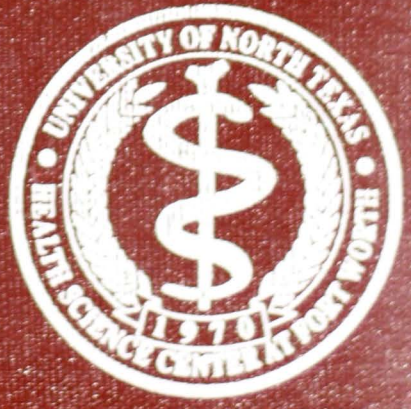

UNIVERSITY OF NORTH TEXAS
HEALTH SCIENCE CENTER AT FORT WORTH
1970

ABSTRACT

It has been hypothesized that there is a common dopaminergic pathway mediating the reward properties of abused drugs, and that dopamine is involved in tolerance to the reinforcing effect of cocaine. The progressive-ratio (PR) schedule can be used to test both potentiation and reduction of the reinforcing effects of cocaine by other factors. Under the PR schedule, an increasing number of responses is required to obtain each subsequent cocaine injection, and failure to complete the required number of responses within 1 h of the previous cocaine injection terminates the session. The number of total reinforcers obtained during a session is defined as "the breaking point" and was used as the primary dependent measure. Fisher F344 male rats acquired the self-administration task under the PR schedule within forty sessions and showed a stable daily acquisition baseline. The breaking point and inter-reinforcer time (IS^RT) were positively correlated within each ratio. A motor-incapacitating side effect of a pretreatment can be determined by a change in the relationship between the IS^RT and the breaking point. *d*-Amphetamine pretreatment (0.32-3.2 mg/kg, i.p., 30 min) potentiates the reinforcing effect of cocaine as demonstrated by a higher breaking point of self-administration without changing the IS^RT. Morphine pretreatment (0.32-3.2 mg/kg, i.p., 30 min) failed to change the breaking point of cocaine self-administration but it did increase the IS^RT. These results support an additive reinforcing effect for amphetamines and cocaine, but do not support an additive reinforcing effect of morphine and cocaine. The reinforcing effect of cocaine was reduced by pretreatment with ketamine (0.032-0.32 mg/kg, i.p., 20 min) as indicated by a reduction in the breaking point. In a concurrent experiment, animals were trained to self-administer cocaine under a fixed ratio 2 schedule (FR2). Ketamine pretreatment did not modify the IS^RT in FR2 trained animals except at the highest dose (0.32 mg/kg, i.p., 20 min), where significant motor incoordination was observed. Both chronic

treatment with cocaine (20 mg/kg/ 8hr x 7 days, iv) or amphetamine (3.2 mg/kg /12hr x 7 days, i.p.) resulted in a reduction in breaking point at any given dose, providing direct evidence of tolerance and cross-tolerance to the reinforcing effects of cocaine. Chronic treatment with ketamine (0.32 mg/kg/8hr x 7days, i.v.) failed to modify either the breaking point under a PR schedule of reinforcement or the IS^RT under a FR2 schedule of reinforcement. Co-administration of ketamine (0.32 mg/kg/8h x 7days, i.v.) with chronic cocaine (20 mg/kg/8hr x 7days, i.v.) failed to prevent tolerance to the reinforcing effect of cocaine as indicated by either the breaking point under a PR schedule of reinforcement or the IS^RT under an FR2 schedule of reinforcement. These data indicate that the breaking point in the PR schedule is more sensitive to changes in the dopamine reward system, whereas changes in rate of response are not consistently related to the changes in the dopamine reward system. These data support the use of the PR schedule as a better method than FR schedule for determining reward properties of drugs of abuse with fewer complications due to the central nervous system inhibitory effects of some drugs of abuse.

THE INFLUENCE OF CNS STIMULANTS, OPIOID ANTAGONISTS AND AN NMDA ANTAGONIST ON THE REINFORCING EFFECT OF COCAINE USING A PROGRESSIVE-RATIO SCHEDULE

Donghang Li, B.S.

APPROVED:

Cleatus J. Wallis
Major Professor

Michael J. Fanton
Committee Member

GLDr
Committee Member

Michael W. Martin
Committee Member

R R Luedtke
Committee Member

Edward Miller
Committee Member

Chair, Department of Pharmacology

Thomas Yorio
Dean, Graduate School of Biomedical Sciences

THE INFLUENCE OF CNS STIMULANTS, OPIOID ANTAGONISTS AND AN NMDA ANTAGONIST ON THE REINFORCING EFFECT OF COCAINE USING A PROGRESSIVE-RATIO SCHEDULE

DISSERTATION

Presented to the Graduate Council of the

Graduate School of Biomedical Sciences

University of North Texas Health Science Center at Fort Worth

in Partial Fulfillment of the Requirements

For the Degree of

DOCTOR OF PHILOSOPHY

By

Donghang Li, B.S.

Fort Worth Texas

July 25, 1996

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AMY	Amygdaloid
d-A or AMPH	*d*-amphetamine
ARC	Arcuate nucleus
DRL	Differential reinforcement of low rates
PAG	Periaqueductal gray
BUP	Buprenorphine
COC	Cocaine
DA	Dopamine
EEA	Excitatory amino acid
FR	Fixed-ratio
inj	Injection
IS^RT	Inter-reinforcer time
KET	Ketamine
LPO	Lateral preoptic area
NAc	Nucleus accumbens
NMDA	N-methyl-D-aspartate
METH	Methamphetamine
MOR	Morphine
PR	Progressive-ratio
Reinf	Reinforcer
S^D	Discriminative Stimulus
SI	Substantia innominata
S^R	Reinforcing stimulus
VTA	Ventral tegmental area

INTRODUCTION

A reinforcer can be defined operationally as any event that increases the probability of a response. Drugs of abuse are very powerful reinforcers, and even in conditions of limited access (where the organism is not dependent) these drugs will motivate high rates of operant responding (Koob 1992 a and b). Three major brain neurotransmitter systems are involved in drug reward: dopamine (DA), opioids and GABA (Koob 1992b). Data implicating the dopamine and opioid systems in indirect sympathomimetic and opiate reward include critical elements in both the nucleus accumbens (NAc) and ventral tegmental areas (VTA). Recently, new information about the anatomical connections of these systems has stimulated argument as to whether there is a common mechanism mediating the reinforcing effect of these drugs (Ettenberg *et al.*, 1982; Koob 1992 a and b; Wise and Rompre 1989). Therefore, we chose to study the interaction between opioids and cocaine in order to understand the interaction between the reinforcing effects of these two classes of drugs. The study of tolerance was applied to characterize the factors that modify the reinforcing effect of cocaine after repeated exposure to cocaine. Tolerance development was also used as a tool to modify the reinforcing effect of drugs of abuse, such as opioids, ketamine, and amphetamine. We also sought to determine if this modification also produces cross-tolerance to cocaine. The goal of this series of studies was to test the hypothesis that the DA rewarding system is the fundamental basis for the reinforcing effect of cocaine and the other drugs of abuse being studied. The study of the impact of a N-methyl-D-aspartate (NMDA) antagonist on both acute and chronic cocaine administration supplied some evidence that the NMDA neurotransmitter system is involved in the DA reward pathway modulating the reinforcing effect of drugs. In summary, the following experiments were performed:

1. To determine and evaluate self-administration of cocaine under the PR schedule. The relationship between the dose of cocaine administered, the number of reinforcers obtained and the inter-reinforcer time were evaluated. Then D1 and D2 receptor

antagonists were applied to confirm the model of DA mediated reinforcing effect of cocaine.

2. To determine the role of opioid receptors in mediating the reinforcing effects of cocaine. The effect of acute administration of morphine and buprenorphine on cocaine self-administration was determined and compared with direct stimulation of the dopamine system with amphetamine (AMPH). The inter-reinforcer time under the PR schedule was also examined to evaluate a potential role of motor inhibition in the modulation of the proposed dopaminergic common pathway of reward.
3. To determine the effect of chronic treatment of cocaine and other indirect dopamine agonists on tolerance to the reinforcing effect of cocaine. In addition, we characterized an acute antagonist of cocaine's reinforcing effect (ketamine) and used this antagonist to attempt to block the development of tolerance to the reinforcing effects of cocaine.

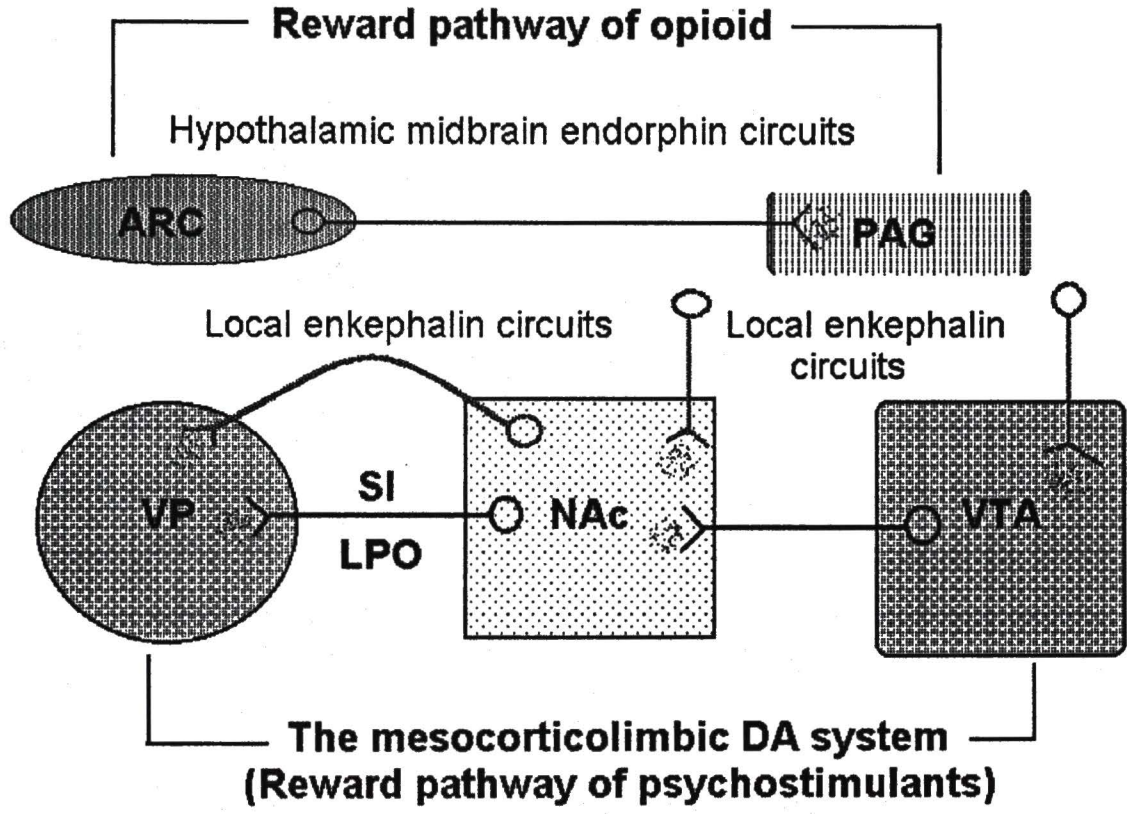
Cocaine, a psychostimulant drug, has a variety of physiological effects. Cocaine is known to produce behavioral activation (Scheel-Kruger *et al.*, 1977), to serve as a discriminative stimulus (Wood and Emmett-Oglesby, 1986), and to support self-administration (Woolverton & Johnson, 1992). Its powerful, seductive reinforcing properties have made it a widely abused drug and a severe sociological, medical and personal problem (Kuhar *et al.*, 1992). Cocaine has an affinity for dopamine, serotonin (5-HT), and norepinephrine transporters (Ritz *et al.*, 1990). The ability of cocaine to elevate synaptic levels of monoamines by blocking their reuptake apparently produces the behavioral and subjective effects of the drug in animals and humans (Hubner & Koob 1990; Kuhar *et al.*, 1992). Neurobiological studies demonstrate that cocaine and the other major classes of drugs of abuse (alcohol and opiates) increase the functional release of dopamine, which activates the ventral tegmental-nucleus accumbens (VTA-NAc) pathway, that is known to be a major anatomical component of the reward system in brain (Figure 1). Activation of this pathway is essential for the reinforcing actions of psychomotor stimulants (Withers *et al.* 1995). Although the nucleus accumbens (NAc) appears to be a critical site for some of the

actions of cocaine within the central nervous system, the nucleus does not operate in isolation within the limbic system. Neuroanatomical studies indicate that first-order efferents of the NAc project to a region of the substantia innominata/lateral preoptic area (SI/LPO), medial to the ventral pallidum (Figure 1; Hubner and Koob, 1990). Behavioral evidence also suggests that these areas are important for the activating properties of the psychostimulant drugs (LeMoal and Simon, 1991). The VTA-NAc-SI/LPO circuitry appear to be an important part of the neural network underlying the behavioral activation induced by cocaine or other psychostimulant drugs (Mogenson *et al.*, 1989).

Among cocaine addicts, poly-drug abuse is a common phenomenon (Sample 1977), and drugs representing a wide range of pharmacological classes are frequently combined with cocaine. On the street, in an attempt to reduce ("cut") the amount of "active" agent being sold, amphetamines are frequently used to substitute for a reduced amount of cocaine. In addition, the cocaine abuse epidemic of the 1980s was paralleled by an increase in cocaine use by opiate abusers (Kosten *et al.*, 1986; Kozel and Adams, 1986). Dual dependence on cocaine and opiates is now commonly reported by drug abuse treatment programs (Gastfriend *et al.*, 1993), and cocaine was involved in approximately 29% of heroin-related hospital emergencies in 1991 (NIDA 1991). Because concurrent cocaine and heroin abuse is a predominant form of poly-drug abuse, characterization of drug abusers in terms of a single or exclusive drug preference has become increasingly problematic. Practically, co-abuse of amphetamine and cocaine may be related since both drugs have reinforcing effects and both drugs elevate synaptic levels of biogenic amines, in particular dopamine, from their storage sites in the nerve terminals. There is no simple explanation for poly-drug abuse that involves substances from different pharmacological classes having different, sometimes antithetical, pharmacological effects. Clinical reports of preferred poly-drug use patterns are inconsistent, and cocaine and heroin abusers do not report a uniform

Figure 1. A HYPOTHETICAL MODEL OF THE COMMON REWARD PATHWAY I

(The interaction between dopamine and opioid system)



ACR	arcuate nucleus	NAc	nucleus accumbens
AMG	amygdala	SI	substantia innominata
Hippo	hippocampus	VP	ventral pallidum
LPO	lateral preoptic area	VTA	ventral tegmental area
PAG	periaqueductal gray		

This graph is adapted from the figure of Koob GF (1992) Drug of abuse: anatomy, pharmacology and function of reward pathways. TIPS 13:175-184

sequence of drug use (Kosten 1986; Teoh *et al.*, 1994). Simultaneous injection of cocaine and heroin, known as a "speedball", is one frequent type of poly-drug abuse (Mello *et al.*, 1995). Speedball effects have been variously described as enhancing the positive effects of opiates and decreasing the aversive agitation produced by cocaine (Kosten *et al.*, 1986; Tutton and Crayton, 1993). One controlled clinical study of morphine and cocaine combinations suggested that speedballs produced a unique profile of opiate and stimulant effects rather than an enhancement of the subjective effects of either drug alone (Foltin and Fischman, 1992). The subjective effects of the cocaine-morphine combinations appeared to be different from those reported after administration of only cocaine or heroin (Foltin and Fischman, 1992).

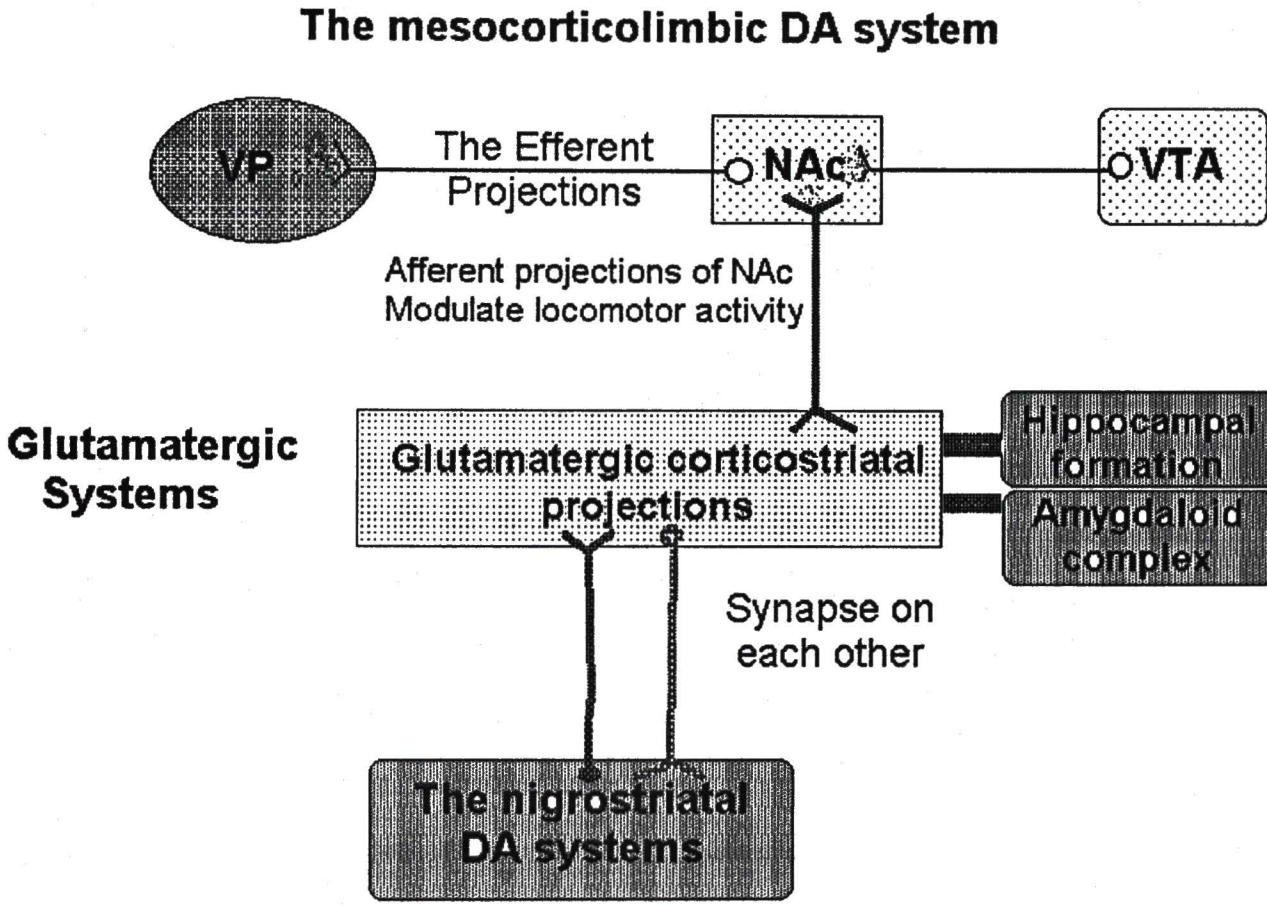
Various studies have indicated that the dopaminergic projection from the ventral tegmental area (VTA) to NAc is an anatomical substrate of the rewarding effect of opiates (Figure 1.). The demonstration that opiates at behaviorally relevant doses activate both firing of VTA dopaminergic neurons and the release of dopamine in NAc supports the dopaminergic hypothesis of opiate reward (Koob, 1992). Experiments demonstrate that both cocaine and opiates increase extracellular dopamine content in the NAc (Di Chiara and Imperato, 1988). Therefore, the administration of both cocaine and opiates enhances NAc dopaminergic activity. The effect of the opioid antagonist naloxone to increase the threshold for rewarding self-stimulation with cocaine (Bain and Kornetsky, 1987) also supplied evidence that endogenous opioid systems are involved in cocaine-induced reinforcement. It has been suggested that brain regions receiving NAc projections may function as a common reward pathway during both opiate and cocaine reinforcement (Koob *et al.*, 1987). Based upon the above experiments and hypotheses, a potentiation in the reinforcing effect of cocaine is expected when cocaine is co-administered with either amphetamines or opiates. The experiments presented in this thesis were designed to examine the interaction between amphetamines, opiates and cocaine. If there is a potentiation effect on the reinforcing effect of cocaine among these classes of drugs, an increase in the breaking points of cocaine self-administration can be expected after co-administration with either amphetamines or opiates. The breaking point is a dependent measure

of the reinforcing effect of cocaine. If the potentiation is only related with the reinforcing effect of the test drug (e.g., amphetamine), then there should be a facilitation of the self-administration of saline.

Dopamine-containing neurons within the VTA are hyperpolarized by dopamine acting at D_2 receptors or by GABA acting at $GABA_A$ receptors, whereas GABA-containing interneurons are hyperpolarized by opioids acting at μ-receptors. Thus, opioids reduce the frequency of GABA-mediated inhibitory synaptic potentials recorded from dopaminergic cells. Cells of both types also receive synaptic inputs from excitatory amino acid receptors, and from $GABA_B$ receptors. Although the physiological factors that affect either the release or the re-uptake of dopamine from nerve terminals are not clearly defined, there is evidence from a wide variety of experimental approaches to suggest that the excitatory amino acids (EAA) participate in regulating the release of dopamine in the striatum (Figure 2; Freed and Cannon-Spoor, 1990; Rao *et al.*, 1991). There is indeed an interaction between EAA, specifically glutamate via N-methyl-D-aspartate (NMDA) type ligand gated ion channels and the cocaine reward system (Karler and Calder 1992). Glutamate increases dopamine release in the nNAc and this effect is blocked by MK-801, a NMDA antagonist. Karler and co-workers indicated that pretreatment with NMDA receptor antagonists prevents the initiation of behavioral sensitization to cocaine or amphetamine (Karler *et al.*, 1989). This finding has been generally replicated (Wolf and Khansa, 1991; Kalivas and Alesdatter, 1993; Stewart and Druhan, 1993) and non-NMDA glutamate antagonists have been found to prevent the initiation and expression of behavioral sensitization (Karler *et al.*, 1991). In addition, antagonists of the NMDA type EAA receptors blocked cocaine-induced stereotypy, locomotor stimulation and convulsions (Karler and Calder, 1992). In De Montis' experiment (De Montis *et al.*, 1992), MK-801 (0.25 mg/kg i.p.) successfully prevented the development of both tolerance and sensitization to the stimulation of locomotor activity induced by cocaine and by the dopamine D_2 agonist quinpirole. His interpretation of the results was that both tolerance and sensitization phenomena are different aspects of a common neuronal response in which NMDA

Figure 2. A HYPOTHETICAL MODEL OF THE COMMON REWARD PATHWAY II

(The interaction between dopamine and glutamatergic system)



AMG amygdala

Hippo hippocampus

NAc nucleus accumbens

VP ventral pallidum

VTA ventral tegmental area

This graph is designed based on the description of Karler and Calder (1992) Excitatory amino acids and the actions of cocaine. Brain Research, 582:143-146

transmission plays a crucial role. My experiment measured the effects of a chronic NMDA antagonist, ketamine, on the reinforcing effect of cocaine. The study also focused on blocking the tolerance produced by chronic cocaine by using the NMDA antagonist to reduce the acute reinforcing effects of cocaine. Finding a drug treatment which can block the acute reinforcing effect of cocaine over an extended period would be the first step in developing a clinical strategy for reducing cocaine intake in a manner similar to methadone substitute treatment in opioid abuser in clinics.

Drug self-administration by a variety of species is considered to be a useful model of abuse liability in humans. Most drugs that humans abuse are self-administered by animals in these studies. Because drug self-administration is widely believed to be the best model for studying abuse liability of drugs, this method has been utilized to identify the relative efficacy of abused drugs (Iwamoto and Martin 1988). The self-administration environment is thought to be complex because all of the effects of a drug on the animal are present (Cunningham *et al.*, 1992). The drug taking experience includes response-contingent presentation stimuli as well as the cognitive stimulus properties of the drug, and motor behaviors produced by the drugs. The self-administered drug has positive stimulus properties that facilitate the emission of additional responses. Other properties of the drug can increase or decrease the rate of drug taking behavior. They can also modulate the intake of food or water which, in turn, further increases or decreases drug intake; or they can modulate other unspecified physiological functions in either a direct or indirect manner that may alter drug intake. In spite of these complexities, experiments utilizing self-administration methodologies have provided significant information with regard to the neurobiological basis of drug reinforcement (Cunningham *et al.*, 1992). Therefore, self-administration is currently the most useful nonhuman laboratory model that can directly address the reinforcing effects of a drug (Cunningham *et al.*, 1992).

