstatement when they received the No Drug pretreatment than were rats that received No Drug pretreatment first. It has been shown previously that diazepam interferes in the conditioning of fear and analgesia to footshock stress by one previous exposure to social defeat stress (Hotsenpiller & Williams ,1996). On the basis of this finding, it is possible that in the pilot study, diazepam interfered in the conditioning of fear to footshock in animals given the No Drug pretreatment first. Since the primary purpose of the present experiment was to determine whether footshock-induced reinstatement of cocaine-trained behaviour could be altered by pretreatment with diazepam, and since in Experiment 1 the number of responses animals made between the first and second footshock tests (means [±SEM] of 36.75 [±9.33] and 32.00 [±9.42] responses, respectively) or between the first and second cocaine tests (means [±SEM] of 26.00 [±4.65] and 21.50 [±3.77] responses, respectively) were similar, a decision was made not to counterbalance the order in which animals received the Pretreatment Conditions.

Test Conditions: footshock and saline

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-Value	p-Value
Dose	1	200.62	200.62	5.87	.03
error	21	717.79	34.18		
Pretreatment	1	173.62	173.62	5.59	.03
D X P	1	150.36	150.36	4.84	.04
error	21	652.79	31.09		
Test Condition	1	245.92	245.92	6.66	.02
D X TC	1	197.01	197.01	5.34	.03
error	21	775.32	36.92		
P X TC	1	117.01	117.01	3.81	.06
D X P X TC	1	174.10	174.10	5.67	.03
error	21	645.22	30.72		

Test Conditions: cocaine and saline

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-Value	p-Value
Dose	1	525.71	525.71	1.42	.25
error	12	4443.09	370.26		
Pretreatment	1	518.43	518.43	7.72	.02
D X P	1	23.43	23.43	.35	.57
error	12	805.80	67.15		
Test Condition	1	7354.38	7354.38	25.90	.00
D X TC	1	270.09	270.09	.95	.35
error	12	3407.00	283.92		
P X TC	1	599.38	599.38	9.26	.01
D X P X TC	1	234.67	234.67	3.63	.08
error	12	776.42	64.70		

Test Conditions: footshock and saline

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-Value	p-Value
Pretreatment	1	7754.96	7754.96	12.54	.00
error	22	13604.96	618.41		
Test Condition	1	648.29	648.29	3.40	.08
error	22	4198.96	190.86		
Hour of Testing	2	6141.66	3070.83	10.92	.00
error	44	12378.51	281.33		
P X TC	1	1592.64	1592.64	6.07	.02
error	22	5770.28	262.29		
P X H	2	6144.49	3072.24	8.59	.00
error	44	15743.35	357.80		
TC X H	2	14067.41	7033.71	12.58	.00
error	44	24608.09	559.27		
P X TC X H	2	9693.02	4846.51	9.38	.00
error	44	22730.81	516.61		

Test Conditions: cocaine and saline

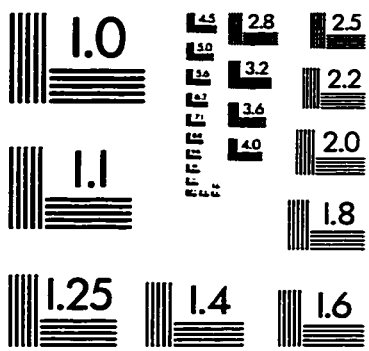
Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-Value	p-Value
Pretreatment	1	2332.60	2332.60	40.86	.00
error	13	742.07	57.08		
Test Condition	1	2044.02	2044.02	35.00	.00
error	13	746.31	57.41		
Hour of Testing	2	744.25	372.12	11.32	.00
error	26	845.42	32.86		
P X TC	1	2499.43	2499.43	27.25	.00
error	13	1192.57	91.74		
P X H	2	1306.58	653.29	12.75	.00
error	26	1331.75	51.22		
TC X H	2	1064.30	532.15	10.66	.00
error	26	1297.37	49.90		
P X TC X H	2	521.68	260.84	7.27	.00
error	26	932.32	35.86		

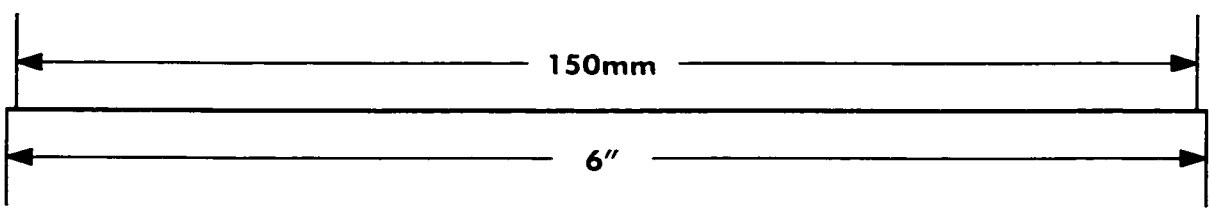
Test Conditions: footshock and saline

Hour of Testing	Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-Value	p-Val
1	Pretreatment	1	3618.79	3618.79	5.20	.03
	error	22	15309.46	695.88		
	Test Condition	1	21640.45	21640.45	11.81	.00
	error	22	40295.80	1831.63		
	P X TC	1	4111.14	4111.14	6.43	.02
	error	22	14058.11	639.00		
2	Pretreatment	1	706.53	706.53	3.69	.07
	error	22	635.22	28.87		
	Test Condition	1	164.45	164.45	4.65	.04
	error	22	777.30	35.33		
	P X TC	1	175.32	175.32	5.38	.03
	error	22	716.43	32.57		
3	Pretreatment	1	10.45	10.45	1.10	.31
	error	22	209.80	9.54		
	Test Condition	1	14.88	14.88	1.86	.19
	error	22	176.37	8.02		
	P X TC	1	.10	.10	.01	.91
	error	22	175.15	7.96		

Test Conditions: cocaine and saline

Hour of Testing	Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-Value	p-Valu
1	Pretreatment	1	5.16	5.16	.05	.83
	error	13	1456.09	112.01		
	Test Condition	1	5304.02	5304.02	35.76	.00
	error	13	1928.23	148.33		
	P X TC	1	.88	.88	.01	.93
	error	13	1221.37	93.95		
2	Pretreatment	1	5.79	5.79	1.24	.29
	error	13	60.71	4.67		
	Test Condition	1	1.79	1.79	.31	.58
	error	13	73.71	5.67		
	P X TC	1	3.50	3.50	1.47	.25
	error	13	31.00	2.38		
3	Pretreatment	1	39.45	39.45	2.33	.15
	error	13	220.30	16.95		
	Test Condition	1	27.16	27.16	2.12	.17
	error	13	166.95	12.81		
	P X TC	1	54.02	54.02	4.57	.05
	error	13	153.73	11.83		





APPLIED IMAGE, Inc
1653 East Main Street
Rochester, NY 14609 USA
Phone: 716/482-0300
Fax: 716/288-5989