A wide variety of schedules of reinforcement have been used in self-administration studies using primates as subjects. By contrast, studies using rats have typically been limited to simple schedules of reinforcement, most commonly a fixed ratio (FR). Under a FR schedule,

reinforcement is contingent upon a fixed number of responses. Responding under this schedule is typically characterized as "break-and-run," with pauses in behavior observed immediately after reinforcement followed by an abrupt transition to high rates of responding that continue until delivery of the next reinforcer. Numerous investigators have shown that injections of psychoactive drugs can maintain responding under FR schedules and that behavior characteristic of this schedule of reinforcement is maintained (Johanson and Schuster, 1981; Young and Herling, 1986). Although FR schedules have the advantage of being easily implemented and readily learned by rodents, there is one major difficulty associated with their use: the rate of self-administration is the only dependent variable that can be derived from simple schedules. Measures of response rate have utility for determining whether drugs can serve as reinforcers. Katz (1990) has argued that, at best, paradigms which use these schedules can only predict that a drug will have abuse potential in humans and they cannot predict the relative degree of abuse liability that a particular compound might possess.

Of the few studies using a more complex reinforcement schedule, a number have employed a progressive-ratio (PR) schedule. Hodos (1961) originally suggested that a PR schedule could be employed to assess the relative strength of food reward. This schedule has been used to assess the reinforcing effects of electrical brain stimulation and drugs, using primates (Bedford *et al.*, 1978; Griffiths *et al.*, 1980; Winger and Woods 1985), pigs (Dantzer 1976), dogs (Risner and Cone 1986) and rats (Hubner and Moreton 1991; Loh and Roberts, 1990; Roberts 1989; Roberts *et al.*, 1989). Under a PR schedule, each reinforcer is delivered after completion of a progressively greater number of responses. Eventually the subject fails to emit the required number of responses in the designated period (this highest ratio completed within the time limit is usually designated as the breaking point). The breaking point produced by cocaine self-administration has been shown to increase as a function of cocaine dose (Griffiths *et al.*, 1978, Risner and Cone, 1986; Roberts *et al.*, 1989), until toxic effects of the drug reduce responding at high doses (Bedford *et al.*, 1978; Risner and Silcox 1981). Under many experimental conditions, the PR schedule does not produce data different from low-value FR

procedures (Winger & Woods, 1985). However, low-value FR schedules (low and constant response requirements in which the rate of drug intake is used as dependent measure) provide only nominal data (yes / no) about the abuse liability of a particular compound (Iwamoto and Martin, 1988; Katz 1990). The PR schedule provides ordinal data (a>b) about the abuse liability between compounds. In addition, the data produced under the PR schedule are less effected by motor incoordination because they are not response rate dependent. We have applied a modified PR schedule based on the methodology of Roberts (1989) to test the reinforcing effect of cocaine in groups of rats.

Another significant approach used in this work is the study of tolerance to the reinforcing effect of cocaine. Tolerance to the subjective effects of drugs is widely regarded as a key component of the dependence process and, as such, it has been extensively studied (Khanna *et al.*, 1982; Jaffe, 1990). Tolerance to the reinforcing effects of drugs is expected because humans escalate the dose of drugs being consumed as a result of reduced drug efficacy. Yanagita (1973) used a PR schedule to determine whether the intensity of drug-seeking behavior of monkeys would increase following development of physical dependence on cocaine. After daily treatment of primates with a high-dose of cocaine for 1 month, physical dependence failed to be induced by cocaine and there was no increase of the reinforcing properties of cocaine to indicate increased craving for cocaine. However, a 50% reduction of total responses emitted in the primate subjects supplied the first evidence of tolerance to the reinforcing effect of cocaine. Based on the above experiments, Emmett-Oglesby (1992) first demonstrated that tolerance to the reinforcing effects of cocaine occurred in a rodent model of i.v. (intravenous) drug self-administration under a FR 2 schedule. The tolerance was induced by repeatedly infusing rats with a high dose of cocaine. Since then, the time-course of tolerance development to the reinforcing effects of cocaine was also established (Emmett-Oglesby *et al.*, 1993). In this study, particular attention was given to the role of time because the time elapsed until the last exposure to cocaine seemed a critical variable in determining the occurrence of tolerance. Another important contribution in the study of tolerance was the demonstration that high dose treatment

of CNS stimulants caused cross-tolerance to the reinforcing effect of cocaine under the FR 2 schedule (Peltier & Emmett-Oglesby, 1994). At a practical level, these experiments provided valuable information about the interaction between drugs of abuse, specifically whether high-dose treatment with various drugs of abuse can lead to an increase or decrease of cocaine intake. Since the measurement of the reinforcing effect of cocaine is rate dependent under the FR2 schedule of reinforcement, a variety of hypotheses have been put forward to explain the phenomenon of tolerance under this schedule. Wilson (Wilson and Schuster 1973) hypothesized that animals take as much drug as possible until the disruptive effects of the drug temporarily preclude further administration. Alternatively, subjects may titrate the blood concentration of drug that produces an optimal effect (Wise *et al.*, 1977). If the former hypothesis were correct, changes in the rate of cocaine self-administration in the low-value FR schedule would reflect tolerance to the disruptive effects of the drug. If the latter hypothesis were correct, these changes would reflect the change of blood concentration of cocaine. However, previous experiments failed to demonstrate the change in pharmacokinetics of cocaine after chronic treatment with cocaine (Katz *et al.*, 1993; Misra 1976), which indicated that tolerance is not due to a decrease in cocaine blood concentration. My experiment used rats trained on a PR schedule to determine whether a chronic dosing regimen of cocaine or amphetamines that produced tolerance or cross-tolerance to cocaine self-administration in FR2 schedules would result in a decrease in the breaking point, which would indicate tolerance to the reinforcing effect of cocaine. Because PR does not depend on a rate dependent measure, like IS^RT, a reduction in breaking point would be a definitive demonstration of tolerance to the reinforcing effects of cocaine. In addition, the failure to increase breaking point under experimental conditions that resulted in a higher IS^RT would indicate that the reinforcing effect of cocaine was unchanged and the higher IS^RT was due to motor effects of the drug rather than sensitization to the reinforcing effects of cocaine.

Chapter 1

The experiments in this chapter were designed to systematically evaluate cocaine self-administration under a progressive-ratio schedule. The experiments determined the rate of acquisition and stability of the breaking point baseline within forty sessions. The experiments were also designed to establish the relationship between the dose of cocaine self-administered, the number of injections obtained and inter-reinforcer time (IS^RT). To test the sensitivity of the PR method to measure the changes caused by the other drugs and support the hypothesis that cocaine produces reinforcing effect through a DA-mediated reward pathway, the effects of D1 and D2 receptor antagonists on cocaine self-administration were also determined.

The original version of this manuscript was published in *Pharmacol Biochem Behav 45:539-548, 1993* witten by Depoortere. The whole work was completed by D.H. Li, the current author of the dissertation. The current author performed all experiments from training the animals to the antagonist testing. The current manuscript is modified based on Depoortere's writing as follows: the results of the pretreatment of D2 antagonist eticlopride on the breaking point; the comparison of the effect of SCH 23390 and eticlopride on the breaking point is also shown in Figure 6. Appropriate changes were made to the methods section and additions were made to the discussion as necessary to include the conclusions from this additional data. Changes to the original text are indicated in italics.

Parameters of self-administration of cocaine in rats under a progressive-ratio schedule: acquisition, stability, effect of changing the dose of cocaine, and blocking by SCH 23390 and eticlopride but not by ondansetron[1]

R. Y. Depoortere, D.H. Li[2], J. D. Lane and M. W. Emmett-Oglesby

Department of Pharmacology

University of North Texas HSC at Fort Worth

3500 Camp Bowie Blvd

Fort Worth TX 76107-2699 USA

Running Title: Parameters of PR

1 Supported by NIDA grant RO1 4137 (MWE-O), State of Texas Advanced Technology Program Grants 3711 and 9768 (JDL), and by GLAXO Group Research, Ltd (MWE-O)

2This project is taken from a dissertation submitted to the University of North Texas Health Science Center at Fort Worth in partial fulfillment of the requirements for the degree Doctor of Philosophy

Abbreviations:

Progressive-ratio schedule (PR)

Fixed-ratio schedule (FR)

Injection (inj)

Inter-reinforcer time (IS^RT)

ABSTRACT

Progressive-ratio (PR) schedules may provide a more direct measure of drug reinforcing efficacy than the more traditionally used fixed-ratio schedule. Under a PR schedule, an increasing number of lever presses is required for the delivery of each successive reinforcer. However, there have been few studies of fundamental parameters of cocaine self-administration under a PR schedule. This study was undertaken to assess if PR responding using cocaine reinforcement in rats would: 1) be acquired rapidly, 2) be maintained on a stable baseline for long periods and 3) provide data on the effect of changing the dose of cocaine that are amenable to statistical analysis. In addition, the effects of pretreatments with SCH 23390, a D1 receptor antagonist, *or eticlopride, a D2 receptor antagonist*, or ondansetron, a 5-HT_3 receptor antagonist, were tested against several doses of cocaine. Stable performance of PR cocaine self-administration (0.25 mg/inj) was acquired within 10 training sessions and was maintained for over 50 training sessions. Increasing the dose of cocaine from 0.028 to 0.75 mg/inj resulted in a directly related increase in 1) the number of reinforcers obtained, 2) the highest ratio completed, and 3) the inter-reinforcer time (IS^RT: time between each cocaine infusion). In terms of statistical analysis, the number of reinforcers obtained was found to be preferable to the highest ratio completed as a measure of breaking point. Pretreatment with SCH 23390 significantly reduced the breaking point; this reduction was not due to a motor-incapacitating effect of SCH 23390, since the IS^RT showed a tendency to be shortened by SCH 23390. *Pretreatment of eticlopride also reduced the breaking point.* Pretreatment with ondansetron failed to significantly affect either the number of reinforcers obtained or the IS^RT. These results show that rats can readily acquire the task of self-administration of cocaine under a PR schedule and maintain a stable baseline for an extended period. Furthermore, a PR schedule appears to be suitable for the study of pharmacological treatments that might affect cocaine self-administration. Simultaneous monitoring of the breaking point and of the IS^RT determines if a decrease in the breaking point is the result of a motor-incapacitating side-effect of the pretreatment.

INTRODUCTION

Drug self-administration studies are valuable in part because a high concordance exists between drugs that serve as reinforcers in animals and drugs that humans abuse (Griffiths et al, 1975; Shuster and Thompson, 1969; Young and Herling, 1986). Many studies of animal drug taking have used procedures in which drug is available under a simple fixed-ratio (FR) schedule, and often the value of the ratio is relatively low. Such schedules have utility for determining whether drugs can serve as reinforcers, but they provide only nominal information concerning the relative reinforcing efficacy of different drugs (Iwamoto and Martin, 1988; Katz 1990). For example, Katz (1990) has argued that at best these schedules can only predict that a drug will have abuse potential in humans but that they cannot predict the relative degree of abuse liability that a particular compound might possess.

One procedure that has been claimed to provide an index of reinforcing efficacy is the progressive-ratio (PR) procedure. Initially introduced by Hodos (1961) to study liquid reinforcement in rats, this schedule has since been used to assess the reinforcing effects of electrical brain stimulation or drugs, using primates, pigs, dogs and rats as subjects (Bedfor et al, 1978; Dantzer 1976;Griffiths et al, 1980;Hubner and Moreton 1991;Loh and Roberts, 1990; Risner and Cone 1986; Roberts 1989; Roberts et al, 1989; Winger and Woods 1985). Under a PR schedule, each reinforcer is delivered under a ratio schedule, but the ratio value for each successive reinforcer is increased progressively until eventually the subject fails to emit the required number of responses in the designated period (this highest ratio completed within the time limit is usually deemed the breaking point). The breaking point maintained by cocaine has been shown generally to increase as a function of cocaine dose (Griffiths et al, 1978, Risner and Cone, 1986; Roberts et al, 1989), although at high doses of cocaine this relationship may not hold (Bedford et al, 1978; Risner and Silcox 1981). Most of these studies have used relatively few subjects and have presented data for individual animals without statistical analyses.

More recently, Roberts (Roberts 1989; Roberts et al, 1989) and others (Hubner and Moreton, 1991; Schenk and Peltier 1991) have used rats as subjects in the PR procedure. The establishment of the PR procedure in rats has several appealing aspects. For example, rodents are inexpensive as subjects, and it should be easier to obtain data from enough subjects to permit statistical evaluation. To date, however, little information is available concerning the parameters of self-administration by rats under this schedule. If dose-effect data from different drugs are to be compared, or if several doses of a blocking drug are to be tested against several doses of cocaine, then it will be critical that baseline stability is maintained across an extended period of time. At least one laboratory has reported difficulties in maintaining adequate baseline stability under this procedure (Schenk and Peltier, 1991). Thus, we have investigated the acquisition and stability of PR responding using cocaine as a reinforcer to determine the viability of this technique in rats.

When cocaine self-administration is tested under low-value FR schedules, rate of responding is frequently the dependent variable. If this variable is expressed as the time between successive reinforcers (inter-reinforcer time, IS^RT), then as the dose of cocaine is increased in those procedures, the IS^RT also increases. Indeed, in many cases the IS^RT increases with dose such that essentially constant intake of cocaine occurs per unit time, independent of the dose tested (Lane et al, 1992; Pickens and Thompson, 1968, Wilson et al 1071). For the PR schedule, little information exists concerning changes that occur in the IS^RT as a function of the dose of drug tested. Most studies using PR methodology have simply reported breaking point (highest ratio completed) data without providing a measure of time between individual reinforcers. Thus, only in the study by Roberts *et al.* (1989), in which the overall rate of cocaine intake was measured during PR responding, has this relationship been examined. Those authors showed that pretreatment with the dopamine antagonist haloperidol resulted in a faster intake of cocaine at the same time that the breaking point was reduced. However, that study used only a single dose of cocaine, and no studies have systematically varied the dose of cocaine to determine the relationship between breaking point and IS^RT. We

have studied the effects of a wide range of doses of cocaine on both the number of reinforcers obtained, the highest ratio completed, and the IS^RT. We have also determined which of the two measures, the number of reinforcers obtained or the highest ratio completed, would be the most appropriate measure of the breaking point for statistical analysis.

Although the primary purpose of this study was to provide a detailed analysis of the parameters of PR responding, we also assessed the effect of pretreatment with two drugs that we have recently tested on cocaine self-administration under a low-value FR schedule (Lane et al 1992). In that study, i.p. pretreatment with the dopamine type-1 receptor (D1) antagonist SCH 23390 (50 and 100 μg/kg) decreased the IS^RT for cocaine self-administration, whereas i.p. pretreatment with ondansetron, a serotonergic type-3 receptor ($5HT_3$) antagonist, had no significant effect upon cocaine self-administration. Based upon these results in the low-value FR procedure, we hypothesized that SCH 23390 would reduce the number of reinforcers obtained and shorten the IS^RT for cocaine self-administration under the PR schedule. Consequently, the effect of s.c. injection of SCH 23390 (10 μg/kg) was tested against a 27-fold range of doses of cocaine (0.028, 0.083, 0.25 and 0.75 mg/inj). In addition, SCH 23390 was tested at the doses of 5.6, 10, 17.8 and 32 μg/kg, this time against the training dose of cocaine (0.25 mg/inj). The s.c. route was chosen because SCH 23390 has a relatively short duration of action, and because a cocaine self-administration session under the type of PR schedule we used typically runs for about twice as long as an FR session, for the same dose of infused cocaine. *Eticlopride was tested s.c. at the dose of 0.032 and 0.1 mg/kg against 0.028, 0.083 and 0.25 mg/inj of cocaine.* Ondansetron was tested i.p. at the dose of 0.1 mg/kg against the same dose range of cocaine. Ondansetron was also tested i.p., using a 1000-fold range of doses (0.001 to 1.0 mg/kg), against a single dose of cocaine (0.25 mg/inj).

METHODS

Animals Adult male rats of Fisher F344 strain (Harlan, Indianapolis, IN) were subjects. They were maintained at approximately 270 g body weight (range: 264 to 291 g) by restricting daily access to food; over the course of the experiments their weight corresponded to approximately 85% of their free-feeding body weight. Rats were weighed every second day. Body weights were restricted because this procedure produces higher rates of drug intake in self-administration procedures (Carroll et al, 1979).

Apparatus Lever-press shaping was done with food as a reinforcer in standard two-lever operant chambers (model E-10-10TC, Coulbourn Instruments, Lehigh Valley, PA), using 45 mg food pellets (BioServ, Frenchtown, NJ). Self-administration experiments were performed in chambers that were custom-designed and locally made. They contained a single lever, 5.0 cm above the floor and centered on the back wall, which could be depressed (force of 0.15 N) to close a micro-switch. These chambers were also equipped with two stimulus lights (3 W), one located on the ceiling and the other 9 cm above the lever. The ceiling was constructed of Plexiglas, and was fitted with a 1.0 cm hole in the center through which a catheter/spring assembly was passed. The arrangement of the counterbalanced swivel was essentially identical to that reported by Dworkin *et al.* (1990). The chambers were placed in sound- and light-attenuating enclosures. Syringe pumps (Razel, Model A; driven at 3.33 RPM) were located outside the enclosures and were used to drive 10 ml syringes that contained a cocaine or a saline-vehicle solution. Lines (0.06 inch O.D. Tygon Microbore Tubing, Norton Performance Plastics, Akron, OH) from these pumps were connected via a stainless steel Y connector (part D-TYC-316-20, Small Parts, Miami, FL) placed just before the swivel, and a single line left the Y connector to enter the swivel (Part 50500, Stoelting, Chicago, IL). The exit line from the swivel immediately entered the chamber; this line was protected inside a steel spring that was 24 cm in length and 0.4 cm in diameter. The free end of the spring was ended by a nut that could be locked onto the threaded part of the rat's head mount (see the Surgical Procedure section). All experimental contingencies were controlled and data were recorded via

MS-DOS-compatible microcomputers using software described previously (Emmett-Oglesby et al, 1982; Spencer and Emmett-Oglesby, 1985).

Lever-press shaping Sixteen rats were first daily trained to press a lever using food (45 mg pellets) as a reinforcer. Under a series of FR schedules, increasing from FR1 to FR10 and with no time limit, they were shaped until they emitted at least 200 presses during one hour under a FR10 schedule during two consecutive daily sessions. Rats were then trained under a PR schedule, in which a progressively greater number of lever presses had to be emitted for each successive reinforcer (one food pellet). The progression in the number of presses required (ratio) was a modified version of the exponential equation used by Roberts (personal communication):

Ratio = A * exp(Reinforcer number*B) - C

where A and C both equal 5, and B equals 0.2. We modified the progression to produce a more rapid increase in the size of the ratio required by replacing the first six values (1, 2, 4, 6, 9 and 12) with the values 3, 6 and 10, and then continued with the progression given by the equation (15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 603, 737, 901, 1102, and 1347). The session was ended when a rat failed to complete the ratio for a particular reinforcer within one hour since the delivery of the previous reinforcer. After seven daily training sessions on this PR schedule, surgery was performed for catheter implantation.

Surgical procedure As described in detail in Lane *et al.* (1992), under anesthesia (ketamine, 100 mg/kg; chlordiazepoxide, 20 mg/kg; and nalbuphine, 10 mg/kg), a Silastic catheter (0.025 inch O.D.) was inserted into the right external jugular and its tip advanced into the right atrium. The free end of the catheter was run subcutaneously to an incision in the skin at the top of the skull. This free end was then connected to a modified C313G cannula assembly (Plastic One, Roanoke, VA). The cannula assembly was modified by the vendor such that 5.0 mm of the 22 ga stainless steel cannula guide was left above the plastic threads. Below the plastic threads, this guide cannula was bent at a right angle; the catheter was connected to this bent portion of the 22 ga tube, and the entire unit consisting of catheter, guide

cannula and lower-half of the plastic threads was then embedded in dental acrylic cement and fixed to the skull via three stainless steel screws. Immediately following surgery, subjects were injected i.v. with 0.1 ml of a solution containing 1 U/ml heparin and 1000 U/ml streptokinase (Kabivitrum Inc., Franklin, OH) and an antibiotic (ticarcillin plus clavulanate: Timentin, Beecham Laboratories, Bristol, TN), 3.3 mg in 0.1 ml. This treatment was repeated every 12 hours for five days after surgery. This antibiotic regimen was also administered for five days at the start of each month. Catheter patency was assessed immediately before starting each self-administration session by first checking that blood could be drawn into the catheter and second by flushing the catheter with 0.1 ml of heparinized saline (10 U/ml). At the conclusion of the session, the catheter was flushed with an additional 0.1 ml of heparinized saline (30 U/ml) containing 1000 U/ml streptokinase.

Cocaine self-administration shaping on a fixed-ratio schedule On the fifth day following surgery, subjects were shaped to self-administer cocaine once daily. At the start of each session, a priming injection of cocaine was administered. Subjects were first shaped on a FR1 schedule with a maximum of 25 self-injections per session and with no time limit. The priming injection and each infusion of cocaine consisted of 0.25 mg/inj of cocaine in 0.1 ml of saline-vehicle delivered over 5.6 sec. Priming and each infusion were accompanied by a flashing of the two stimulus lights followed by a 30-sec time-out in the dark; a press during this time-out had no consequences. Here and for the progressive-ratio schedule of cocaine self-administration (see below), the 30-sec time-out was included to prevent overdose. Once stability in the rate of self-administration was acquired (i.e., the IS^RT did not vary by more than 20 percent between two consecutive sessions) rats were switched to a series of FR2 schedule sessions. Each FR2 session was limited to 15 self-injections of cocaine (0.25 mg/inj), again with no time limit. The same stability criterion as the one used for the FR1 schedule was applied before switching the rats to the PR schedule. Once the rats had been switched to the progressive-ratio schedule, they were never returned to the fixed-ratio schedule.

Cocaine self-administration training on a progressive-ratio schedule Rats were trained or tested (see paragraphs below) once daily. The PR schedule used for cocaine self-administration was the same as the one used with food pellets as reinforcers except that at the start of each session, a priming injection of cocaine was administered. The priming injection and each infusion of cocaine were in all respects similar to the ones used for the FR schedule. The PR schedule described in "Lever-press shaping" governed the relationship between current reinforcer number and ratio to be completed, and there was a one-hour time limit to obtain each reinforcer. Data from each self-administration session were scored as number of reinforcers obtained, highest ratio completed, and IS^RT.

Acquisition and stability of cocaine-self-administration under a PR schedule Rats were allowed to self-administer the training dose of cocaine (0.25 mg/inj) for 50 sessions to permit determination of acquisition and stability of the number of reinforcers obtained under the PR schedule. At the end of this 50 training sessions period, three different doses of cocaine (0.028, 0.083 or 0.75 mg/inj) or saline-vehicle, tested in a randomized order, were substituted for the training dose of cocaine. For these tests, the priming injection consisted of a 0.1 ml volume of the saline-vehicle or of the solution of cocaine (0.028, 0.083 or 0.75 mg/inj) to be tested. Saline-vehicle or dose of cocaine were tested in a randomized order. Test sessions were separated by at least one training session, and the number of reinforcers obtained during these training sessions had to be within two of the baseline value before subjects were used in the next test session. Failure to meet this criterion resulted in supplemental training sessions until the criterion was fulfilled. This criterion was also applied during the SCH 23390 and ondansetron pretreatment experiments.

Relationship between the dose of cocaine infused, number of reinforcers obtained, highest ratio completed and IS^RT recorded These parameters of cocaine self-administration were determined for saline-vehicle and four doses of cocaine (0.028, 0.083, 0.25 and 0.75 mg/inj) following a saline i.p. pretreatment, which occurred 30 min prior to the start of the session. Results from this experiment were first analyzed separately to determine the

appropriate parameter (i.e., either number of reinforcers obtained or highest ratio completed) for subsequent analysis of the effect of SCH 23390, eticlopride or ondansetron pretreatments. Mean IS^RTs were calculated by first averaging each subject's individual IS^RT, excluding the first and the last IS^RT. These two IS^RTs were excluded because they were not representative of the general pattern of IS^RTs: the first IS^RT was variable because some rats took much longer than others to start self-administering following the infusion of the priming dose; the last IS^RT also showed greater variability because at this final ratio value, some rats occasionally displayed a pattern of lever pressing in which responding was interrupted by periods of non-responding.

Effects of pretreatment with SCH 23390, eticlopride or ondansetron Nine rats were used to study the effect of s.c. pretreatment with saline or SCH 23390 (10 μg/kg) against four doses of cocaine (0.028, 0.083, and 0.25 mg/inj). The antagonists were not tested against the highest dose of cocaine used in initial dose-response testing (0.75 mg/inj) because baseline test session with this dose typically lasted 4-6 hours, during which time any effect of the antagonist would have diminished. Saline or SCH 23390 was administered 20 min pre-session. In addition, the effect of s.c. pretreatment with saline or SCH 23390 (5.6, 10, 17.8 or 32 μg/kg) against the training dose of cocaine (0.25 mg/inj) was evaluated in ten rats. Saline, SCH 23390, as well as the dose of infused cocaine were tested in a randomized block-design. *Ten rats were used to determine the effect of saline or eticlopride (0.032, or 0.1 mg/kg) pretreatment (30 min pre-session, s.c. administration) against the training dose of cocaine.* Six rats were used to determine the effect of saline or ondansetron (0.001, 0.01, 0.1 and 1.0 mg/kg) pretreatment (30 min pre-session, i.p. administration) against the training dose of cocaine (0.25 mg/inj). Eight rats were used to assess the effect of 0.1 mg/kg i.p. ondansetron against 0.028, 0.083 and 0.25 mg/inj of cocaine. Saline, ondansetron, as well as the dose of cocaine, were tested in a randomized order.

Drugs Cocaine HCl (Sigma, St. Louis, MO; and NID, Research Triangle Park, NC) was dissolved in saline-vehicle (1.0 U/ml heparinized 0.9% saline) and filtered through 0.22

micron filters (Millipore, Bedford, MA) into sterile 10 ml syringes immediately before use. Ondansetron HCl (gift of Glaxo Group Research, Ware, UNITED KINGDOM), eticlopride Hcl (research Biochemicals Inc., Natick, MA) and SCH 23390 HCl (Research Biochemicals, Natick, MA) were all prepared freshly before each test session and diluted in 0.9% saline.

Data analysis All data reported in the text or plotted in the figures are expressed as mean ± S.E.M., except for Fig. 2 where data are plotted as mean ± Std. Dev. For the analysis of the cocaine dose-effect data following saline pretreatment, the number of reinforcers obtained, highest ratio completed, and IS^RT recorded were subjected to a Cochran's test (Winer 1962) to determine whether data violated assumptions of homogeneity of variance. Data that did not violate these assumptions were further subjected to a one-way ANOVA for repeated measures. For the SCH 23390, eticlopride and ondansetron experiments that used several doses of cocaine, the number of reinforcers obtained was subjected to a two-way ANOVA for repeated measures, with the dose of cocaine and pretreatment (saline or drug) as the two within factors. For the experiments where several doses of SCH 23390, eticlopride or ondansetron were tested against the training dose of cocaine, the number of reinforcers obtained was subjected to a one-way ANOVA for repeated measures, with the doses of the tested drug as the within factor.

IS^RTs from the SCH 23390 or ondansetron experiment were subjected to a two-way or one-way ANOVA for repeated measures, respectively. Analysis of IS^RTs for ondansetron was performed on data collected during the experiment where saline or four doses of ondansetron were assessed against the training doses of cocaine, as opposed to using values from the single dose of ondansetron against three doses of cocaine, since this yielded more ondansetron IS^RT values.

For all ANOVA's, when F values showed a significant effect ($p < 0.05$), a planned comparison analysis was performed, with significance reported whenever the p values were less than 0.05 (for clarity sake, the F and p values for the post-hoc tests are not reported in the text). All ANOVA's were performed with Systat software (Winer 1962).

RESULTS

Acquisition and stability of cocaine self-administration under a PR schedule

Stable self-administration of cocaine (training dose of 0.25 mg/inj) under a PR schedule was acquired within approximately 10 sessions (Fig. 1). Once PR responding was acquired, there was a high degree of stability in the number of reinforcers obtained over the period covering the next 41 sessions; the average number of reinforcers obtained during these last 41 sessions was 19.0±0.1. In addition, for each individual session over this period, the S.E.M.'s ranged from 0.8 to 1.8 reinforcers. The low values of these S.E.M.'s reflect the small inter-subject differences in the number of reinforcers obtained within the same training session.

Relationship between the dose of cocaine infused, the number of reinforcers obtained and the highest ratio completed

Varying the dose of infused cocaine over a 27-fold range (from 0.028 to 0.75 mg/inj) resulted in a 2.7-fold dose-related increase in the number of reinforcers obtained (Fig 2, top panel; $F(3,30)=46.57$, $p<<0.001$). However, this was not a linear effect across the entire range of doses. Tripling the training dose (from 0.25 to 0.75 mg/inj) did not markedly increase the number of reinforcers obtained but instead the curve plateaued (which may be caused by overdose intoxication. All but the 0.25 and 0.75 mg/inj doses differed significantly from one another. Substituting the saline-vehicle solution for the training dose of cocaine yielded a very low number of reinforcer obtained (1.1±0.3). Note that the average number of reinforcers earned under the 0.25 mg/inj dose (19.5±1.2) was very close to the average calculated for the period covering the last 41 training sessions (19.0±0.1; ref. to Fig. 1).

As the dose of cocaine increased, the highest ratio completed also increased (Fig 2, bottom panel). Increasing the dose of cocaine from 0.028 to 0.75 mg/inj resulted in a 16.6-fold increase in the highest ratio completed. However, as shown by the different magnitudes of Std. Dev.'s on the figure, these data violate assumptions of homogeneity of variance (Cochran's test: $C=0.70$, $p<<0.01$). Violation of homogeneity of variance can be a serious

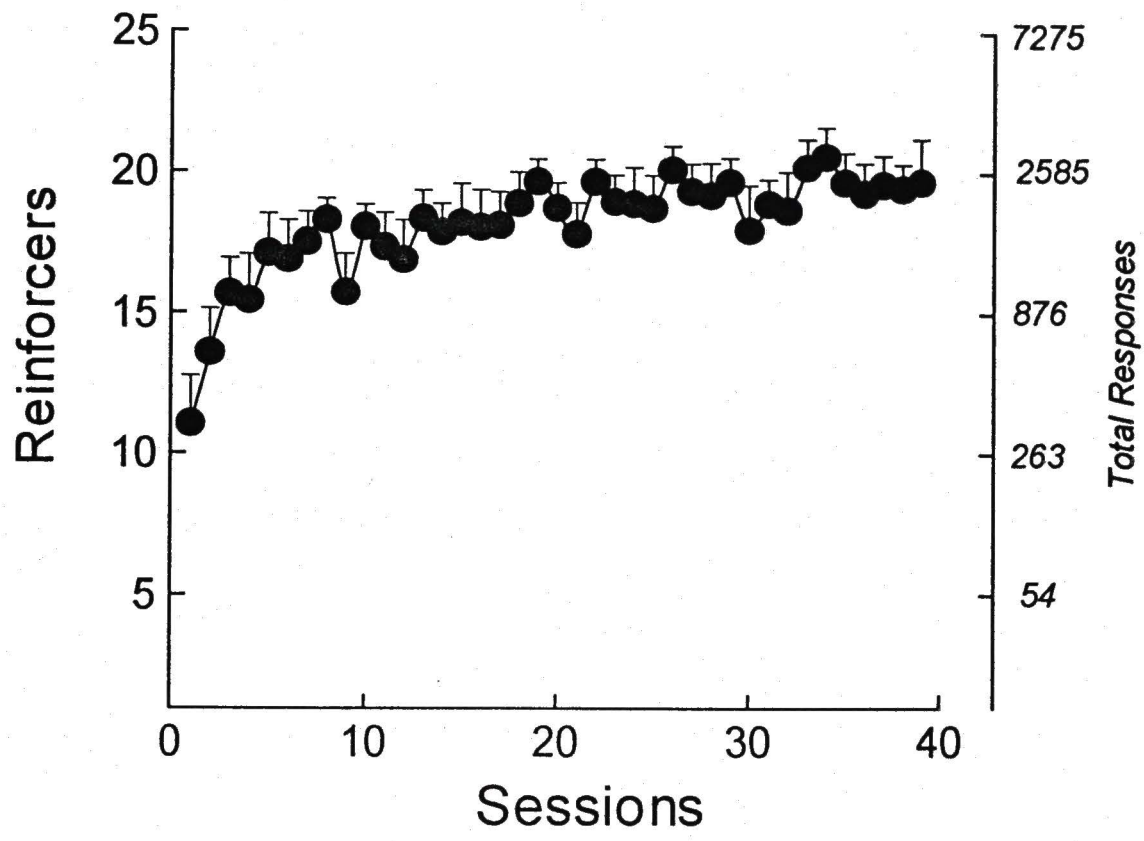


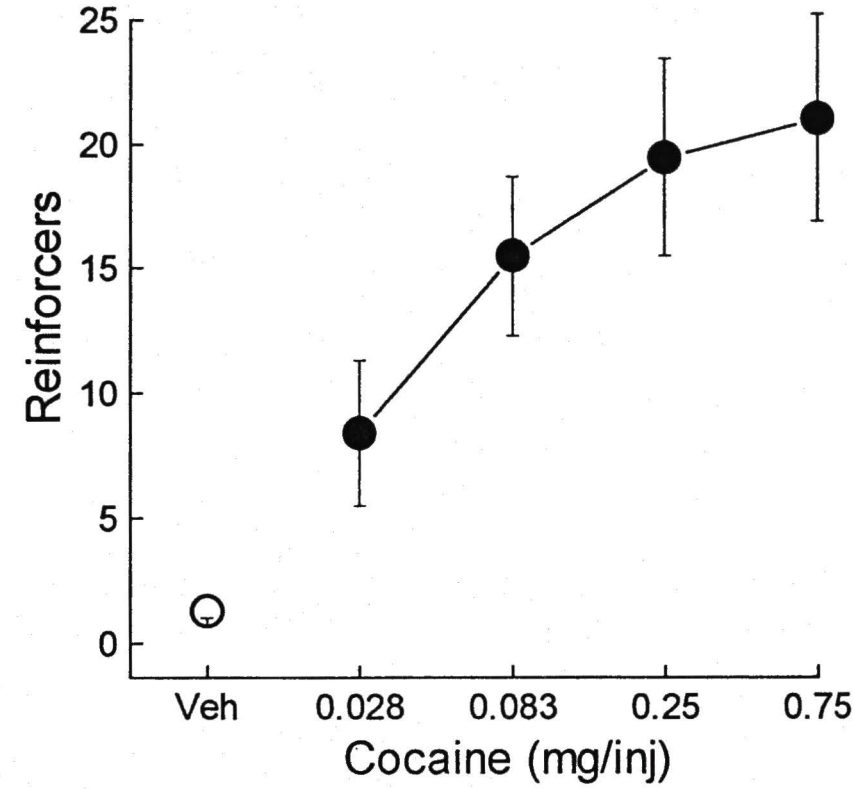
Figure 1. *Acquisition and stability of self-administration of cocaine under a PR schedule*

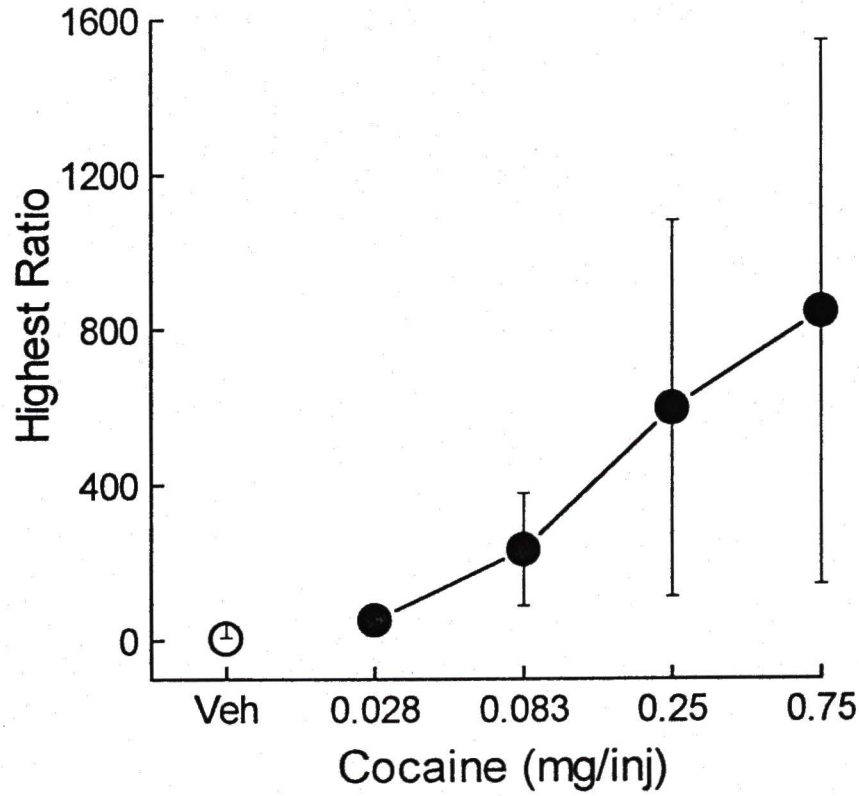
Abscissa: training session number; ordinate: number of reinforcers obtained during the training session. Each circle represents the average number of reinforcers obtained as a function of the training session shown on the abscissa. The dose of cocaine infused as a reinforcer was 0.25 mg/inj. Vertical bars represent S.E.M. 's. N= 13.

Figure 2. *Relationship between the dose of cocaine infused and the number of reinforcers obtained (top panel) or the highest ratio completed (bottom panel)*

Top panel: abscissa: dose of cocaine available for self-administration; ordinate: number of reinforcers obtained during the session. Each filled circle represents the average ± Std. Dev. The open circle represents the average number of reinforcers obtained when saline-vehicle (VEH) in a volume of 0.1 ml per infusion was substituted for cocaine.

Bottom panel: same legend as top panel except for the ordinate, which is the highest ratio completed during the session. Refer to "Lever-press shaping" for the relationship between reinforcer number and the associated ratio. For both panels, some Std. Dev. bars do not show because they are smaller than the symbol size. N=11.


25
20
15
10
5
0
Reinforcers
Veh
0.028
0.083
0.25
0.75
Cocaine (mg/inj)


1600
1200
800
400
0
Highest Ratio
Veh
0.028
0.083
0.25
0.75
Cocaine (mg/inj)

confound in an ANOVA for repeated measures (Winer, 1962), and for this reason, highest ratio completed was not considered further for statistical analysis. When a data set violates assumptions of homogeneity of variance, a means of circumventing this problem is to transform the data through square root or logarithmic calculation (Winer 1962). Here, the number of reinforcers obtained is a natural logarithmic function of the highest ratio completed, and the reinforcer data do not violate assumptions of homogeneity of variance. For these reasons, subsequent data analyses for the SCH 23390 and ondansetron experiments were performed on the number of reinforcers obtained instead of highest ratio completed.

Relationship between the dose of cocaine infused, the reinforcer number, and the inter-reinforcer time

The relationship between the dose of cocaine infused, the number of reinforcers obtained and the IS^RT is presented in the three dimensional graph of Fig. 3. Two relationships concerning the IS^RT can be seen in this graph: first, the IS^RT increases as a function of the dose of cocaine infused; second, for any given dose of cocaine, the IS^RT increases during the session from reinforcer to reinforcer.

The mean IS^RT (Table 1) increased 7.7-fold ($F(3,30)=157.71$, $p<<0.001$) as the dose of infused cocaine increased 27-fold, and all four mean IS^RT s were significantly different from one another. Note that IS^RTs obtained under saline-vehicle were not analyzed because most subjects (10 out of 11) took less than three reinforcers.

Effect of pretreatment with saline or SCH 23390 on the number of reinforcers obtained and the inter-reinforcer time

For all three doses of cocaine, pretreatment with SCH 23390 (10 μg/kg) decreased the highest ratio completed (data not shown and not amenable to statistical analysis, see above) as well as decreased the number of reinforcers obtained (Fig. 4, top panel; $F(1,6)=32.66$,

Figure 3. *Relationship between the dose of cocaine infused, the reinforcer number, and the inter-reinforcer time*

X axis: reinforcer number; Y axis: dose of cocaine available for self-administration; Z axis: inter-reinforcer time (IS^RT) in min. Each column represents the mean IS^RT. IS^RTs were calculated for individual subjects by excluding the first and the last IS^RT, resulting data for each remaining reinforcer were then averaged across subjects. For each dose of cocaine, the number of IS^RTs plotted corresponds to the average number of reinforcers obtained rounded to the nearest integer (see Fig. 2, top panel). For clarity, S.E.M.'s have been omitted. N=11.

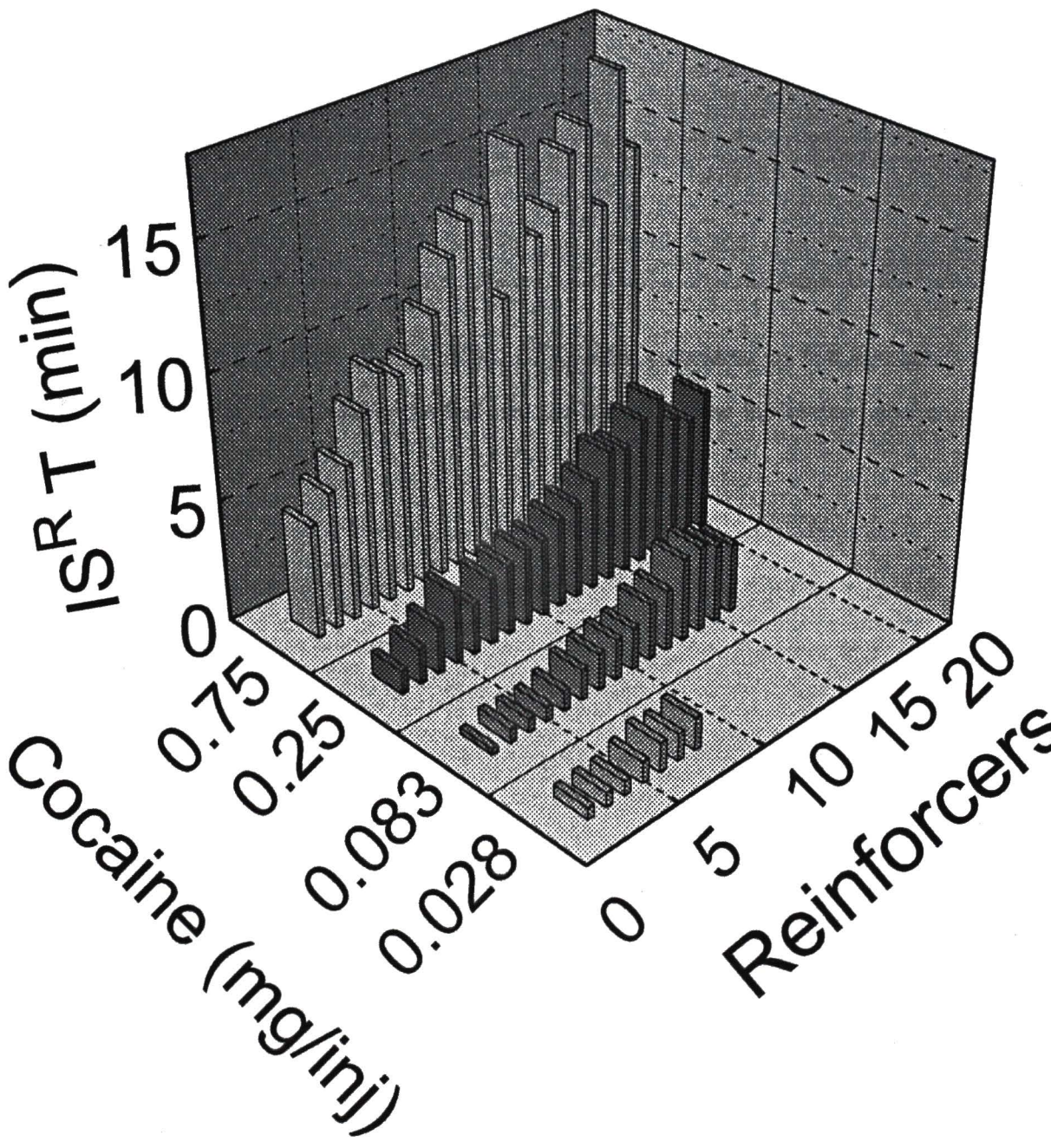

$IS^R T$ (min)
15
10
5
0
0.75
0.25
0.083
0.028
Cocaine (mg/inj)
0
5
10
15
20
Reinforcers

TABLE 1

RELATIONSHIP BETWEEN THE DOSE OF COCAINE INFUSED AND THE MEAN INTER-REINFORCER TIME (IS^RT)

	Dose of Cocaine (mg/inj)			
	0.028	0.083	0.25	0.75
IS^RT	1.57 ± 0.16	2.35 ± 0.13	4.65 ± 0.52	12.14 ± 0.58

Values are expressed as mean (min) ± SEM. IS^RTs were calculated by first averaging each subject's individual IS^RTs, excluding the first and the last IS^RT (see the Method section). n = 11.

$p<<0.001$). The ability of SCH 23390 to decrease the number of reinforcers obtained was significant for the three lowest doses of cocaine. The significant interaction between the dose of cocaine infused and the pretreatment ($F(3,18)=7.14$, $p<<0.01$) suggests that SCH 23390 or saline pretreatment affected the cocaine dose-effect curve in a different way. This differential effect of SCH 23390 can be seen in the graph where the SCH 23390 pretreatment curve is not parallel to the saline pretreatment curve.

When tested against the training dose of cocaine (0.25 mg/inj), SCH 23390 was found to decrease the number of reinforcers in a dose-dependent manner (Fig. 4, bottom panel; $F(3,27)=14.18$, $p<<0.001$). A dose of 32 ug/kg of SCH 23390 completely blocked self-administration of 0.25 mg/inj of cocaine. Differences in the number of reinforcers were significant for all pairs of doses of SCH 23390 and saline compared, except for two pairs: the saline versus 3.2 μg/kg, and the 17.8 μg/kg versus 32 μg/kg. For all subjects, SCH 23390, markedly decreased the number of reinforcers obtained in a dose-dependent manner.

SCH 23390 at the dose of 10 μg/kg showed a tendency to reduce the IS^RT (Table 2), but this tendency was not significant, nor was the interaction between the dose of infused cocaine and pretreatment. The results at cocaine doses of 0.028 and 0.083 mg/inj were not included in this table since when these dose of cocaine was combined with SCH 23390, three (0.028 mg/inj) and four (0.083 mg/inj) out of the seven rats did not self-administer cocaine. These subjects were omitted from the whole IS^RT analysis since missing data points in a within-subjects ANOVA prevent analysis.

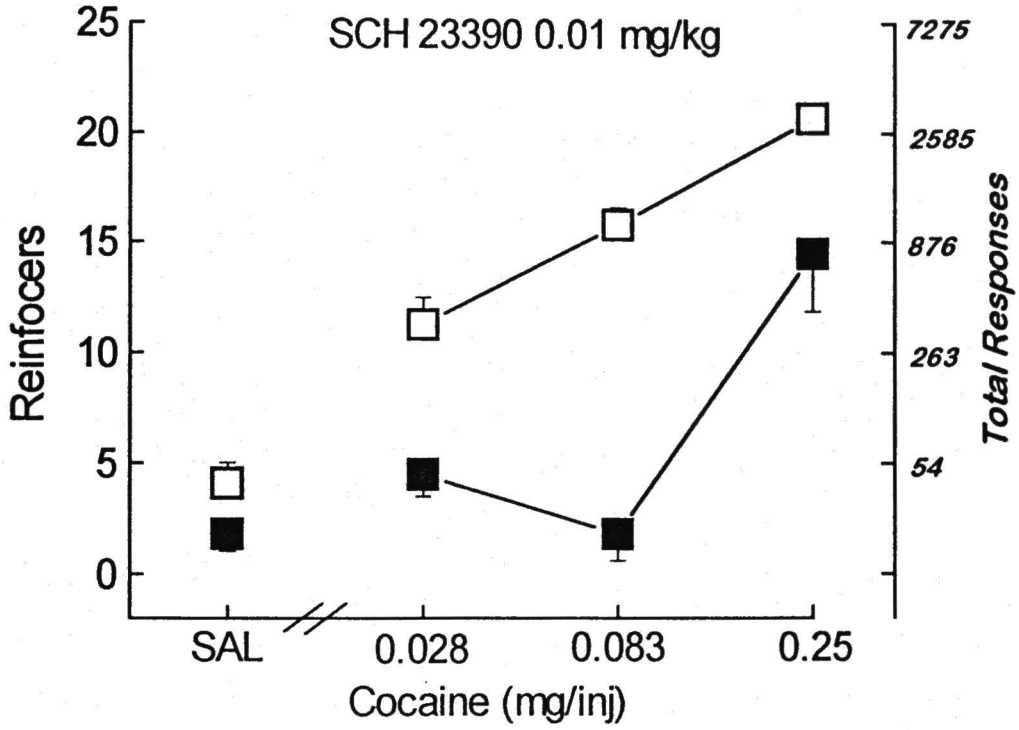
Effect of pretreatment with saline or eticlopride on the number of reinforcers obtained and the inter-reinforcer time

For all three doses of cocaine, pretreatment with eticlopride (32 μg/kg) decreased the highest ratio completed (data not shown and not amenable to statistical analysis, see above) as well as decreased the number of reinforcers obtained (Fig.5, top panel; $F(1,32)=39.80$,

Figure 4. *Effect of SCH 23390 pretreatment on the number of reinforcers obtained*

Top panel: abscissa and ordinate same as in top panel of Fig. 2. Each symbol represents the average number of reinforcers obtained in a session with pretreatment of saline (hollow squares), or SCH 23390 (10 μg/kg, s.c.; filled squares). Vertical bars represent S.E.M.'s. N=7.

Bottom panel: abscissa: dose of SCH 23390; ordinate: number of reinforcers obtained during the session. Each symbol represents the average number of reinforcers obtained in a session with pretreatment of saline (hollow square), or SCH 23390 (filled squares). Saline or SCH 23390 were tested against the training dose of cocaine (0.25 mg/inj). Vertical bars represent S.E.M.'s. N=10.


SCH 23390 0.01 mg/kg
Reinfocers
25
20
15
10
5
0
7275
2585
876
263
54
Total Responses
SAL
0.028
0.083
0.25
Cocaine (mg/inj)

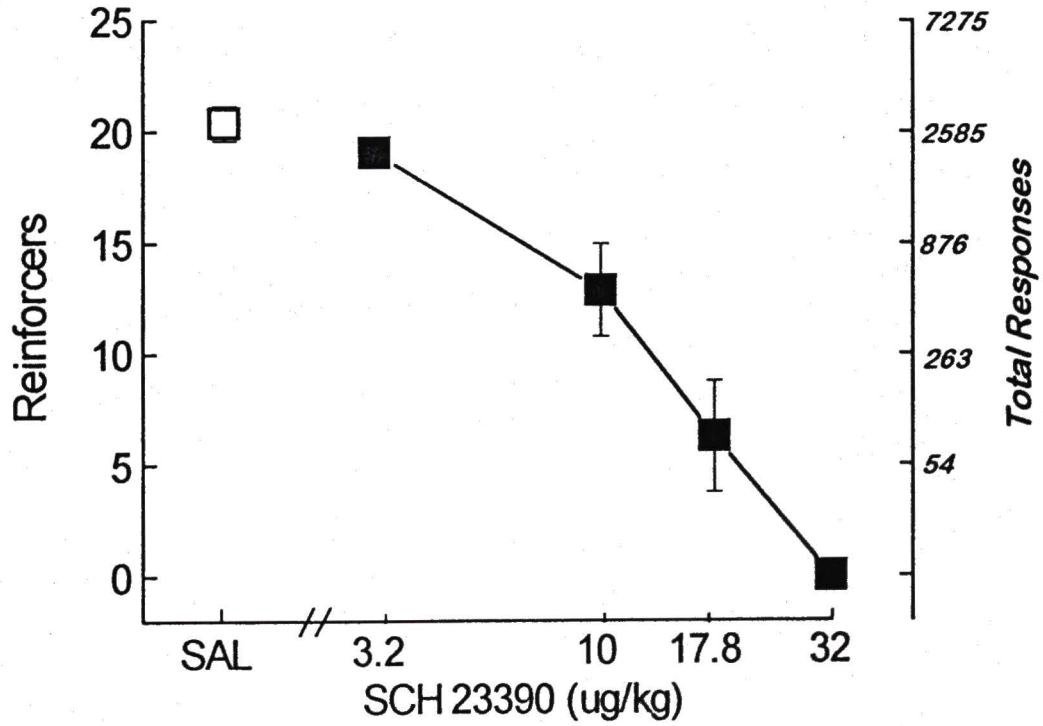

Reinforcers
25
20
15
10
5
0
7275
2585
876
263
54
Total Responses
SAL
3.2
10
17.8
32
SCH 23390 (ug/kg)

TABLE 2

EFFECT OF SALINE OR SCH23390 (10 μg/kg) PRETREATMENT ON THE MEAN INTER-REINFORCER TIME (IS^RT)

	Cocaine (0.25 mg/inj)
Saline	2.60 ± 0.46
SCH23390	2.37 ± 0.22

Values are expressed as mean (min) ± SEM. IS^RTs were calculated by first averaging each subject's individual's, excluding the first and the last IS^RT (see the Method section). In addition to excluding these two IS^RTs, the IS^RT distribution was further truncated for each subject such that equal numbers of IS^RTs were available for comparison for a given dose of cocaine. For example, if a subject tested under the 0.25-mg/inj dose of cocaine obtained 20 and 15 reinforcers under saline and SCH23390 pretreatment, respectively, then the calculation of the mean IS^RT would use only reinforcer 2 to 14 for both pretreatments. This was done to have identical numbers of IS^RTs for each subject across both pretreatments for a given dose of cocaine. If this procedure is not adopted, the additional IS^RTs occurring under saline bias the data by making the saline IS^RTs appear to be longer than is actually the case. n = 7.

p<<0.001). The ability of eticlopride to decrease the number of reinforcers obtained was significant for the three lowest doses of cocaine. The significant interaction between the dose of cocaine infused and the pretreatment (F(2,32)=5.44, p<0.01) suggests that eticlopride or saline pretreatment affected the cocaine dose-effect curve in a different way. This differential effect of eticlopride can be seen in the graph where the eticlopride pretreatment curve is not parallel to the saline pretreatment curve.

When tested against the training dose of cocaine (0.25 mg/inj), eticlopride was found to decrease the number of reinforcers in a dose-dependent manner (Fig. 5, bottom panel; F(3,32)=12.32, p<0.001). A dose of 32 μg/kg of eticlopride completely blocked self-administration of 0.25 mg/inj of cocaine. For all subjects, eticlopride markedly decreased the number of reinforcers obtained in a dose-dependent manner.

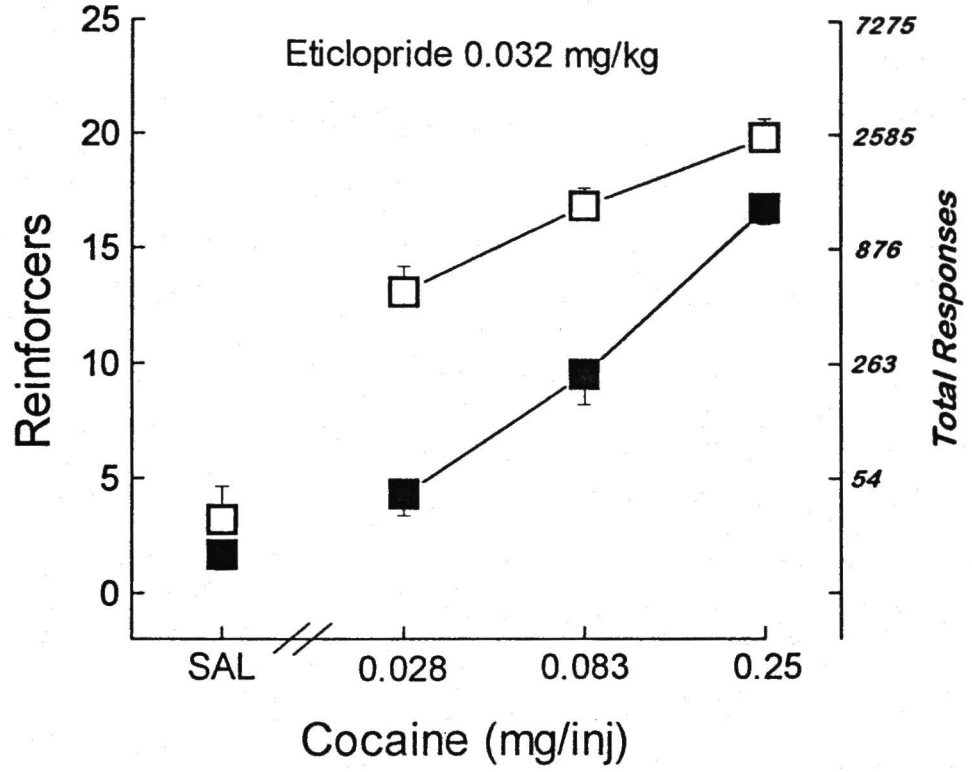
Comparison the reduction of pretreatment with SCH 23390 or eticlopride on the number of reinforcers obtained

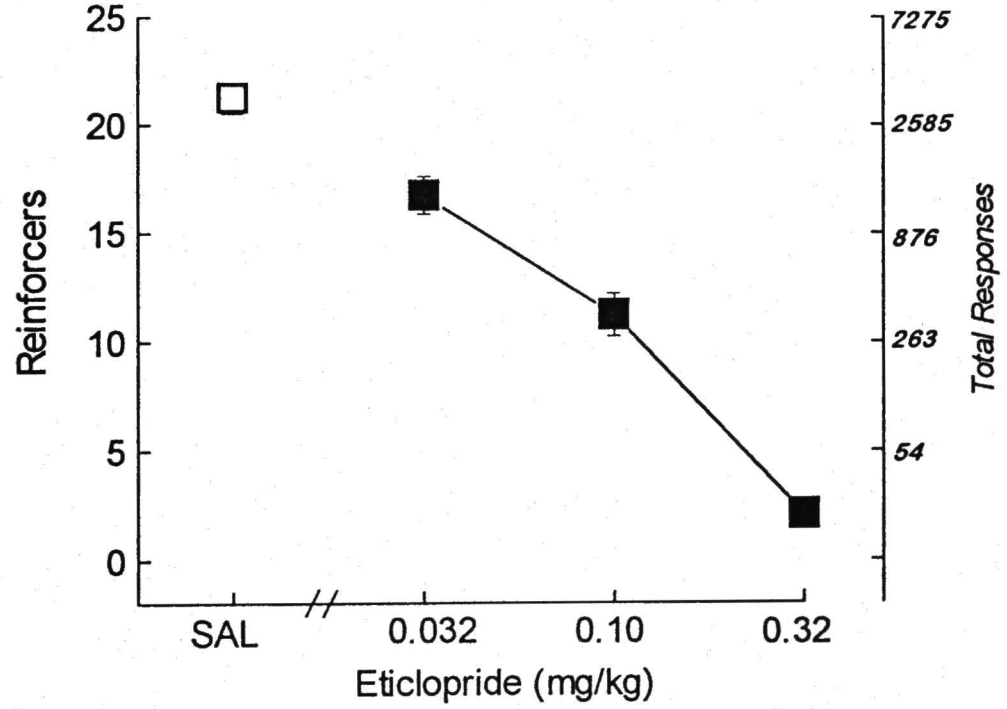
A group of animals (N=12) were tested for the comparison of the effect of reduction in the breaking point between a D1 antagonist (SCH 23390) and a D2 antagonist (eticlopride). SCH 23390 (10 μg/kg) pretreatment reduced the number of cocaine injections taken (Fig 6; F(1,11)=106.8, P<0.001), and this effect was significant for all doses of cocaine tested. Low-dose eticlopride (0.032 mg/kg) pretreatment also reduced the number of reinforcers obtained, but by post-hoc tests this was significant for the medium (0.3 mg/kg/inj; F(1,11)=43.7, P<0.01) and the high dose of cocaine (0.25 mg/inj; F 1,11=17.8, p<0.01). High-dose eticlopride (0.1 mg/kg) pretreatment reduced cocaine self-administration at all doses of cocaine tested (F(1,11)=150.5, P<0.01).

Figure 5. *Effect of eticlopride pretreatment on the number of reinforcers obtained*

Top panel: abscissa and ordinate same as in top panel of Fig. 2. Each symbol represents the average number of reinforcers obtained in a session with pretreatment of saline (hollow squares), or eticlopride (32 μg/kg, s.c.; filled squares). Vertical bars represent S.E.M.'s. N=12.

Bottom panel: abscissa: dose of eticlopride; ordinate: number of reinforcers obtained during the session. Each symbol represents the average number of reinforcers obtained in a session with pretreatment of saline (hollow square), or eticlopride (filled squares). Saline or eticlopride were tested against the training dose of cocaine (0.25 mg/inj). Vertical bars represent S.E.M.'s. N=12.


Eticlopride 0.032 mg/kg
Reinforcers
Total Responses
25
20
15
10
5
0
7275
2585
876
263
54
SAL
0.028
0.083
0.25
Cocaine (mg/inj)


Reinforcers
Total Responses
25
20
15
10
5
0
7275
2585
876
263
54
SAL
0.032
0.10
0.32
Eticlopride (mg/kg)

Effect of pretreatment with saline or ondansetron on the number of reinforcers obtained and the inter-reinforcer time

For the three doses of cocaine tested (0.028, 0.083 and 0.25 mg/inj), pretreatment with 0.1 mg/kg of ondansetron failed to significantly affect the number of reinforcers obtained (Fig. 7, top panel). Indeed, this dose of ondansetron had virtually no effect on the number of reinforcers obtained. Interaction between the dose of cocaine infused and the pretreatment was not significant. Ondansetron was not tested against the dose of 0.75 mg/inj of cocaine since the cocaine-dose response relationships obtained under saline in the SCH 23390 experiment showed that 0.25 and 0.75 mg/inj yielded comparable numbers of reinforcers obtained.

When tested against the training dose of cocaine (0.25 mg/inj), pretreatment with ondansetron, over a 1,000-fold range of doses, neither increased nor decreased significantly the number of reinforcers obtained (Fig. 7, bottom panel). For all four doses of ondansetron, the number of reinforcers obtained was changed by a maximum of five percent as compared to control (saline pretreatment).

Although ondansetron showed a tendency to shorten the mean IS^RT (Table 3), this was not statistically significant. The mean IS^RT for the training dose of 0.25 mg/inj (5.44 min) is higher than the one reported in Table 2 for the SCH 23390 experiments (2.60 min) because the mean IS^RTs in the SCH 23390 experiments were calculated on the basis of a smaller number of reinforcers (see Table 2 for more information).

DISCUSSION

Self-administration of cocaine by rats under this PR schedule presented several interesting features. Its acquisition was rapid and it showed long-term stability. This study provides data analysis showing that for statistical purpose, the number of reinforcers obtained instead of the highest ratio completed is the appropriate measure of the breaking point. As the dose of cocaine is increased, both the breaking point and the inter-reinforcer time (IS^RT)

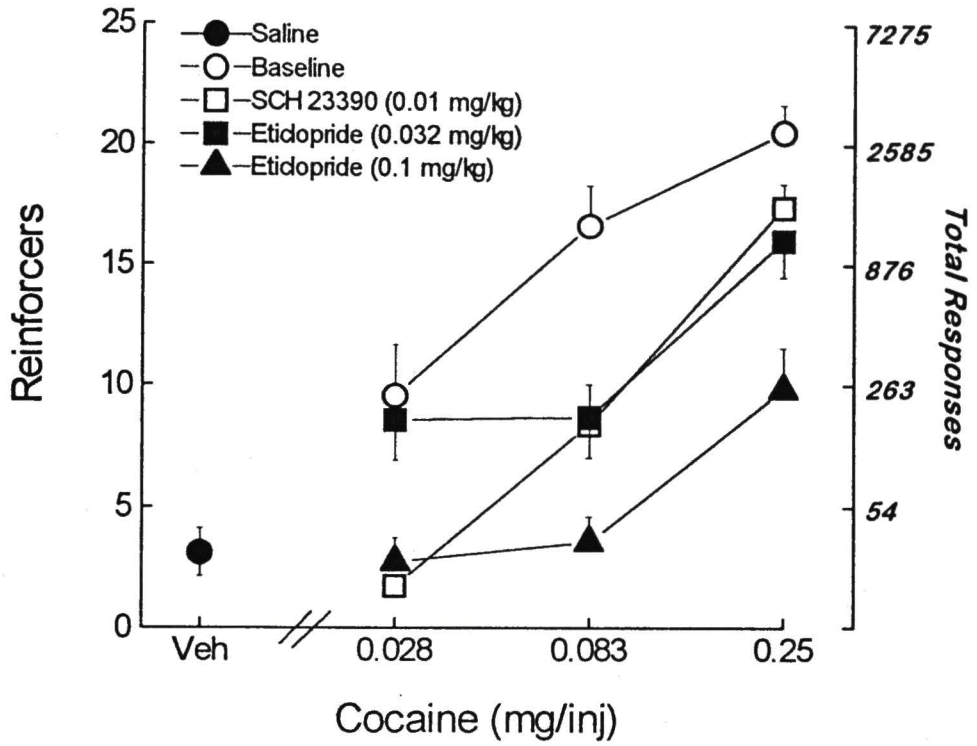


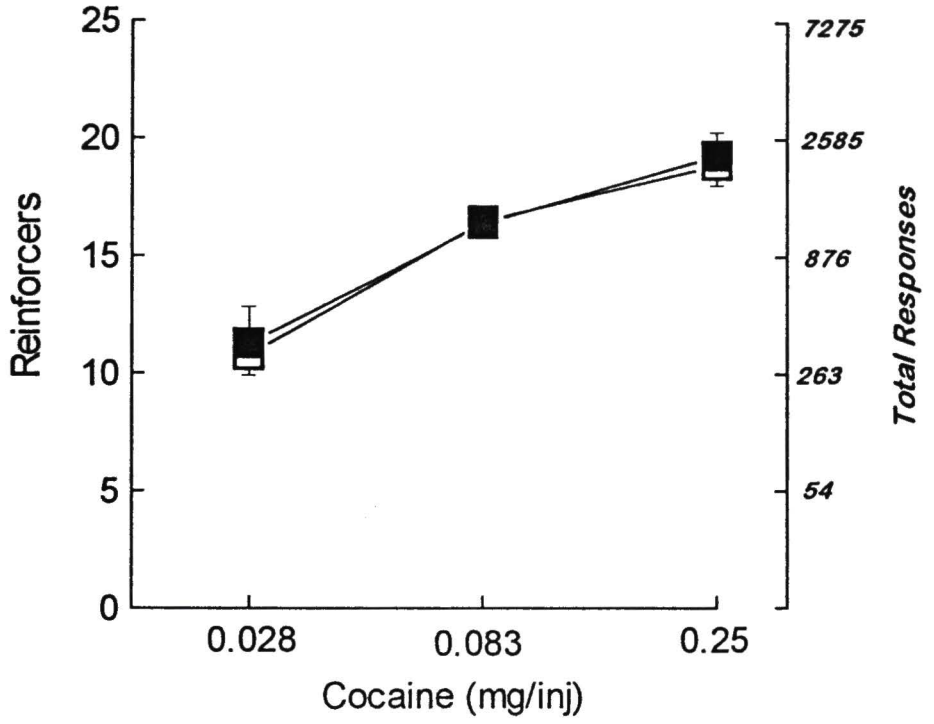
Figure 6. *Comparison of the pretreatment of SCH 23390 and Eticlopride on the number of reinfocers obtained*

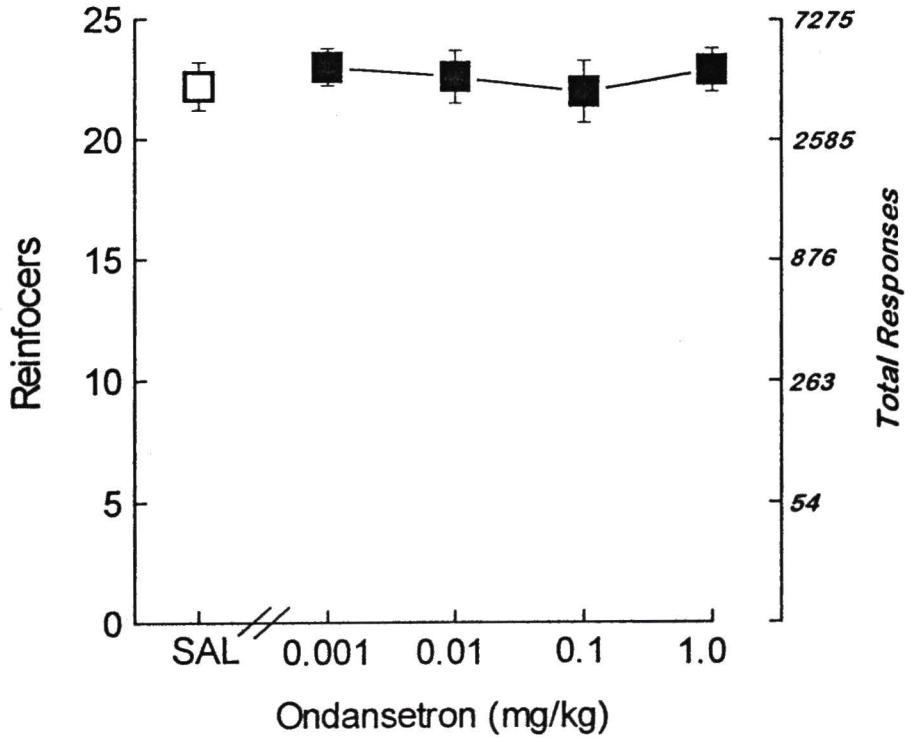
Effects of SCH 23390 (0.01 mg/kg), eticlopride (0.032 mg/kg), and eticlopride (0.1 mg/kg) pretreatment on cocaine self-administration. Left ordinate: average number of cocaine injections self-administered during the session. Right ordinate: Cumulative number of lever pressed emitted during the session. Abscissa: dose of cocaine tested. Each symbol represents the average number of reinforcers obtained in a session with saline (filled circle), SCH 23390 (0.01 mg/kg, s.c.; hollow squares), eticlopride (0.032 mg/kg, s.c.; filled squares), eticlopride (0.1 mg/kg, s.c.; filled triangles) pretreatment. Hollow circle indicated cocaine dose-response curve baseline (without pretreatment). Vertical bars represent S.E.M.'s. N=12.

Figure 7. *Effect of ondansetron pretreatment on the number of reinforcers obtained*

Top panel: Abscissa and ordinate same as in top panel of Fig. 2. Each symbol represents the average number of reinforcers obtained in a session with saline (hollow squares) or ondansetron (0.1 mg/kg, i.p.; filled squares) pretreatment. Vertical bars represent S.E.M.'s. N=6.

Bottom panel: Abscissa: dose of ondansetron. Ordinate: number of reinforcers obtained during the session. Each symbol represents the average number of reinforcers obtained in a session with pretreatment of saline (hollow square), or ondansetron (filled squares). Saline or ondansetron were tested against the training dose of cocaine (0.25 mg/inj). Vertical bars represent S.E.M.'s. N=8.


25
20
15
10
5
0
Reinforcers
7275
2585
876
263
54
Total Responses
0.028
0.083
0.25
Cocaine (mg/inj)


25
20
15
10
5
0
Reinfocers
7275
2585
876
263
54
Total Responses
SAL
0.001
0.01
0.1
1.0
Ondansetron (mg/kg)

increase in an orderly manner. The breaking point was found to be sensitive to the effects of SCH 23390, a drug previously shown to increase the rate of cocaine self-administration in other schedules, and was not affected by ondansetron, a drug that has not previously been shown to affect cocaine self-administration in rats. Finally, monitoring of the IS^RT along with the breaking point allows for control of motor incapacitating effects of drug pretreatment on cocaine self-administration, without having to resort to controls using other schedules or others reinforcers such as food. Our data overall suggest that the rat is an excellent subject for the production of PR schedule data amenable to statistical analysis.

Stability of cocaine self-administration under this PR paradigm can be maintained routinely for more than three months, allowing one to conduct a series of parametric experiments. To our knowledge, long-term stability of PR responding, particularly for large groups of animals, has not been demonstrated before. This study also shows that changing the dose of infused cocaine, with or without pretreatment with SCH 23990 or ondansetron, does not affect baseline stability of PR responding. This is particularly important if one wants to conduct multiple drug tests against several doses of infused cocaine rather than solely against the training dose of cocaine. In addition, rapid acquisition of cocaine self-administration under this type of PR schedule allowed testing to begin within two weeks of initiation of training. We attribute the longevity and viability of the subjects to semi-sterile surgical procedures and post-operative care, and to a fastidious regimen of anticoagulants and antibiotics that was designed to minimize infection and maximize catheter patency (see "Surgical Procedure").

Both the number of reinforcers obtained and the highest ratio completed increased in an orderly manner as a function of an increase in the dose of infused cocaine. These increases are thought to reflect an increase in the reinforcing efficacy of the reinforcer (Hodos and Kalman 1963). Inverted U-shaped curves have been observed by others at the highest doses of drug intake (Griffiths et al, 1979; Risner and Goldberg, 1983; Yanagita 1973). Doses of cocaine above 0.75 mg/inj were not used in this study because of the inherent toxicity of the

TABLE 3

EFFECTS OF SALINE OR ONDANSETRON PRETREATMENT ON THE MEAN INTER-REINFORCER TIME (IS^RT)

Dose of Ondansetron (mg/kg)

	Saline	0.001	0.01	0.1	1.0
IS^RT	5.44 ± 0.27	5.00 ± 0.20	4.84 ± 0.26	4.98 ± 0.24	4.55 ± 0.12

Values are expressed as mean (min) ± SEM. Saline or Ondansetron was tested against the training doses of cocaine (0.25 mg/inj). IS^RTs were calculated by excluding the first and the last IS^RT (see the Methods section). n = 6

drug (e.g., in pilot studies, when doses of 3.7 mg/inj and above were used, seizures and/or death were occasionally observed).

Independent of the highest dose utilized, the values for the highest ratio completed were routinely higher in this study than the values reported by others using rats as subjects. For example, Roberts *et al.* (Roberts et al, 1989) reported average highest ratios of 38 and 92 for doses of cocaine of 0.083 and 0.25 mg/inj, respectively. Hubner and Moreton (Hubner and Moreton, 1991) reported two different averages for a 0.225 mg/kg dose of cocaine: in one, the highest ratio was 88 and in the other the highest ratio was 168. In contrast, our animals reached average highest ratio values of 599 and 848 for 0.25 and 0.75 mg/inj, respectively. We do not know why our animals worked to such high values, but perhaps the difference between our results and those of Hubner and Moreton (Hubner and Moreton, 1991) may be found in differences between paradigms (i.e., progression of the ratio, force required to produce a lever-press, duration of the time-out, etc.) or differences in the strains, ages and body weights of the rats. However, a difference in the progression of the ratio is unlikely to account for differences between this study and that of Roberts *et al.* (1989), who used essentially the same progression.

As a rate measure, inter-reinforcer time (IS^RT, in minutes) was used. The IS^RT increased approximately 8-fold and in a direct relationship to the dose of cocaine tested. For example, for cocaine doses of 0.25 and 0.75 mg/inj, associated IS^RTs were approximately 4.5 and 12 min, respectively. Interestingly, the IS^RT value found with the training dose of cocaine (0.25 mg/inj) is similar to the IS^RT found in a FR2 procedure (IS^RT: 4.5 min) for this same dose in our laboratory (Lane et al, 1992). From data in which an FR5 was used with a cocaine dose of 0.75 mg/inj (Hubner and Moreton, 1991) we calculated that these authors obtained an IS^RT of approximately 3.4 min; and similarly, using an FR1 schedule and a 0.5 mg/inj dose, we calculated that Peltier and Schenk (Peltier and Schenk, 1991) obtained an IS^RT of approximately 4.8 min. Although additional data are necessary to reach a firm conclusion, the available data suggest that independent of the schedule that governs cocaine delivery, rats

respond such that across strains and different laboratories, cocaine self-injections occur at comparable average intervals that are a function of the dose of cocaine. We would further note, however, that the average IS^RT in this PR procedure could be misleading since IS^RTs are not uniformly distributed across the session.

With this PR schedule, increasing doses of cocaine resulted in a greater number of responses emitted (highest ratio completed) and reinforcers obtained. Historically, breaking point under a PR schedule has been arbitrarily defined as the highest ratio completed (Hodos, 1961). However, the use of the highest ratio completed as a measure of the breaking point presents a problem for statistical analysis. Because the enormous range of ratio values produces unequal variance across different doses of the reinforcer, the highest ratio completed is not amenable to ANOVA. This difficulty with analyzing PR data may have been recognized previously; for example, although Roberts *et al.* (1989) present data in the form of highest ratio completed, they note that the data were log-transformed for statistical analysis. In the present case, the reinforcer number is a natural logarithmic function of the ratio value, and indeed the reinforcer data in the top panel of Fig. 2 are amenable to ANOVA because they do not violate assumptions of homogeneity of variance. If the PR schedule is to be used for parametric statistical comparisons of the effects of pretreatments, then the logarithmic transformation of the highest ratio completed appears to be the appropriate measure of breaking point. We suggest that an exponential formula should be used to construct the relationship between ratio and reinforcer number, because the reinforcer number then provides a ready logarithmic transformation of the ratio value.

Cocaine is thought to act at dopamine and serotonin transporter sites (Reith et al, 1983; Ritz et al, 1987), which suggests that compounds interacting with the receptors for these neurotransmitters might be expected to modify the rate of cocaine self-administration. A substantial body of evidence is accumulating that implicates brain dopamine in the reinforcing effects of cocaine (for a review, see Kuhar et al, 1991). Consistent with this putative role of dopamine is the observation that both dopamine D1 (Hubner and Moreton, 1991; Kleven and

Woolverton, 1990;; Lane et al, 1992; Woolverton 1986) and D2 (Hubner and Moreton, 1991; Koob et al, 1987; Peltier and Schenk 1991; Woolverton 1986) antagonists are efficacious in shifting cocaine self-administration to faster intake under low-value FR procedures. Using a second-order schedule, Bergman *et al.* (1990) have also shown that SCH 23390 blocks the reinforcing effects of cocaine. Because faster intake of cocaine is associated with lower doses of cocaine in FR schedules, these shifts to faster intake of cocaine have been interpreted to mean that D1 and D2 antagonists block the reinforcing effects of cocaine.

Little work has been published concerning effects of dopamine antagonists under PR schedules. Using a single dose of cocaine, Roberts *et al.*, (1989) reported that haloperidol reduced the highest ratio completed and increased the rate of cocaine intake. Hubner and Moreton (1991) reported that SCH 23390, and the D2 antagonist spiperone, also decreased the highest ratio completed. In the present study, SCH 23390 decreased the breaking point (number of reinforcers obtained) over the whole range of doses of cocaine tested. This would suggest that SCH 23390 blocked the reinforcing effects of cocaine. This is also in agreement with the well documented action of dopamine in the initiation and/or maintenance of cocaine self-administration (Johanson and Fischman, 1989; Weiss et al, 1993), and the results of these studies provide evidence that the PR schedule is sensitive to the blocking effects of drugs interfering with dopamine transmission on cocaine self-administration.

One alternative explanation for the observed decrease in the number of reinforcers earned under SCH 23390 could be that SCH 23390 simply blocked the discriminative properties of cocaine. According to this hypothesis, the priming dose would provide a discriminative stimulus which would be necessary for the initiation of self-administration. Thus, under SCH 23390, some rats would have failed to self-administer (no reinforcers earned), while other rats would have self-administered a number of reinforcers comparable to control (saline pretreatment) levels. As the dose of SCH 23390 increased, one would have expected to see an increasing number of rats failing to initiate self-administration. Instead, we found that for each rat, as the dose of SCH 23390 increased, the number of reinforcers obtained was

progressively decreased. This pattern of results with SCH 23390 argues in favor of a blocking effect of cocaine self-administration rather than a blocking effect of a cocaine discriminative cue.

Like Hubner and Moreton (1991), we also observed in a previous study that SCH 23390 increased the rate of responding in rats self-administering cocaine under a low-value FR schedule (Lane et al, 1992). On the basis of our previous data and those of Hubner and Moreton, one hypothesis is that SCH 23390 blocks the effects of cocaine by making a bigger dose of cocaine have the effects of a smaller dose. Thus, SCH 23390 would be expected to produce shorter IS^RTs (as found in low-value FR procedures), and to reduce the number of reinforcers obtained (as found in PR procedures). This simple hypothesis, however, is not supported by the present set of IS^RT data. In the absence of SCH 23390, the effect of lowering the cocaine dose is to produce shorter IS^RTs. Although SCH 23390 decreased the number of reinforcers, it showed no capacity to significantly shorten the IS^RT under this PR schedule.

On the other hand, SCH 23390 did not reduce the breaking point simply because of motor-impairment. Had that been the case, we might have expected to see longer IS^RTs, whereas we observed that the IS^RT showed a tendency to be shortened by SCH 23390. Hubner and Moreton (1991) proposed that in their group of rats trained on a PR schedule, SCH 23390 did not decrease the highest ratio completed through motor impairment. Those authors drew their conclusion regarding PR responding indirectly from the observation that SCH 23390 increased the rate of responding in a separate group of rats trained to self-administer cocaine on an FR5 schedule. In the present study, simultaneous monitoring of both the number of reinforcers obtained and the IS^RT allowed us to reach this conclusion directly, in the same animals, and using solely the PR procedure.

The effect of D2 selective antagonists on cocaine reward has supported the proposition that D2-like receptors are critical in regulating the reinforcing effect of cocaine (de Wit and Wise, 1977; Roberts and Vickers, 1984; Koob et al, 1987). Using a PR schedule, D2-like receptors have also been found to play a role in mediating the reinforcing effects of cocaine

(Roberts et al, 1989). The present study used eticlopride, a high-affinity antagonist at D2 and D3 receptors. Low-dose (0.032 mg/kg) pretreatment with eticlopride reduced responding for the medium and high dose of cocaine. Eticlopride (0.1 mg/kg) reduced responding to a greater extent than did SCH 23390 for all doses tested. This finding is consistent with results from previous studies (Roberts et al, 1989) indicating that D2-like DA receptors are involved in regulating the reinforcing effect of cocaine.

Ondansetron, a selective 5-HT_3 antagonist, had no significant effect either on the number of reinforcers or on the IS^RT. An absence of effect of ondansetron on cocaine self-administration using low-value FR procedures has been reported by Lane *et al.*, (1992) as well as Peltier and Schenk (1991). Using a PR procedure, Lacosta and Roberts (1991) have provided preliminary evidence that another selective 5-HT_3 antagonist, MDL 72222, was without effect on the highest ratio completed in their PR procedure. We provide evidence that ondansetron, across a 1,000-fold range of doses tested against the training dose of cocaine, as well as at the dose of 0.1 mg/kg tested against three doses of cocaine (0.028, 0.083 or 0.25 mg/inj), has no significant effect on either the number of reinforcers or the IS^RT. The range of doses investigated more than spanned the doses that have been reported to be active via the i.p. route in other behavioral tests (Costall et al, 1990; Peltier and Schenk, 1991). Thus, if serotonergic mechanisms are indeed important in cocaine self-administration (Carroll et al, 1990; Loh and Roberts, 1990) it is likely that these mechanisms do not involve the 5-HT_3 site.

ACKNOWLEDGMENTS

We appreciate the technical assistance of Clay Pickering, Mickey Hooper, Doug Lytle and Scott Boone.

REFERENCES

Bedford JA, Bailey LP, and Wilson MC (1978) Cocaine reinforced progressive-ratio performance in the rhesus monkey. Pharmacol. Biochem. Behav. 9: 631-638.

Bergman J, Kamien JB, and Spealman RD (1990) Antagonism of cocaine self-administration by selective dopamine D1 and D2 antagonists. Behav. Pharmacol. 1: 355-363.

Carroll ME, France CP, and Meisch RA (1979) Food deprivation increases oral and intravenous drug intake in rats. Science. 205: 319-321.

Carroll ME, Lac ST, Ascencio M, and Kragh R (1990) Fluoxetine reduces intravenous cocaine self-administration in rats. Pharmacol. Biochem. Behav. 35: 237-244.

Costall B, Jones BJ, Kelly ME, Naylor RJ, Onaivi ES, and Tyers MB (1990) Ondansetron inhibits a behavioral consequence of withdrawing from drugs of abuse. Pharmacol. Biochem. Behav. 36: 339-344.

Dantzer R (1976) Effect of diazepam on performance of pigs in a progressive-ratio schedule. Physiol. Behav. 17:161-163.

Deneau G, Yanagita T, and Seevers MH (1969) Self-administration of psychoactive substances by the monkey. A measure of psychological dependence. Psychopharmacologia. 16: 30-48.

Dworkin SI, Mirkis S, Smith JE (1990) Reinforcer interactions under concurrent schedules of food, water, and intravenous cocaine. Behav. Pharmacol. 1: 327-338.

Emmett-Oglesby MW, Spencer Jr DG, and Arnoult DE (1982) A TRS-80-based system for the control of behavioral experiments. Pharmacol. Biochem. Behav. 17: 583-587.

Griffiths RR, Findley JD, Brady JV, DplanGutcher K, and Robinson WW (1975) Comparison of progressive-ratio performance maintained by cocaine, methylphenidate and secobarbital. Psychopharmacologia. 43: 81-83.

Griffiths RR, Brady JV, and Snell JD (1978) Progressive-ratio performance maintained by drug infusions: comparison of cocaine, diethylpropion, chlorphentermine, and fenfluramine. Psychopharmacology. 56: 5-13.

Griffiths RR, Bradford LD, and Brady JV (1979) Progressive-ratio and fixed-ratio schedules of cocaine-maintained responding in baboons. Psychopharmacology. 65: 125-136.

Griffiths RR, Bigelow GE, and Henningfield JE (1980) Similarities in animal and human drug-taking behavior. In: Mello, N.K., ed. Advances in Substance Abuse: Behavioral and Biological Research, vol. 1. Greenwich: JAI Press; 1-90.

Hodos W (1961) Progressive-ratio as a measure of reward strength. Science 134: 943-944.

Hodos W and Kalman G (1963) Effects of increment size and reinforcer volume on progressive-ratio performance. J. Exp. Anal. Behav. 6: 387-392.

Hoffmeister F (1979) Progressive-ratio performance in the rhesus monkey maintained by opiate infusions. Psychopharmacology. 62: 181-186.

Hubner CB and Moreton JE (1991) Effects of selective D1 and D2 dopamine antagonists on cocaine self-administration in the rat. Psychopharmacology. 105: 151-156.

Iwamoto ME and Martin W (1988) A critique of drug self-administration as a method for predicting abuse potential of drugs. In: Harris, L.S., ed. Problems of Drug Dependence. Proceedings of the Committee on Problems of Drug Dependence. Washington: U S. Government Printing Office; pp. 457-465.

Johanson CE and Fischman MW (1989) The pharmacology of cocaine related to its abuse. Pharmacol. Rev. 41: 3-52.

Katz JL (1990) Models of relative reinforcing efficacy of drugs and their predictive utility. Behav. Pharmacol. 1: 283-301.

Keesey RE and Goldstein MD (1968) Use of progressive fixed-ratio procedures in the assessment of intracranial reinforcement . J. Exp. Anal. Behav. 11: 293-301.

Kleven MS and Woolverton WL (1990) Effects of continuous infusion of SCH 23390 on cocaine or food-maintained behavior in rhesus monkeys. Behav. Pharmacol. 1: 365-373.

Koob F, Le HT, and Creese I (1987) The D1 dopamine receptor antagonist SCH 23390 increases cocaine self-administration in the rat. Neurosci. Let. 79:315-320.

Kuhar MJ, Ritz MC, and Boja JW (1991) The dopamine hypothesis of the reinforcing properties of cocaine. Trends Neurosci. 14: 299-302.

Lacosta S and Roberts DCS (1991) Specific 5-HT2 and 5-HT3 receptor antagonists fail to alter breaking points on a progressive-ratio schedule reinforced by intravenous cocaine in the rat. Abst. Soc. Neurosci. 17: 888.

Lane JD, Pickering CL, Hooper ML, Fagan K, Tyers MB, and Emmett-Oglesby MW (1992) Failure of ondansetron to block the discriminative or reinforcing stimulus effects of cocaine in the rat. Drug Alc. Dep. 30:151-162.

Loh EA and Roberts DCS (1990) Breakpoints on a progressive-ratio schedule reinforced by intravenous cocaine increase following depletion of forebrain serotonin. Psychopharmacology. 101: 262-266.

Peltier R and Schenk S (1991) GR38032F, a serotonin 5HT3 antagonist, fails to alter cocaine self-administration in rats. Pharmacol. Biochem. Behav. 39: 133-136.

Pickens R and Thompson T (1968) Cocaine-reinforced behavior in rats: effects of reinforcement magnitude and fixed-ratio size. J. Pharmacol. Exp. Ther. 161: 122-129

Reith M, Sershen H, Allen DL, and Lajtha A (1963) A portion of [3H]cocaine binding in brain is associated with serotonergic neurons. Mol. Pharmacol. 23: 600-610.

Risner ME and Jones BE (1975) Self-administration of CNS stimulants by dog. Psychopharmacology. 43: 207-213.

Risner ME and Silcox DL (1981) Psychostimulant self-administration by beagle dogs in a progressive-ratio paradigm. Psychopharmacology. 75: 25-30.

Risner ME and Goldberg SR (1983) A comparison of nicotine and cocaine self-administration in the dog: fixed-ratio and progressive-ratio schedules of intravenous drug infusion. J. Pharmacol. Exp. Ther. 224: 319-326.

Risner ME and Cone EJ (1986) Intravenous self-administration of fencamfamine and cocaine by beagle dogs under fixed-ratio and progressive-ratio schedules of reinforcement. Drug Alc. Dep. 17: 93-101.

Ritz MC, Lamb RJ, Goldberg SR, and Kuhar MJ (1987) Cocaine receptors on dopamine transporters are related to self-administration of cocaine. Science. 237: 1219-1223.

Roberts DCS (1989) Breaking points on a progressive-ratio schedule reinforced by intravenous apomorphine increase daily following 6-hydroxydopamine lesions of the nucleus accumbens. Pharmacol. Biochem. Behav. 32: 43-47.

Roberts DCS, Loh EA, and Vickers G (1989) Self-administration of cocaine on a progressive-ratio schedule in rats: dose-response relationship and effect of haloperidol pretreatment. Psychopharmacology. 97: 535-538.

Roberts DCS, Bennett SAL, and Vickers GJ (1989) The estrous cycle affects cocaine self-administration on a progressive-ratio schedule in rats. Psychopharmacology. 98: 408-411.

Schenk S and Peltier R (1991) The progressive-ratio paradigm as a measurement of the reinforcement strength of cocaine: an analysis of longterm stability of breaking points and dose/response relations. Abst. Soc. Neurosci. 17: 1427.

Schuster CR and Thompson T (1969) Self-administration of and behavioral dependence on drugs. Ann. Rev. Pharmacol. 9: 483-502.

Spencer Jr. DG and Emmett-Oglesby MW (1985) Parallel processing strategies in the application of microcomputers to the behavioral laboratory. Behav. Res. Meth. Inst. Comput. 17: 294-300.

Weiss F, Hurd YL, Ungersted U, Markou A, Plotsky PM, and Koob GF (1992) In: Neurochemical correlates of cocaine and ethanol self-administration. In: Kalivas, P.W. and Samson, H. H., eds. Annals of the New York Academy of Sciences: The neurobiology of Drug and Alcohol Addiction, In Press.

Wilkinson L (1990) SYSTAT: The system for statistics. Evanston: SYSTAT, Inc.

Wilson MC, Hitomi M, and Schuster CR (1971) Psychomotor stimulant self-administration as a function of dosage per injection in the rhesus monkey. Psychopharmacologia. 22: 271-281.

Winer BJ (1962) Statistical Principles in experimental design. New York: McGraw-Hill Book Company.

Winger G and Woods JH (1985) Comparison of fixed-ratio and progressive-ratio schedules of maintenance of stimulant drug-reinforced responding. Drug Alc. Dep. 15: 123-130.

de Wit H, Wise RA (1977) Blockade of cocaine reinforcement in rats with the dopamine receptor blocker pimozide, but not the noradrenergic blockers phentolamine and phenoxybenzamine. Can J Psychol 31:195-203

Woolverton WL (1986) Effects of a D1 and a D2 dopamine antagonist on the self-administration of cocaine and piribedil by rhesus monkeys. Pharmacol. Biochem. Behav. 24:531-535.

Yanagita T (1973) An experimental framework for evaluation of dependence liability of various types of drugs in monkeys. Bull. Narc. 25: 57-64.

Young AM and Herling S (1986) Drugs are reinforcers: studies in laboratory animals. In: Goldberg, S.R. and Stolerman, I.P., eds. Behavioral analysis of drug dependence. New York: Academic Press; 9-68.

Chapter 2

In this manuscript, the hypothesis that a common reward pathway mediates the reinforcing effect of both cocaine and opioids was tested. The experiments in this chapter were designed to examine the interactions between *d*-amphetamine or opiates with cocaine. This hypothesis was based on the anatomical evidence that the dopaminergic projection from the ventral tegmental area (VTA) to nucleus accumben (NAc) is an anatomical substrate of the rewarding effect of opiates. Therefore, an increase in the breaking point or the inter-reinforcer time (IS^RT) of cocaine self-administration is expected when cocaine is co-administered with either *d*-amphetamine or opiates. Since both cocaine and amphetamine are dopamine indirect agonists, a synergistic effect on the breaking point of cocaine self-administration is expected when amphetamine and cocaine are co-administered.

This manuscript has been submitted to Pharmacol Biochem Behav and is currently undergoing review.

A study using a progressive-ratio schedule to assess potentiation of cocaine self-administration[1]

D.-H. Li[2], R. Y. Depoortere, and M. W. Emmett-Oglesby

Department of Pharmacology

University of North Texas HSC at Fort Worth

3500 Camp Bowie Blvd

Fort Worth TX 76107-2699 USA

Running Title: Potentiation of Cocaine

1 Supported by grants RO1-4137 and Texas Advanced Technology Award 3711

2 This project is taken from a dissertation submitted to the University of North Texas Health Science Center at Fort Worth in partial fulfillment of the requirements for the degree Doctor of Philosophy

Corresponding Author:

M.W. Emmett-Oglesby

Department of Pharmacology

University of North Texas HSC at Fort Worth

3500 Camp Bowie Blvd.

Fort Worth TX 76107-2699 USA

Phone: (817) 735-2056

FAX: (817) 735-2091

Abbreviations:

Amphetamine (AMPH)

Morphine (MOR)

Buprenorphine (BUP)

Injection (inj)

Inter-reinforcer time (IS^RT)

ABSTRACT

The hypothesis that the progressive-ratio (PR) schedule could be employed to determine the potentiating effects of other abused agents on cocaine reinforcing effect was tested in this experiment. The effects of pretreatment with buprenorphine (BUP; the partial *mu*-agonist), morphine (MOR; the full *mu*-agonist), or *d*-amphetamine (AMPH; the dopamine releaser) were investigated in Fisher F-344 rats trained to self-administer cocaine (0.25mg/injection of 100μl) under the progressive-ratio schedule of reinforcement. When pre-treated with vehicle (s.c.), increasing doses of cocaine (0.028, 0.083 and 0.25 mg/injection) resulted in higher breaking points (responding ceased at 12, 17 and 22 reinforcers, respectively). Pretreatment with AMPH (0.32, 1.0 or 1.8 mg/kg) significantly increased the number of reinforcers obtained for all three doses of cocaine and for cocaine-vehicle. Pretreatment with morphine (1.0, 3.2 or 5.6 mg/kg, s.c., given 30 min before the session) did not increase the breaking point in any of these doses of cocaine. Indeed, at the highest dose of morphine tested, several subjects failed to initiate response for cocaine, and of the remaining subjects, the breaking points for all doses of cocaine were unchanged. The effect of the pretreatment with buprenorphine was tested against the intermediate dose of cocaine (0.083 mg/injection), and it also failed to enhance the breaking point. The absence of the effect of BUP pretreatment does not appear to be due to a lack of sensitivity of this PR schedule, since AMPH pretreatment resulted in an increase in the number of reinforcers obtained. To date, we are unaware of evidence which successfully demonstrates that breaking points maintained by cocaine can be enhanced by pretreatment with any drug. Supported by NIDA grant RO1-4137 and Texas Advanced Technology Award 3711.

INTRODUCTION

Among drug self-administration methods, the progressive-ratio (PR) schedule is a method that has been claimed to provide an index of reinforcing effect of a psychoactive drug (Hodos, 1961). The PR schedule has been demonstrated to supply a sensitive measure in evaluating the effects of lesions and pharmacological pretreatments on drug reinforcement (Roberts and Richardson 1992). Several studies have used the PR schedule to determine the effects of dopamine and serotonin antagonists (Roberts et al., 1989b; Lacosta & Roberts, 1993; Depoortere et al., 1993), and serotonin up-take inhibitors (McGregor et al., 1993) on the reinforcing effect of cocaine. This work showed that the PR schedule is a sensitive tool to test the inhibitory effect of various substances on cocaine self-administration. In another study, intracerebral injections of 5,7-dihydroxy-tryptamine (5,7-DHT) were applied to demonstrate that the depletion of forebrain serotonin could significantly increase breaking points (Loh & Roberts, 1990). Although some doses of clozapine also increased the breaking point of cocaine self-administration in rats (Loh et al., 1992), there was a lack of systematic evaluation of this action (see details in Discussion).

Among cocaine addicts, polydrug abuse is a common phenomenon (Sample 1977), and drugs representing a wide range of pharmacological classes are frequently combined with cocaine. The primary reasons expressed by users for polydrug use ranged from: combating unpleasant side effects, to alleviating feelings of anxiety when cocaine's initial euphoria dissipates, to reducing the intensity of the cocaine high, and to enhancing the cocaine high (Siegel 1984). Amphetamines are weak inhibitors of monoamine oxidase, an action that would increase catecholaminergic activity. They also appear to exert the stimulus effects in the CNS by releasing biogenic amines from their storage sites in the nerve terminals. On the street, in an attempt to reduce ("cut") the amount of "active" agent being sold, amphetamines are frequently used to substitute for a reduced amount of cocaine. Animal studies indicated that there are some greater than additive effects between cocaine and *d*-amphetamine in some animal models (Scheckel and Boff, 1964; Foltin et al. 1983).

On the street, the combination of narcotics with cocaine, "speed balling," is also quite common and was reported to be used to produce a more intensely pleasurable "rush" (Wesson & Smith, 1977). Cocaine was demonstrated to enhance morphine-induced analgesia and to attenuate morphine-induced side-effects in rats (Kauppila et al. 1992). Among the opioid family, the effects of buprenorphine on cocaine are also of interest. Mello et al. (1989) first demonstrated that buprenorphine could be used to suppress cocaine self-administration by rhesus monkeys. Later, a series of studies concerned with both acute and chronic suppression effects of buprenorphine treatment on cocaine self-administration were published (Mello et al., 1990; Mello et al., 1992). These experiments were performed based on the rate of drug taking in self-administration. However, the rate of drug taking in FR schedule is at best an ambiguous measure of a drug's reinforcing effect and may not be appropriate for predicting relative reinforcing effect of different drugs or different doses of the same drug (Iwamoto and Martin, 1988; Katz, 1990). Another report used both a conditioned place preference procedure and *in vivo* microdialysis to demonstrate that either buprenorphine or cocaine increased both conditioned place preference and extracellular dopamine in the nucleus accumbens and together acted synergistically on both measures (Brown et al., 1991). It has also been demonstrated that both morphine and buprenorphine could potentiate the discriminative stimulus effects of cocaine (Spealman & Bergman, 1992).

On the basis of the above work, our experiments used the PR schedule as a behavioral tool to test the effect of *d*-amphetamine, morphine or buprenorphine on the breaking points of cocaine self-administration in rats. Our main purpose was to determine if PR could be used to determine a potentiation effect of these drugs on cocaine self-administration as determined by an increase in the breaking point.

METHODS

Subjects and Apparatus. Adult male Fisher F344 rats (Harlan, Indianapolis IN) were housed individually and maintained at approximately 270g body weight (range: 260-290g) by daily feeding of measured quantities of food. Lever-press shaping was done with food as a reinforcer in single lever operant chambers (Model E-10-10TC, Coulbourn Instruments, Lehigh Valley, PA) using 45-mg food pellets (BioServ, Frenchtwon, NJ). Initially, rats were trained to press a lever under a FR1 schedule using food pellets as a reinforcer. After the responding was stable, each subject was implanted with a permanently indwelling jugular catheter (for details, see Depoortere *et al.*, 1993). After surgery the catheter was flushed with a combination of heparin, streptokinase, and Timentin (ticarcillin plus clavulinic acid) every 12 h for 5 days. On the 6th day, Timentin was removed from the regimen.

Preliminary Training Phase. Self-administration experiments were performed in chambers that contained a single lever, 5.0 cm above the floor and centered on the back wall, that could be pressed (force of 0.15 N) to close a microswitch. The chambers were also equipped with two stimulus lights (3W), one located on the ceiling (house light) and the other 9 cm above the lever (lever light). (for details see Depoortere *et al.*, 1993). Five days following surgery, animals began training under a fixed-ratio one schedule (FR 1) of reinforcement, with cocaine, 0.25 mg/0.1ml/injection serving as the reinforcer. Sessions lasted either until 25 injections were self-administered or until approximately 12-hr elapsed. Each session started with a priming injection of cocaine (0.5 mg) delivered by the experimenter. Priming and subsequent injections were accompanied by a flashing of two stimulus lights in the chamber; each injection was followed by a 30-s time-out in the dark, during which responding had no programmed consequences.

If a rat self-administered cocaine at a constant rate ($\pm$ 20%) over the course of 3-5 days, then the subjects were selected for training under the progressive ratio (PR) schedule. Daily PR sessions began with a 0.25 mg priming infusion. Each subsequent cocaine injection, 0.25 mg/0.1ml, occurred upon completion of each of the ratios in the following sequence: 3, 6,

10, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 603, 737, 901, 1102, and 1347. There was a 1-hr time limit to obtain each reinforcer, and failure to obtain the reinforcer terminated the session. The last reinforcer the subject obtained during the session was termed as breaking point, which indicated the total number of injections of cocaine the animal obtained during that session. Typically, when rats failed to complete a ratio, they always ceased responding within 30 min after the previous reinforcer was obtained, and they subsequently emitted few if any additional responses. Stability under the PR schedule for this 0.25mg/inj dose was defined as at least 7 sessions in which the breaking point did not differ by more than 3 injections.

Drug interaction data. After this stability criterion was obtained, rats were tested based on the following design:

1) pretreatment with *d*-amphetamine (s.c., 0.32, 1.0 or 1.8 mg/kg) or saline against self-administration of cocaine on 3 separate doses (0.028, 0.083 and 0.25 mg/inj) or its vehicle (saline; 4x4 design). The doses were chosen based on the results of the disruptive effect on food intake by psychomotorstimulants (Foltin et al., 1983).

2) pretreatment with buprenorphine (s.c., 0.033, 0.1 or 0.33 mg/kg) or water as its vehicle against self-administration of cocaine at 0.083 mg/inj or its vehicle (4x2 design). The reason this dose was chosen was that dose-response curve showed a very sharp decline in the breaking point at this dose which indicated that this dose is very sensitive to the change of reinforcing effect of cocaine (see figures and detail in Depoortere et al., 1993).

3) pretreatment with Morphine (s.c., 1.0, 3.2 or 5.6 mg/kg) or its vehicle saline against self-administration of cocaine on 3 separate doses or saline (4x4 design). The choice of doses of buprenorphine and morphine was based on Spealman's discriminative stimulus study and Brown's buprenorphine study. The doses in this study covered the most effective dose range of BUP and MOR on the effect of cocaine from previous studies (Brown et al., 1991; Spealman & Bergman, 1992).

Each dose of cocaine or saline combined with each pre-treatment drug or its vehicle was tested on a different day. Each pretreatment was performed 30 minutes before the PR session started. After the test of the combination of each interaction dose, the rats were retrained using the baseline dose of cocaine (0.25 mg/0.1ml) without pretreatment until the pre-treatment baseline was obtained for at least two days. Only the animals who returned to their normal baselines (baseline $\pm$ 3 reinf.) were subjected to further experiments. Since some subjects occasionally failed to start responding at the lowest dose of cocaine tested (0.028 mg/injection), a slightly larger priming injection (0.05 mg) was introduced. The treatment was performed during the session of baseline testing and interaction testing.

Data Analysis: The number of reinforcers obtained was used as the dependent measure, which is termed the breaking point. This measure of the breaking point was used rather than the final ratio completed or the total number of responses emitted because those variables are not amenable to parametric analysis (for a discussion of this problem in analysis of response and reinforcer data from PR procedures, see Depoortere *et al.*, 1993; and Roberts and Richardson, 1992. For ready comparison, the right-hand ordinate of figures shows cumulative number of responses associated with the number of reinforcers on the left-hand ordinate). The influence of pretreatment drugs on cocaine dose-response data were subjected to two-way ANOVA for repeated measures, with the vehicle pre-treatment (control) and drug pre-treatment as the two within factors.

Effect of drug pretreatments on the mean Inter-Reinforcer Time (IS^RT) between each reinforcer was used as another dependent measure, and was used to determine the response rate related to response pattern. The first step to calculate this variable was to exclude each subjects' first and last IS^RT. These two IS^RTs were excluded because they were not representative of the general pattern of IS^RTs: the first IS^RT was variable because some rats took much longer than others to start self-administering following the infusion of the priming dose; the last IS^RT also showed greater variability because at this final ratio value, some rats occasionally displayed a pattern of lever pressing in which responding was interrupted by

periods of non-responding. The second step to analyze the IS^RT s was to perform a logit transform the data according to the formula 2 + log (IS^RT). The purpose of this treatment is to minimize the variation introduced by the dramatically increase in number of responses as the number of reinforcers increased. This variation lead the failure of parametric analysis of the IS^RT under the PR schedule (Beitinger 1993).

Since the breaking points of some animals at the low dose of cocaine (0.028 mg/inj) were very low (< 4), the sample size of the experiment will be significantly reduced. To avoid the data analysis being biased by the low dose cocaine self-administration, the treatment of IS^RT was concerned only on 0.0833 and 0.25 mg/inj cocaine self-administration dosage. Based on the transformed data, IS^RT of different pretreatments on either 0.083 or 0.25 mg/inj cocaine were further subjected to fully factorial ANOVA (MANOVA) for repeated measures with the value of IS^RT as dependent variables and pretreatments as factors.

All MANOVA were performed with SYSTAT software (Wilkinson, 1990), and all ANOVAs were performed with Statistic Analysis Systems (SAS, 1987) software.

Drugs. Cocaine HCl was obtained from the National Institute on Drug Abuse (Research Triangle Park, NC). Amphetamine sulfate was obtained from Sigma, St. Louis, MO. Buprenorphine HCl was obtained from Reckitt & Colman Pharmaceuticals. Morphine Sulfate injection (15 mg/ml, 1ml/vial) was obtained from Elkin Sinn Inc.

All doses refer to the weight of the salt for the convenience.

RESULTS

The number of subjects involved in the experiments varied from group to group. Individual animals demonstrated stable daily breaking points during the period of each interaction testing under the PR schedule. There was also a quite low level degree of variability in baseline breaking points between animals (ranged among *d*-AMPH 17-24; BUP

15-21; MOR 21-26). Therefore, the animals supplied a set of very stable and repeatable data under the different combination treatments.

Effect of d-amphetamine (d-AMPH) pre-treatment on the number of reinforcers obtained and the inter-reinforcer time (IS^RT) of cocaine.

For all three doses of cocaine, pretreatment with *d*-AMPH (0.32, 1.0, or 1.8 mg/kg) significantly ($F_{3,128}$=52.28, p<0.0001) shifted the cocaine dose-response curve to the left (Fig 1). Under baseline conditions, increasing the dose of cocaine also resulted in an orderly increase in the time between injections ($F_{3,24}$=62.68, p<0.0001). The significant interaction between the dose of cocaine infused and all of the three pretreatments ($F_{9,128}$=6.58, p<0.0001) suggests that *d*-AMPH and saline pretreatment affected the cocaine dose-response curve in a different way. This differential effect of *d*-AMPH can be seen in the graph, where the *d*-AMPH pretreatment curve is not parallel to the saline pretreatment curve. However, before the cocaine was available, the animals that were pretreated with *d*-AMPH showed an effect similar to cocaine intoxication. The fact that d-AMPH also increased the breaking point obtained from saline self-administration ($F_{3,24}$=54.77, p=0.0001) indicated that it cannot be concluded that an up-shift of the cocaine dose-response curve is due simply to an effect of *d*-AMPH directly on the reinforcing effect of cocaine.

Although the pretreatment with d-AMPH failed to shifted the IS^RT to longer duration on 0.25 mg/inj ($F_{3,423}$=1.85, p>0.1) cocaine self-administration (Table 1), the pre-treatment showed a significant shift to the left of IS^RT on cocaine 0.083 mg/inj ($F_{3,315}$=4.659, p<0.01) (Fig 2). This significance was contributed by the effect of both 1.0 and 1.8 mg/kg of amphetamine.

Effect of morphine (MOR) pre-treatment on the number of reinforcers obtained and the inter-reinforcer time (IS^RT) of cocaine.

In MOR pre-treatment experiments, when the dose of MOR was lower than 5.6 mg/kg, MOR failed to significantly influence the breaking points of cocaine self-administration

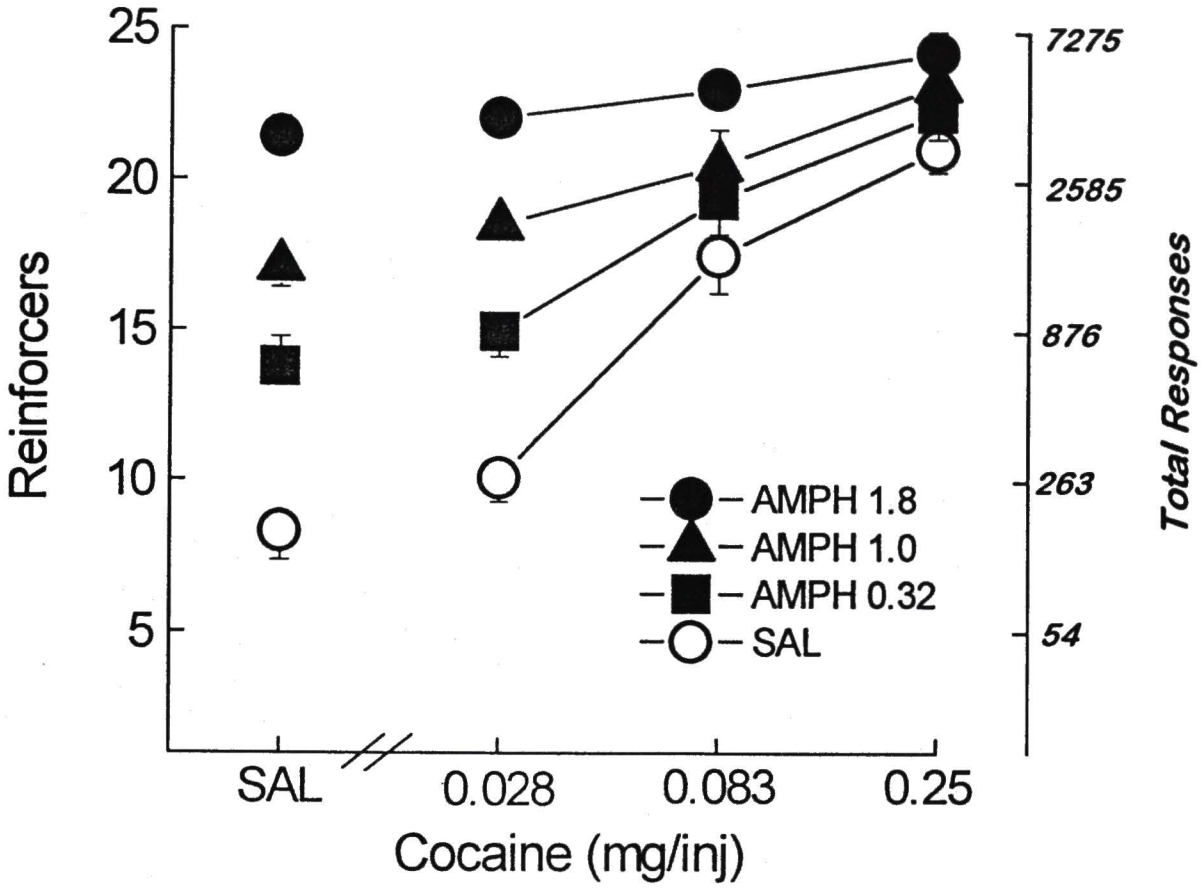


FIG. 1. Effect of *d*-amphetamine pretreatment on the number of reinforcers obtained. Abscissa: dose of cocaine available for self-administration. Ordinate: the number of reinforcers obtained. Each symbol represents the average number of reinforcers obtained in a session, and vertical bars represent SEM. Symbols on the left indicate self-administration of saline. n = 9.

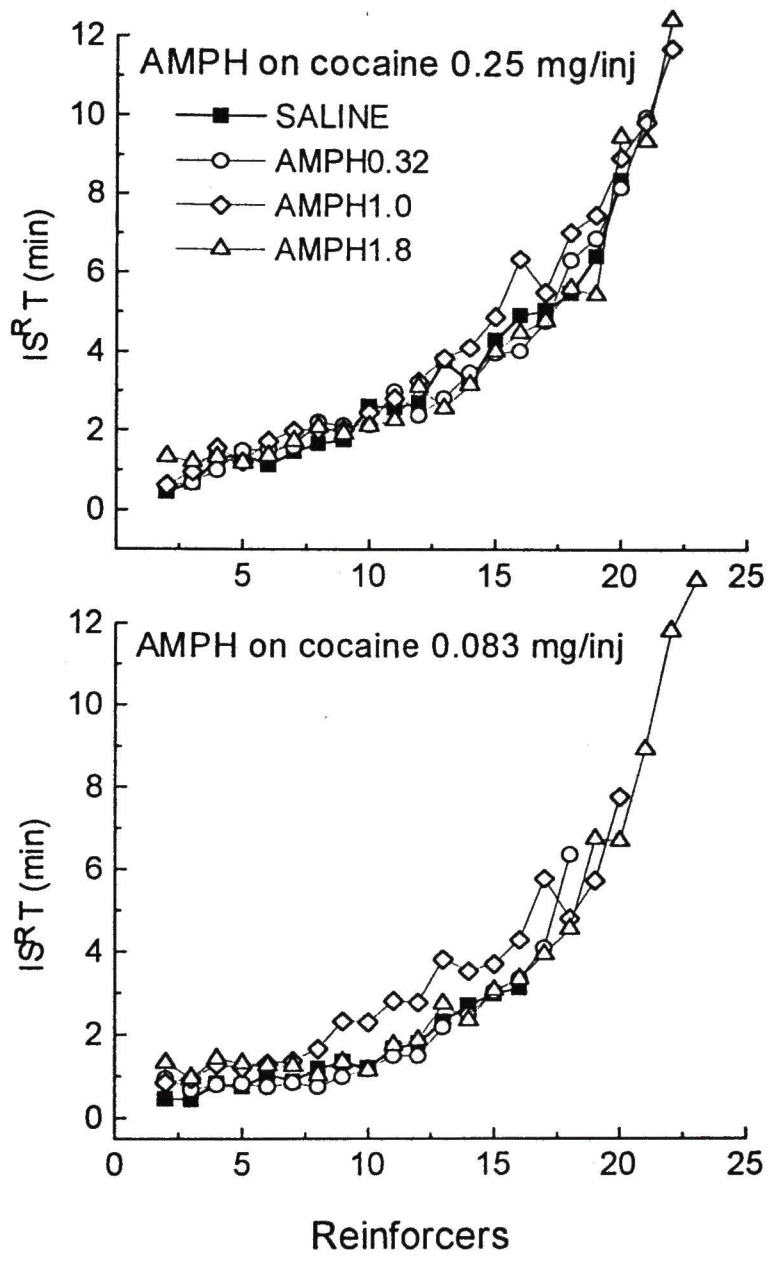
Table 1. The average of individual mean IS^RT of cocaine self-administration after the pretreatment of AMP, BUP and MOR or their vehicle.

Cocaine	Amphetamine (AMPH, mg/kg)			
mg/inj	Saline	0.32	1.0	1.8
0.25	2.25 ± 0.07	2.25 ± 0.07	2.28 ± 0.08	2.22 ± 0.09
0.083	1.97 ± 0.06	1.96 ± 0.06	2.09 ± 0.08	2.03 ± 0.07

Cocaine	Buprenorphine (BUP, mg/kg)			
mg/inj	Water	0.033	0.1	0.33
0.083	1.87 ± 0.05	1.90 ± 0.033	2.12 ± 0.07	1.91 ± 0.1

Cocaine		Morphine (MOR, mg/kg)		
mg/inj	Saline	1.0	3.2	5.6
0.25	1.96 ± 0.2	2.02 ± 0.12	2.20 ± 0.11	2.40 ± 0.22
0.083	1.81± 0.08	1.94 ± 0.07	2.02 ± 0.09	2.09 ± 0.15

FIG. 2. Effect of *d*-amphetamine pretreatment on the inter-reinforcer time of cocaine 0.25 mg//inj (top panel) and cocaine 0.083 mg/inj (bottom panel). Abscissa: the number of reinforcers obtained. Ordinate: inter-reinforcer time (IS^RT). Each symbol represents the mean IS^RT. For each dose of cocaine, the number of IS^RT plotted corresponds to the average number of reinforcers obtained rounded to the nearest integer. To clarity the view of the graph, SEMs have been omitted. n = 9.


AMPH on cocaine 0.25 mg/inj
SALINE
AMPH0.32
AMPH1.0
AMPH1.8
ISRT (min)
AMPH on cocaine 0.083 mg/inj
ISRT (min)
Reinforcers

($F_{2,60}$=0.49, p>0.5). At a dose of 5.6 mg/kg, MOR showed a significant effect of suppressing breaking points. Some of the animals failed to start cocaine self-administration after the treatment. Most of the animals reduced their breaking points significantly (Fig 3). Pretreatment of MOR 5.6 mg/kg, s.c., also successively ceased drug lever pressing behavior of saline self-administration. However, there were still two out of six subjects that failed to show a significant difference on cocaine self-administration after 5.6 mg/kg of MOR or saline pretreatment. One of the animals did not show a significant change on any dose of MOR pretreatment. Although the difference on breaking points of group subjects was significant ($F_{3,80}$=8.43, p<0.01), the effects were varied among individual subjects (Fig 4, notice the larger S.E.M.).

Pretreatment with MOR significantly shifted the IS^RTs to longer duration on both 0.083 mg/inj ($F_{3,108}$=13.92, p<0.0001) and 0.25 mg/inj cocaine self-administration ($F_{3,135}$=27.79, p<0.0001; Fig 5, top and middle panel). Even the rat who failed to show differences on the breaking point by the pretreatment of MOR appeared to have a steady (although slight) increase of its mean IS^RT as the dose of MOR increased.

Effect of buprenorphine (BUP) pre-treatment on the number of reinforcers obtained and the inter-reinforcer time (IS^RT) of cocaine.

Although pretreatment with BUP failed to modify the breaking point of cocaine 0.083mg/inj self-administration ($F_{3,23}$=0.19, p>0.5; Fig 4), pretreatment with BUP, highly significantly increased IS^RT ($F_{3,159}$=14.07, p<0.0001; Fig 5, bottom panel) of cocaine self-administration.

DISCUSSION

These data clearly show that the combination of cocaine and d-amphetamine produce an effect which is greater than cocaine given alone under a progressive-ratio schedule. Doses of

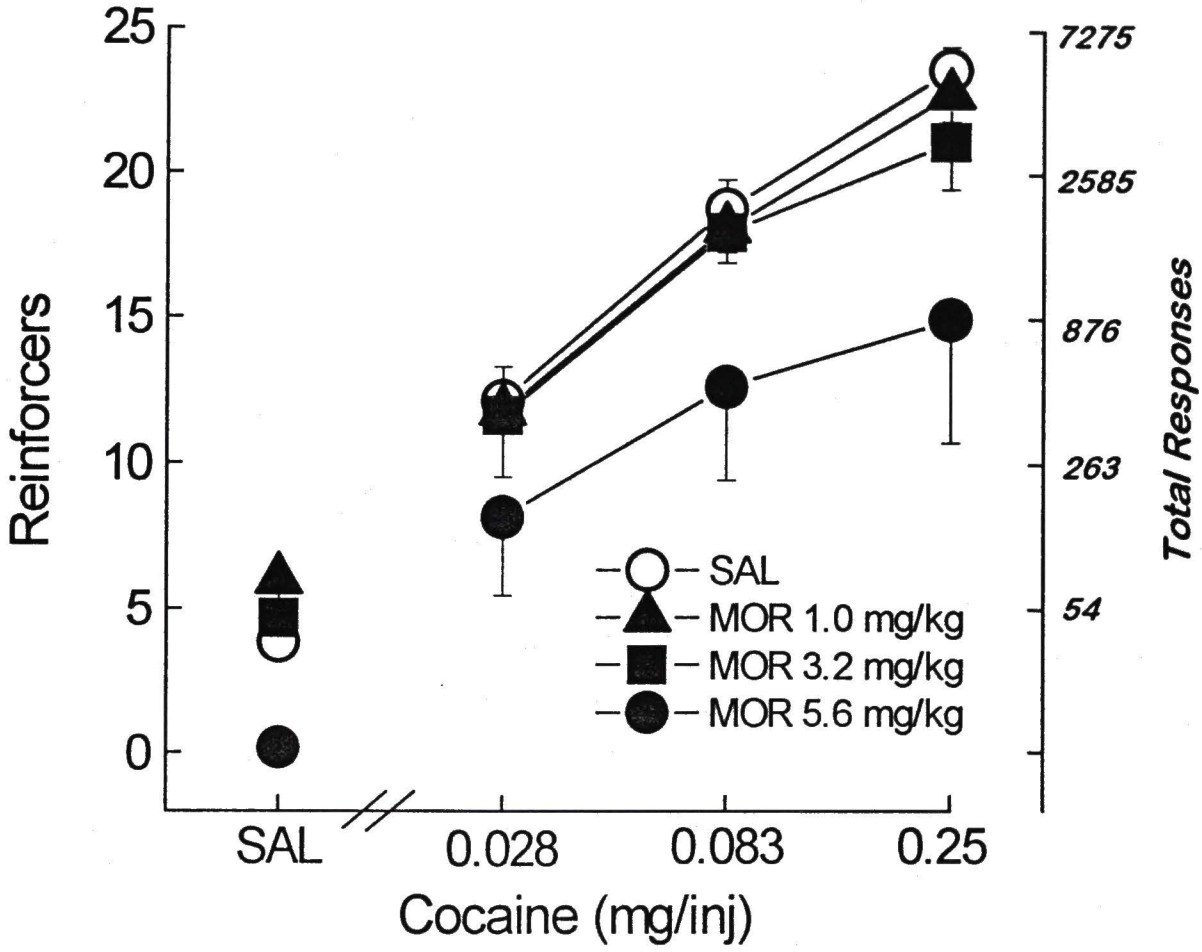


FIG. 3. Effect of morphine pretreatment on the number of reinforcers obtained. Abscissa and ordinate are same as in FIG. 1. Each symbol represents the average number of reinforcers obtained in a session, and vertical bars represent SEM. Symbols on the left indicate self-administration of saline. n = 6.

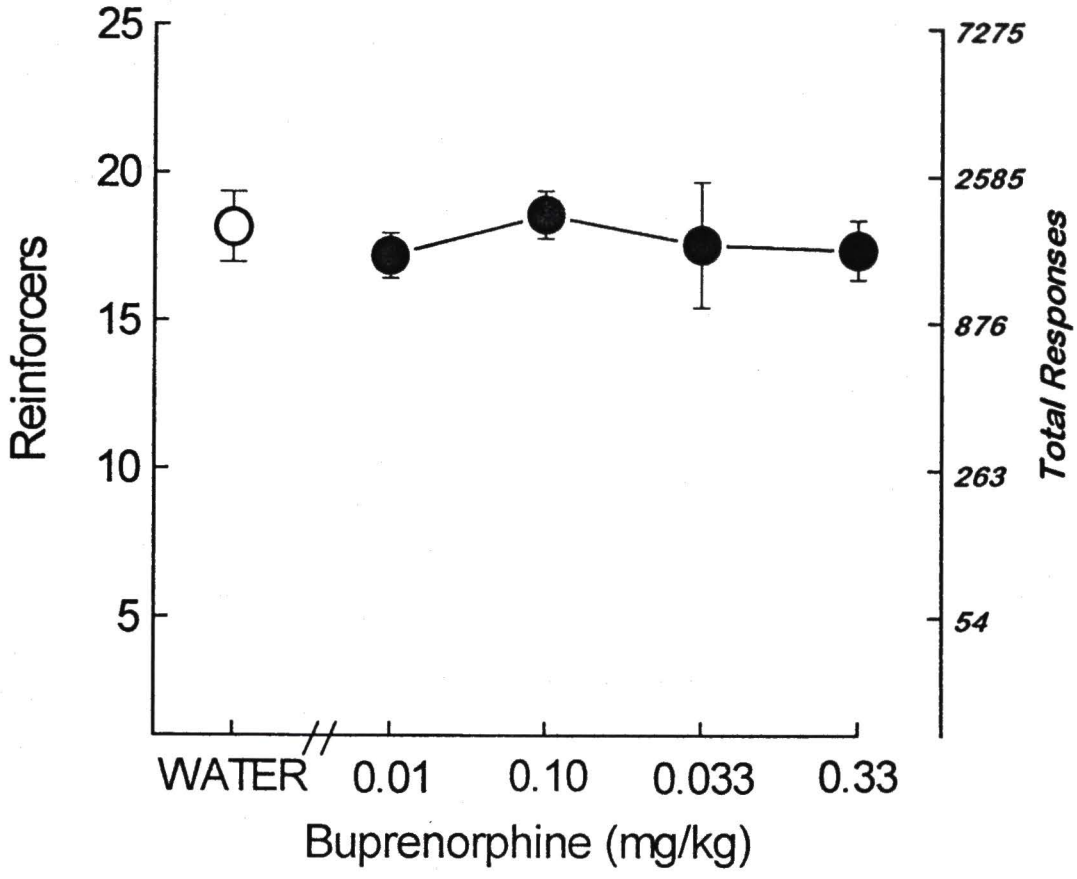
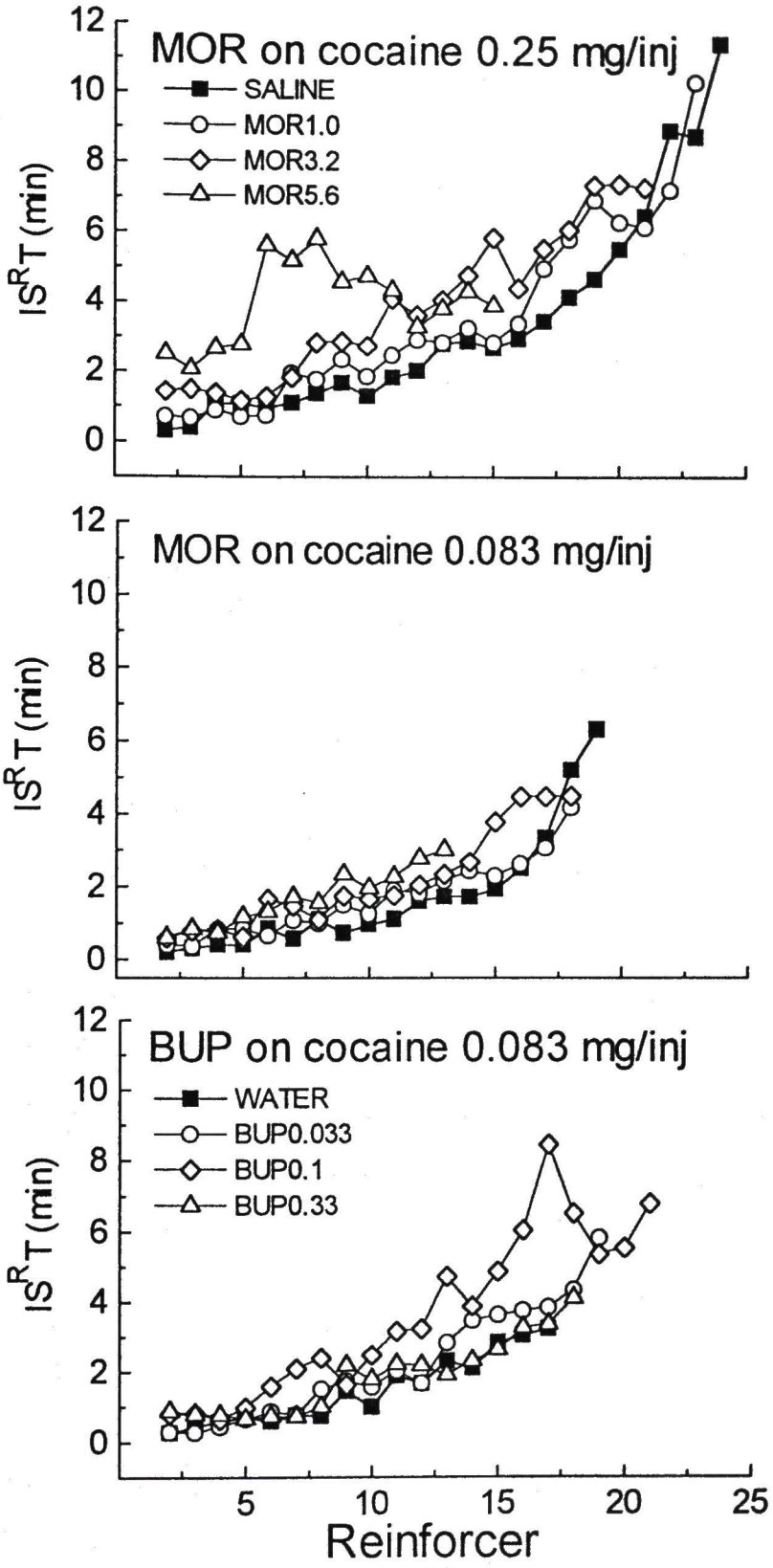


FIG. 4. Effect of buprenorphine pretreatment on the number of reinforcers obtained of cocaine 0.083 mg/inj self-administration. Abscissa and ordinate are same as of FIG. 1. Each symbol represents the average number of reinforcers obtained in a session, and vertical bars represent SEM. Open circle symbol indicates the results obtained with the pretreatment of water as a control; filled circles indicate different doses of BUP pretreatment against 0.083 mg/inj cocaine. n = 6.

FIG. 5. Top panel: Effects of morphine pretreatment on the inter-reinforcer time of cocaine 0.25 mg/inj self-administration. Middle panel: Effects of MOR pretreatment on the inter-reinforcer time of cocaine (0.083 mg/inj) self-administration. Bottom panel: Effect of buprenorphine pretreatment on the inter-reinforcer time of cocaine 0.083 mg/inj self-administration. Abscissa and ordinate are same as in FIG. 2. Each symbol represents the mean IS^RT. For each dose of cocaine, the number of IS^RT plotted corresponds to the average number of reinforcers obtained rounded to the nearest integer. To clarity the view of the graph, SEMs have been omitted. n = 6 for each graph.


MOR on cocaine 0.25 mg/inj
SALINE
MOR1.0
MOR3.2
MOR5.6
IS^RT (min)
MOR on cocaine 0.083 mg/inj
IS^RT (min)
BUP on cocaine 0.083 mg/inj
WATER
BUP0.033
BUP0.1
BUP0.33
IS^RT (min)
0
2
4
6
8
10
12
5
10
15
20
25
Reinforcer

AMPH higher than 1.0 mg/kg significantly shifted the cocaine dose-response curve to the left, which suggested that there may be some potentiation of the effect of cocaine. The result from analysis of IS^RT data indicate that this effect is only related with lower doses of cocaine self-administration. The deviation of results between breaking point and IS^RT may explain the reason for the lack of combination effect of both drugs under a multiple fixed-ratio 30, fixed-interval 600 (mult FR FI) schedule in the pigeon (Evans & Wenger, 1990) and a differential reinforcement of low rates (DRL) schedule in rats (Wenger & Wright, 1990). If we take a closer look at Fig 1, we will find that the dose-response curves between the pretreatment of saline or AMPH 0.32 mg/kg and the pretreatment of AMPH 1.0 or 1.8 mg/kg display different trends. Although this difference is evidence that there may be an interaction between cocaine and AMPH, the significant potentiation of AMPH 1.0 and 1.8 mg/kg pretreatment on saline self-administration indicates that the potentiation may be contributed at least in part by the effect of AMPH alone. This observation indicates that we have to consider additional factors that mediate the reinforcing effect of cocaine when we test them on PR schedule.

The results from earlier work on the study of the interaction between cocaine and amphetamines are quite contradictory. Some studies showed that AMPH enhanced cocaine's effect by increasing avoidance responding under a continuous avoidance procedure (Scheckel & Boff, 1964) Other experiments showed that the combination generally had greater than additive effects on milk drinking in the rat (Foltin et al. 1983). Wenger's experiments (Evans & Wenger, 1990; Wenger & Wright, 1990) demonstrated that under a multiple FR 30 FI 600 schedule and DRL schedule, the consequences of combining cocaine with AMPH at doses that did not affect behavior did not reveal significant changes in the behavioral effect compared to cocaine alone. Similarly, the combination of cocaine with active doses of commonly abused drugs resulted in effects similar to the effects of the interacting drugs alone. Our current results indicate that under the PR schedule, the effect of AMPH on cocaine, if any, is at most an additive effect, even though the mechanisms of the two drugs are expected to produce synergistic effects. Many people who abuse cocaine may

often combine cocaine with another stimulant. As opposed to the result of Wenger's (Wenger & Wright, 1990), our experiments used the PR schedule as an indicator of reinforcing effect and demonstrated that this combination is at least an additive effect. It may be quite reasonable for the abuser that amphetamines are frequently used to substitute for a reduced amount of cocaine to reduce ("cut") the amount of "active" agent on street.

A series of experiments performed by Mello et al. indicated that buprenorphine may successively reduce cocaine self-administration (Mello et al., 1989, 1990, 1992). However, Brown demonstrated that BUP may enhance rather than attenuate the rewarding properties of cocaine under a conditioned place preference procedure and *in vivo* microdialysis (Brown et al., 1991), and Spealman showed that BUP potentiated the discriminative stimulus effects of cocaine (Spealman & Bergman, 1992). The effect of BUP on cocaine 0.083 mg/inj self-administration under the PR schedule tested by our experiments show that BUP has no effect on the breaking points of cocaine self-administration. At this dose of cocaine, pretreatment with BUP caused some animals to fail to start the cocaine self-administration. If this effect is caused by either analgesia or a blockage effect of BUP on the reinforcing effect of cocaine, since these two effects will not modify the reinforcing effect of cocaine, subjects who successfully started the cocaine self-administration would reach breaking points do not differ from control. Therefore, the effect of BUP on cocaine in our case may due to analgesia rather than blockade of the reinforcing effect of cocaine.

Morphine combined with cocaine produced increases in cardiovascular and subjective effects in humans that were less than predicted by an addition model of drug interaction (Foltin & Fischman, 1992). Under a differential reinforcement of low rates (DRL) schedule, the combination of an ineffective dose of cocaine, 1 mg/kg, plus 3 mg/kg morphine produced a shift in the inter-response time (IRT) distribution to the left (an increase in the percentage of short IRT's) (Wenger & Wright, 1990). Under a multiple FR 30 FI 600 schedule, the combination with an active dose of morphine (3 mg/kg) produced less than or approximately equal to an additive effect (Evans & Wenger, 1990). In the current study, under

a PR schedule, pretreatment with morphine (1.0, 3.2 or 5.6 mg/kg, s.c.) did not enhance the breaking point produced by any of these doses of cocaine tested. Indeed, at the highest dose of morphine tested, several subjects failed to initiate responding for cocaine which caused the average breaking point to be decreased. However, among the remaining subjects, the average breaking points for all doses of cocaine were unchanged.

Under the PR schedule, the combination of MOR or BUP with cocaine failed to modify the breaking points of cocaine self-administration. However, this combination caused inter-reinforcer time (IS^RT) of cocaine self-administration to be significantly increased. BUP 0.1 mg/kg caused the greatest increase in the mean IS^RT among the three doses tested. The potentiation of MOR on the mean IS^RT was dose related. Although AMPH caused an increase in both the breaking point and low dose IS^RT of cocaine self-administration, MOR and BUP caused significantly longer IS^RT than AMPH. According to an earlier PR study, the higher the dose of cocaine tested, the higher the breaking point the animal will emit (Depoortere et al., 1993). Furthermore, the increased breaking point always related with a longer IS^RT. After pretreatment with some agents, if the breaking point remained the same but at the same time the IS^RT increased, it is possible that the reinforcing effect of cocaine is in fact potentiated. In the human population, there is also evidence that morphine co-administration with cocaine can increase toxicity. In cocaine-related fatalities, blood cocaine concentrations have been found to be significantly lower in those cases where morphine was also detected (Finkle & McCloskey, 1977). This finding suggests that the presence of morphine was an important factor in toxicity. This view is supported by animal studies where morphine augmented the lethality of cocaine in both rats and mice (Blumberg & Ikeda, 1978). In those cases, the presence of MOR may prolong the effect of cocaine by changing either pharmacokinetic properties or pharmacodynamic effects of cocaine. These possibilities needs to be further investigated.

Although some work using brain lesions indicated that PR could also be applied to the study of potentiation of cocaine self-administration (Loh & Roberts, 1990), little work on

the effect of pharmacological pretreatment has been successfully done. Indeed, Loh in 1992 (Loh et al., 1992) demonstrated that pretreatment with clozapine (10 mg/kg) could increase the breaking points of cocaine self-administration. However, the high variance of the data, the lack of saline control in the experiment, and the steep decline of the breaking point after doubling the dose weaken the conclusion that clozapine potentiate the reinforcing effect of cocaine. Rowlett recently used rhesus monkeys with a progressive fixed ratio schedule observed a left-shifting of the dose-response curve of breaking points after combining cocaine with heroin (Rowlett and Woolverton, 1996). However, heroin alone shifted the dose-response curve to the left. Even though this effect may due to some action of heroin alone, it indicates that interaction of cocaine and heroin may at least have some effect on breaking points. When this combination was used under another progressive ratio schedule similar to our method in rats (Francher et al, 1995), there was no dose dependent relationship found with either heroin alone or in combination studies. In the previous method, the author used Griffiths' method (Griffiths et al, 1975) in which the fixed ratio of lever pressing requirement for the reinforcer increased according to subject's performance of the previous day. In the current method (based on Roberts et al, 1989), a progressively greater number of responses is required for the delivery of each successive reinforcer within a single session. The animal will start to respond based on the priming dose at the beginning of the session. Whether the difference of the two PR methods accounts for the difference in the response patterns needs to be further investigated.

To date, we are unaware of evidence which demonstrates that the progressive-ratio schedule could be used as a tool to test potentiation of the reinforcing effect of cocaine. We need to expand our investigation into wider classes of drugs to evaluate this view of the PR methodology.

REFERENCES

Beitinger TL (1993) Descriptive statistics normality and data presentation. In: Biostatistics Helpbook. UNT Health Science Center handout for the summer course of Bilogy 6220

Blumber H & Ikeda C (1978) Naltrexone, morphine and cocaine interaction in mice and rats. J Pharmacol Exp Ther 206:303-310.

Brown EE, Finlay JM, Wong JT, Damsma G & Fibiger HC. Behavioral and neurochemical interactions between cocaine and buprenorphine: implications for the pharmacotherapy of cocaine abuse. J Pharmacol Exp Ther. 256(1): 119-126,1991

Carroll ME, France CP, Meisch RA (1979) Food deprivation increases oral and intravenous drug intake in rats. Science 205: 319-321

Depoortere RY, Li D-H, Lane JD, Emmett-Oglesby MW (1993) Parameters of self-administration of cocaine in rats under a progressive-ratio schedule. Pharmacol Biochem Behav 45: 539-548

Evans EB & Wenger GR. The effects of cocaine in combination with other drugs of abuse on schedule-controlled behavior in the pigeon. Pharmacol Biochem Behav 37: 349-357, 1990.

Finkle BS, McCloskey KL (1977) The forensic toxicology of cocaine. In: Cocaine: 1977. Washington, DC: National Institute on Drug Abuse Research Monograph 13, Department of Health, Deucation, and Welfare Publication No. (ADM) 79-741, US Government Printing Office; 153-192

Foltin RW & Fischman MW. (1992) The cardiovascular and subjective effects of intravenous cocaine and morphine combinations in humans. J Pharmacol Exp Ther. 261(2):623- .

Foltin RW, Woolverton WL and Schuster CR (1983) Effects of psychomotorstimulants, alone and in pairs, on milk drinking in the rat after intraperitoneal and intragastric administration. J Pharmacol Exp Ther 226:411-418

Francher J, Duvauchelle C, Sapoznik T and Kornetsky C (1995) The progressive-ratio (PR) schedule as a measure of the relative reinforcing properties of the combination of cocaine and heroin ("speedball") with each component alone. NIDA Research Monograph 153: 249.

Hodos W (1961) Progressive ratio as a measure of reward strength. Science 134: 943-944

Hubner CB, Moreton JE (1991) Effects of selective D1 and D2 dopamine antagonists on cocaine self-administration in the rat. Psychopharmacology 105: 151-156

Iwamoto ME, Martin WA (1988) A critique of drug self-administration as a method for predicting abuse potential of drugs. In: Harris LS (ed). Problems of drug dependence. Proceedings of the committee on problems of drug dependence. US Government Printing Office, Washington DC, pp 457-465

Johanson CE, Schuster CR (1981) Animal models of drug self-administration. In: Advances in substance abuse. Behavioral and biological research. Vol II. ed by Mello NK. JAI Press, Greenwich. Conn, pp219-297

Katz JL (1989) Drugs as reinforcers: pharmacological and behavioral factors. In: Liebman JM, Cooper SJ (eds), The neuropharmacological basis of reward. Oxford University Press, Oxford, UK, pp 164-213

Katz JL (1990) Models of relative reinforcing efficacy of drugs and their predictive utility. Behav Pharmacol 1: 283-301

Kauppila T, Mecke E & Pertovaara A. Enhancement of morphine-induced analgesia and attenuation of morphine-induced side-effects by cocaine in rats. Pharmacology & toxicology. 71:173-178, 1992

Lacosta S, Roberts DCS (1993) MDL 72222, ketanserin, and methysergide pretreatments fail to alter breaking points on a progressive ratio schedule reinforced by intravenous cocaine. Pharmacol Biochem Behav, 44:161-165

Loh, EA, Fitch, T, Vickers G & Roberts DCS (1992) Clozapine increases breaking points on a progressive-ratio schedule reinforced by intravenous cocaine. Pharmacol Biochem Behav, 42: 559-562

Loh, Elliot A., & Roberts, D.C.S. (1990) Break-points on a progressive ratio schedule reinforced by intravenous cocaine increase following depletion of forebrain serotonin, Psychopharmacology, 101: 262-266

McGregor A, Lacosta S & Roberts DCS (1993) L-Tryptophan decreases the breaking point under a progressive ratio schedule of intravenous cocaine reinforcement in the rat, Pharmacology Biochem Behav 44: 651-655

Meisch RA, Lemaire GA (1993) Drug self-administration. In: van Haaren F (ed). Methods in behavioral pharmacology. Elsevier Science Publishers, NY, pp 257-300

Mello NK, Lukas SE, Kamien JB, Mendelson JH, Drieze J & Cone EJ. The effects of chronic buprenorphine treatment on cocaine and food self-administration by rhesus monkeys. J Pharmacol Exp Ther 260(3): 1185-1193, 1992

Mello NK, Lukas SE, Kamien JB, Mendelson JH, Drieze J & Cone EJ (1990) Buprenorphine and naltrexone effects on cocaine self-administration by rhesus monkeys. J Pharmacol Exp Ther 254: 926-939.

Mello NK, Jack H. Mendelson, Mark P. Bree, Scott E. Lukas: Buprenorphine suppresses cocaine self-administration by rhesus monkeys. Science, 245:859-862, 1989

Misra AL (1976) Cocaine: Chemical, Biological, Clinical, Social and Treatment Aspects. In: Mule, SJ (ed), CRC Press, Cleveland, pp 73-90

Morbidity and Mortality Weekly Report (1982) Center for Disease Control, Atlanta. 31:265-273

Roberts DCS, Richardson NR (1992) Self-administration of psychomotor stimulants using progressive ratio schedules of reinforcement. In: Boulton AA, Baker G B, Wu PH (eds) (Neuromethods series: vol 24) Animal models of drug addiction, Humana Press, Totowa NJ, pp 233-269

Roberts, DCS (1989a) Breaking points on a progressive ratio schedule reinforced by intravenous apomorphine increase daily following 6-hydroxydopamine lesions of the nucleus accumbens, Pharmacology Biochemistry & Behavior, 32: 43-47

Roberts DCS, Loh EA, Vickers G (1989b) Self-administration of cocaine on a progressive ratio schedule in rats: dose-response relationship and effect of haloperidol pretreatment. Psychopharmacology 97: 535-538

Roberts DCS, Corcoran ME, Fibiger HC (1977) On the role of ascending catecholamine systems in intravenous self-administration of cocaine. Pharmacol Biochem Behav 6: 615-620

Rowlett JK & Woolverton WL Self-administration of cocaine and heroin combinations under a progressive-ratio schedule in rhesus monkeys. NIDA Research Monograph. In press

Sample JC Concept of polydrug use. In: Richards, LG; Blevens, LB, eds. The epidemiology of drug abuse. Research Monograph No. 10. Rockville, MD: National Institute on Drug Abuse; 1977:19-31

SAS System for personal computers, Release 6.03 (1987) SAS Institute Inc., SAS Circle, Cary, NC 27512

Scheckel CL & Boff EB (1964) Behavioral effects of interacting imipramine and other drugs with d-amphetamine, cocaine, and tetrabenazine. Psychopharmacologia 5: 198-208

Siegel, RK (1984) Changing patterns of cocaine use: Longitudinal observations, consequences, and treatment. In: Grabowski, ed. Cocaine: Pharmacology, effects and treatment of abuse. Research Monograph No. 50. Rockville, MD: National Institute on Drug Abuse; 1984:204-220.

Spealman RD & Bergman J. Modulation of the discriminative stimulus effects of cocaine by Mu and Kappa opioids. J Pharmacol Exp Ther 261(2): 607-615, 1992

Wenger GR & Wright DW. Behavioral Effects of cocaine and its interaction with d-amphetamine and morphine in rats. Pharmacol Biochem Behav. 35: 595-600, 1990.

Wesson DR & Smith DE (1977) Cocaine: Its use for central nervous system stimulation including recreational and medical uses. In: Petersen, RC; Stillman, RC, eds. Cocaine: 1977. Research Monograph No. 30. Rockville, MD: National Institute on Drug Abuse; 137-152.

Wilkinson L (1990) SYSTAT: The system for statistics. Evanston IL: SYSTAT, Inc.

Wilson MC, Schuster CR (1973) Cholinergic influence on intravenous cocaine self-administration by rhesus monkeys. Pharmacol Biochem Behav 1: 643-649

Wise RA, Yokel RA, Hansson P and Gerber GJ (1977) Concurrent intracranial self-stimulation and amphetamine self-administration in rats. Pharmacol Biochem Behav 7: 459-461

Wood DM, Emmett-Oglesby MW (1989) Mediation in the nucleus accumbens of the discriminative stimulus produced by cocaine. Pharmacol Biochem Behav 33: 453-457

Chapter 3

If the reinforcing effect of cocaine is determined through a dopamine mediated reward pathway, tolerance to the reinforcing effect of cocaine is expected when the rewarding system is repeatedly exposed to cocaine. The experiment in this chapter was designed to determine whether a chronic treatment regimen with cocaine produces not only a reduction in the inter-reinforcer time (IS^RT) under the FR2 schedule but would also result in a decrease in the breaking point under the PR schedule. The PR schedule supplies direct evidence of the reinforcing effect of a drug.

The original manuscript is published in Psychopharmacology 116:326-332, 1994. The current manuscript is modified as follows: the effect of chronic saline treatment on the breaking point of cocaine; recovery baseline data; and an analysis of inter-reinforcer time after chronic treatment with cocaine under the PR schedule have been added. Appropriate changes were made to the methods section and additions were made to the discussion as necessary to include the conclusions from this additional data. Changes to text are indicated in italics.

Tolerance to the reinforcing effects of cocaine in a progressive-ratio paradigm[1]

D.-H. Li[2], R. Y. Depoortere, and M. W. Emmett-Oglesby

Department of Pharmacology

University of North Texas HSC

3500 Camp Bowie Blvd

Fort Worth TX 76107-2699

USA

Running Title: Tolerance to Cocaine

1 Supported by grants RO1-4137 and Texas Advanced Technology Award 9768031

2 This project is taken from a dissertation submitted to the University of North Texas Health Science Center at Fort Worth in partial fulfillment of the requirements for the degree Doctor of Philosophy

Abbreviations:

Progressive-ratio (PR)

Injection (inj)

Inter-reinforcer time (IS^RT)

ABSTRACT

This experiment used rats to test whether a regimen of chronic cocaine would produce tolerance to cocaine i.v. self-administration under a progressive ratio (PR) schedule of reinforcement. Under this PR schedule, an increasing number of responses was required to complete the ratio for each subsequent cocaine injection, and failure to complete the required ratio for the next injection within one-hr of the previous cocaine injection terminated the session. The number of injections taken in the session was termed the breaking point and used as the dependent variable. Rats were trained under this schedule until breaking point values were stable, after which cocaine dose-effect data were obtained: the breaking point increased as the dose of cocaine increased. Subsequently, rats were assigned to one of two groups for seven days of chronic treatment: one group was infused with cocaine (20 mg/kg, given over 20-min once every 8-hr) and the other group received 0.9% saline. Following termination of chronic treatment, cocaine dose-effect data were determined in both groups. Chronic cocaine treatment significantly decreased breaking point values across the entire dose-effect curve, although the effect was observed in only four of seven subjects. In contrast, chronic saline treatment produced no significant effect on the breaking point. Following a five days of recovery from chronic treatment, cocaine dose-effect curve was determined in both groups; these curves were essentially identical to those obtained before chronic treatments. These data support the hypothesis that tolerance occurs to the reinforcing effects of cocaine, as measured by a decrease in the breaking point, at least for a subset of animals.

Keywords: Cocaine, Progressive-ratio schedule, Rats, Self-administration, Tolerance

INTRODUCTION

Following a chronic regimen with cocaine, there is an increase in the rate of cocaine self-administration in rats trained to self-administer cocaine under a fixed-ratio (FR) schedule of reinforcement (Emmett-Oglesby and Lane, 1992). In that study, rats were trained to self-administer cocaine under a schedule in which two presses on a lever produced a 0.1 ml infusion through an i.v. catheter, and tolerance was produced by suspending training and infusing cocaine, 20 mg/kg/8-hr for a week. As compared to chronic saline administration, treatment with chronic cocaine produced an approximate two-fold shift to the right of the cocaine dose-effect curve, and this tolerance was reversible to baseline when chronic cocaine was terminated. These initial observations were extended in more detailed tests of the time course of tolerance phenomena (Emmett-Oglesby *et al.*, 1993); further, whether cocaine was infused by the experimenter or self-administered by the subjects, comparable tolerance was observed with this chronic regimen of cocaine (Emmett-Oglesby *et al.*, 1993).

Do changes in the rate of cocaine self-administration under low-value FR schedules reflect tolerance to the reinforcing efficacy of cocaine? Unfortunately, the rate of drug taking in such schedules is at best an ambiguous measure of a drug's reinforcing efficacy; indeed, a strong argument can be mounted that low-value FR schedules can only provide a yes/no answer to the question "does a drug have abuse liability?", and these schedules may not be appropriate for predicting relative reinforcing efficacy of different drugs or different doses of the same drug (Iwamoto and Martin, 1988; Katz, 1990). For example, in low-value FR schedules, the intake of cocaine typically is stable over time, independent of the dose that is available for self-administration (e.g., Johanson and Schuster, 1981; Emmett-Oglesby and Lane, 1992); that is, subjects increase or decrease their rate of responding such that a constant quantity of cocaine is taken per unit time, independent of the dose offered for self-administration. A variety of hypotheses have been put forward to explain this phenomenon, including the conjecture that animals take as much drug as possible until the disruptive effects of the drug

temporarily preclude further administration (Wilson and Schuster, 1973). Alternatively, subjects may titrate the blood concentration of drug that produces an optimal effect (Wise *et al.*, 1977). If the former hypothesis were correct, changes in the rate of cocaine taking in the low-value FR schedule would reflect tolerance to the disruptive effects of the drug, whereas if the latter hypothesis were correct, these changes would reflect tolerance to the reinforcing efficacy of cocaine.

One schedule of reinforcement that has been suggested to measure relative efficacy of different reinforcers is the progressive-ratio (PR) schedule (Hodos, 1961). Under this schedule, the number of lever responses required to obtain a reinforcer is increased for each successive reinforcer until the animal fails to meet the demands of the schedule, which is termed a breaking point. The analysis of the change in the breaking point of PR responding has been proposed to offer a reliable and quantitative method for assessing changes in reinforcing efficacy of drugs (Roberts and Richardson, 1992; Roberts *et al.*, 1989); for example, smaller doses of a drug typically produce lower breaking point values than larger doses of the same drug (Depoortere *et al.*, 1993; Roberts *et al.*, 1989), and cocaine, a drug known to have high abuse liability in humans, maintains the highest breaking point values compare with other abused drugs in a variety of subjects tested under PR schedules (Griffiths 1979; Risner 1981).

Our laboratory has studied parameters of cocaine self-administration in rats under a PR schedule (Depoortere *et al.*, 1993). In our experience, responding under this schedule is stable (reproducible breaking points are obtained for in excess of 100 sessions), and the breaking point varies directly as a function of cocaine dose. Because tolerance is defined as the shift of a dose-effect curve of a drug to the right (Cox, 1990), if tolerance does occur to the reinforcing efficacy of cocaine, then breaking points should be decreased following chronic cocaine. Our current experiment used rats trained on a PR schedule to determine whether a chronic dosing regimen of cocaine that produced tolerance to cocaine self-administration in FR2 schedules would result in a decrease in breaking point.

METHODS

Subjects and Apparatus. Adult male Fisher F344 rats (Harlan, Indianapolis IN) were housed individually and maintained at 260-290g by daily feeding of measured quantities of food. Initially, rats were trained to press a lever under a FR1 schedule using food pellets as a reinforcer; after responding was stable, each subject was implanted with a permanently indwelling jugular catheter (for details, see Depoortere *et al.*, 1993). After surgery the catheter was flushed with a combination of heparin, streptokinase, and Timentin (ticarcillin plus clavulinic acid) every 12 h for 5 days. On the 6th day, Timentin was removed from the regimen. Subjects were trained and tested in locally constructed operant chambers that have been described previously (Depoortere et al., 1993).

Preliminary Training Phase. Five days following surgery, animals began training under a fixed-ratio (FR) one schedule of reinforcement, with cocaine, 0.25mg/0.1ml injection serving as the reinforcer. Sessions lasted either until 25 injections were self-administered or until approximately 12-hr elapsed. Each session started with a priming injection of cocaine (2.70 mg/kg) delivered by the experimenter. Priming and subsequent injections were accompanied by a flashing of two stimulus lights in the chamber; each injection was followed by a 30-s time-out in the dark, during which responding had no programmed consequences.

If a rat self-administered cocaine at a constant rate ($\pm$ 20%) over the course of 3-5 days, it was selected for training under the PR schedule. Daily PR sessions began with a 0.90 mg/kg priming infusion. Each subsequent cocaine injection, 0.25mg/inj, occurred upon completion of each of the ratios in the following sequence: 3, 6, 10, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 603, 737, 901, 1102, and 1347. There was a 1-hr time limit to obtain each reinforcer, and failure to obtain the reinforcer terminated the session. Typically, when rats failed to complete a ratio, they ceased responding within 20 to 30 min after the previous reinforcer was obtained, and they subsequently emitted few if any additional responses. Stability under the PR schedule for this 0.25 mg/inj dose was defined as at least 7 sessions in which the breaking point did not differ by more than 3 injections.

Pre-chronic dose-effect data. After this stability criterion was obtained, rats were tested with different doses (0.028, 0.083 and 0.25 mg/inj) of cocaine on several occasions until reproducible dose-effect data were obtained. Subsequently, for the pre-chronic phase of this experiment these same doses were tested, one dose per day, over three consecutive days. In all tests, subjects were first infused with the dose of cocaine available for testing on that day. Because subjects occasionally failed to start responding for the lowest dose of cocaine tested (0.028 mg/inj), a slightly larger priming injection (0.3 mg/inj) was used.

Chronic treatment and testing of cocaine, 0.083 mg/inj. After pre-chronic dose-effect testing was finished and baseline breaking points under 0.25mg/*inj* had returned within three reinforcers of baseline, a final test with 0.083 mg/*inj* was performed. Beginning the next day, all training and testing were terminated, and the chronic dosing regimens were started. For the chronic regimens, subjects lived in the self-administration chambers, with the chambers modified such that no levers were present. Subjects were assigned to two groups (N=9 per group): one group (Saline) received 20 infusions of saline (0.1 ml volume, 1-min. between infusions) at 8-hr intervals for one week; the second group (Cocaine) received 20 infusions of cocaine, 0.25 mg/infusion, in the same volume and time course as the Saline group. The 20th infusion was followed by a heparinized-saline flush (0.4U in 0.1ml), and this flush was repeated 3 and 6 hr later. The cocaine dose was thus approximately 20 mg/kg/8-hr, given for 7 days.

For both the Saline and Cocaine groups, during the chronic administration, rats were tested with a cocaine dose of 0.083 mg/*inj* on days 2, 4 and 6 of chronic treatment. These tests were performed for two reasons: first, to follow the time course for development of tolerance; and second, to maintain rats in contact with the PR schedule (in pilot studies we observed that withholding rats from performing under the PR schedule for periods of 7 days resulted in some subjects showing reduction of breaking points).

Determination of cocaine dose-effect curve immediately upon termination of chronic treatment (post-chronic tests). After the final infusion in the chronic treatment regimens, subjects were returned to their home cages, and on days one, two and three following the last

chronic infusion, breaking points were determined for the cocaine dose-effect curve (0.028, 0.083 and 0.25 mg/*inj*) in both groups. Subjects were tested with these doses in the same order as their pre-chronic dose-effect testing prior to chronic infusions.

Recovery. After these doses were tested, subjects were left without training or testing for 5 days, and then training was resumed using the baseline cocaine concentration (0.25 mg/inj). Recovery from chronic administration was defined as three consecutive sessions in which the breaking point was within three reinforcers of the pre-chronic baseline. When responding was maintained with less than three infusions difference for each rat over at least three successive sessions, dose-effect curves were tested again with doses of cocaine in the same order as for post-chronic tests to determine recovery from tolerance.

Data Analysis: The number of reinforcers obtained was used as the dependent measure, which is termed the breaking point. This measure of breaking point was used rather than the final ratio completed or the total number of responses emitted because these latter variables are not amenable to parametric analysis (for a discussion of this problem in analysis of response and reinforcer data from PR procedures, see Depoortere *et al.*, 1993; and Roberts and Richardson, 1992). Cocaine dose-effect data were subjected to two-way ANOVA for repeated measures, with the dose of cocaine and chronic treatment as the two within factors. The breaking points for 0.083 mg/inj cocaine obtained on days 0 (immediately prior to chronic) and days 2, 4 and 6 of chronic treatment were analyzed by two-way ANOVA with repeated measures. All ANOVAs were performed with Statistic Analysis Systems (SAS, 1987) software.

Effect of drug -treatments on the mean Inter-Reinforcer Time (IS^RTs) was introduced as another dependent measure. To calculate this variable each subjects' first and last IS^RT were excluded, because they were not representative of the general pattern of IS^RTs. The first IS^RT is variable because some rats take much longer than others to start self-administering drug following the infusion of the priming dose. The last IS^RT shows high variability because at this final ratio value an intermittent pattern of lever pressing precedes complete termination of response. The second step to analyze the IS^RT s was to transform the data according to the

formula 2 + log (IS^RT) to minimize the error introduced by an increased number of responses as the number of reinforcers increases. After this conversion, each remained interval between reinforcers will have a similar contribution to the mean IS^RTs.

Since the breaking points of some animals at the low dose of cocaine (0.028 mg/inj) were very low (sometimes, it's less than 4), the sample size of the experiment will be significantly reduced. To avoid the data analysis being biased by the low dose cocaine self-administration, the treatment of IS^RT was concerned only on 0.083 and 0.25 mg/inj cocaine self-administration dosage. Based on the transformed data, IS^RT s of different pre-treatments on either 0.083 or 0.25 mg/inj cocaine were further subjected to fully factorial ANOVA (MANOVA) for repeated measure with the value of IS^RT s as dependent variables and pretreatments as factors.

Drugs. Cocaine HCl was obtained from the National Institute on Drug Abuse (Research Triangle Park, NC). All doses refer to the weight of the salt.

RESULTS

Two subjects were dropped from each group during the course of the experiment. In the Saline group, both rats were terminated during chronic administration because catheters failed to remain patent. In the Cocaine group, one subject was dropped because of catheter problems during chronic administration; the second subject was dropped because the breaking point failed to return to baseline criterion upon termination of chronic administration.

Subjects were originally assigned to the two groups such that the initial dose-effect curves did not differ significantly. After deleting two subjects from each of the groups, there was still no significant difference between the pre-chronic dose-response data for these groups ($F_{1,36}$=0.12, p>0.5; compare open symbols in Figs 1 and 2).

In all tests, the breaking point increased as a function of the dose of cocaine available for testing (Fig 1; $F_{2,36}$=32.8, p<0.001). Immediately upon termination of chronic cocaine, the

breaking point was decreased at all doses (Fig 1; $F_{1,36}$=11.98, p<0.005). The doses of cocaine tested spanned a 9-fold range, and under pre-chronic conditions produced breaking points of approximately 11, 18 and 21 *injections*. These values decreased to approximately 6, 14 and 18 injections following chronic cocaine treatment, which is approximately the effect expected from using only half of the dose tested under pre-chronic conditions (see Fig 1). Because reinforcer numbers occur as a logarithmic function of responses, the change in total number of responses emitted is even more striking. Thus, prior to chronic administration, cumulative responses corresponding to these reinforcer totals were approximately 400, 1,800 and 3,500, and chronic cocaine reduced these numbers to approximately 100, 750 and 1,800. *Finally, tolerance was reversible upon discontinuation of chronic cocaine; after five days without chronic treatment followed by retraining with the 0.25 mg/inj dose for 10 days, the recovery dose-effect curve (Fig 1) was not significantly different from the pre-chronic dose-effect curve ($F_{1,36}$=4.02, p>0.05).*

Although chronic cocaine produced a significant group effect, close inspection of Fig 1 shows that the variance is greater in the tests that occurred immediately after chronic-cocaine. This reflects the fact that three of the seven rats showed little, if any, tolerance. In these three subjects, the total reinforcers obtained over all doses in the pre-chronic test were 149; in tests immediately after terminating chronic cocaine, these subjects received 151 total injections. The remaining four subjects thus account for the significant group effect, and the degree of reduction in reinforcers for these four subjects is much greater than indicated by group averages. Baseline variables such as breaking point and rate of cocaine injections did not account for differential tolerance between these sub-groups of animals (Table 1.; for breaking points: $t_{0.05(2),5}$=0.91, p>0.1; for IS^{R}T: $t_{0.05(2),5}$=0.092, p>0.5).

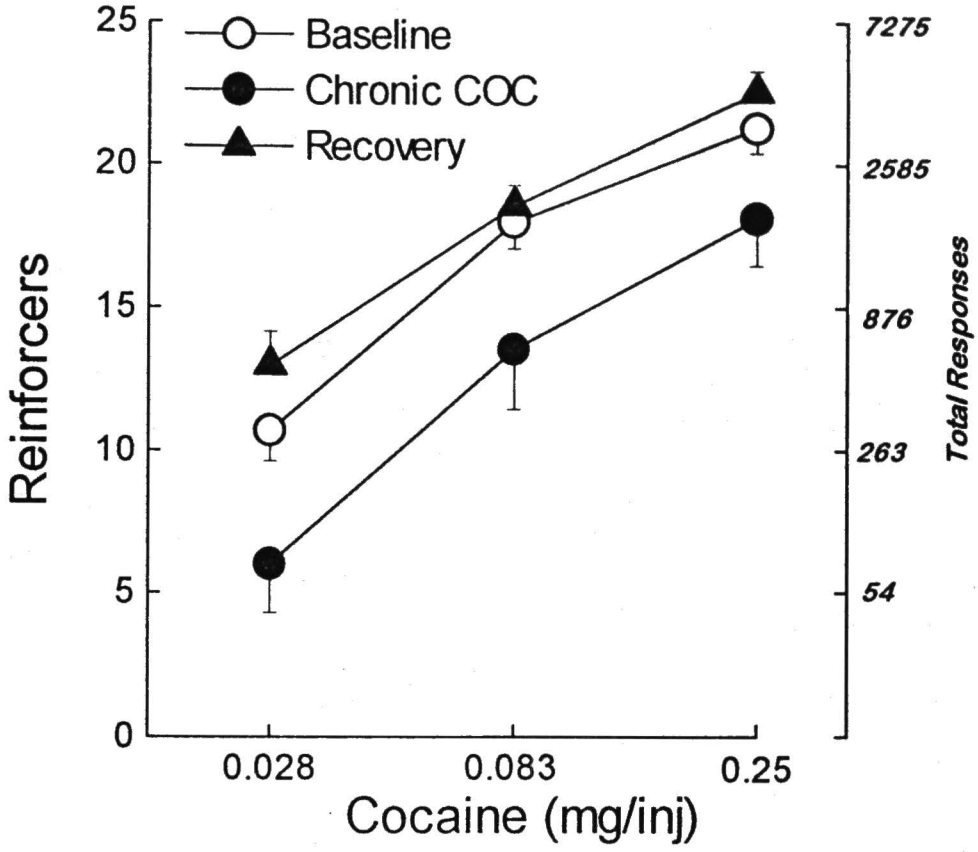


Fig. 1. Effect of chronic treatment with cocaine on the breaking point obtained for various doses of cocaine. Abscissa: dose of cocaine available for self-administration. Ordinate: the number of reinforcers obtained. Each symbol represents the average number of reinforcers obtained in a session, and vertical bars represent S.E.M. Dose-effect data were obtained over a three day period: prior to chronic cocaine (open circle), immediately after chronic cocaine, 18 mg/kg/8-hr for one week (closed circle) and several days after terminating chronic cocaine (closed triangle). N = 7.

TABLE 1. Comparison of pre-chronic parameters for self-administration of cocaine, 0.25 mg/inj, between animals that subsequently were either tolerant or non-tolerant following the chronic cocaine regimen.[a]

	Tolerant	Non-Tolerant
Breaking Points[b]	20.1 ± 1.1	21.4 ± 1.0
Average Time Between Cocaine Injections (Min)[c]	3.5 ± 0.7	3.6 ± 0.8

[a]Values are expressed as mean ± SEM. n=4 for subjects that showed tolerance; n=3 for subjects that did not become tolerant. Data are averages with the 0.25 mg/inj dose that were obtained over three days immediately prior to pre-chronic dose-effect testing.

[b]Breaking points are the total number of cocaine injections.

[c]Average Time Between Cocaine Injections (IS^RT) was calculated from the time between each injection; the times to obtain the first and the last reinforcer were not included in this average because they typically show substantially greater variation from session to session than times associated with all other reinforcers (see Depoortere *et al.*, 1993 for a detailed description of this measure).

In the chronic saline group there was also a significant effect on the dose effect of cocaine ($F_{2,36}$=48.1, p<0.001); however, in contrast to the results obtained with the chronic cocaine regimen, chronic treatment with saline did not significantly modify the cocaine dose-effect curve (Fig. 2.; $F_{1,36}$=0.24; p>0.5). Subjects were then permitted to recover from chronic administration for 5 days and subsequently trained with the 0.25 mg/inj dose until baseline stability was obtained; when a dose-effect curve was determined at this point, it also did not differ significantly from the pre-chronic dose-effect curve ($F_{1,36}$=0.11, p>0.05).

Immediately prior to initiating chronic treatment, all subjects were tested with cocaine, 0.083 mg/inj. During chronic treatment, subjects were given the opportunity to self-administer this dose on days 2, 4 and 6. These injection opportunities occurred approximately 4-hr after the morning infusions of chronic administration. Chronic treatment with cocaine reduced the breaking point on each of days 2, 4 and 6, although this effect did not reach statistical significance as compared to the Saline group (Fig. 3.; $F_{1,12}$=3.81, 0.1>*p*>0.05); in contrast, chronic treatment with saline did not alter the breaking point by even one reinforcer over all tests.

After termination of chronic treatment, testing and an additional 5 days during which rats were neither trained, tested or infused, training on the 0.25 mg/inj baseline dose was resumed. On the first day of training, the breaking points obtained for the two groups did not differ significantly from their pre-chronic baselines nor from one another (Fig 4.; $F_{1,18}$=0.3, p>0.5). Across the next seven sessions, breaking points remained stable.

Although chronic treatment with cocaine shifted baseline cocaine breaking point to the right, this treatment had no significant effect on IS^RT at the cocaine dose of 0.25 mg/inj (Fig 5. top panel; $F_{1,44}$=0.001, p>0.9). However, the chronic treatment caused significant decrease of IS^RT on cocaine administration at dose of 0.083 mg/inj (Fig 5. bottom panel; $F_{1,\ 27}$=5.91, p<0.05). After the recovery period, the IS^RT of cocaine dose of 0.25 mg/inj significantly differed from the pretreatment value ($F_{1,44}$=5.95, p<0.05), but the IS^RT for the cocaine dose of 0.083 mg/inj successfully returned to their pretreatment values ($F_{1,27}$=0.326, p>0.5).

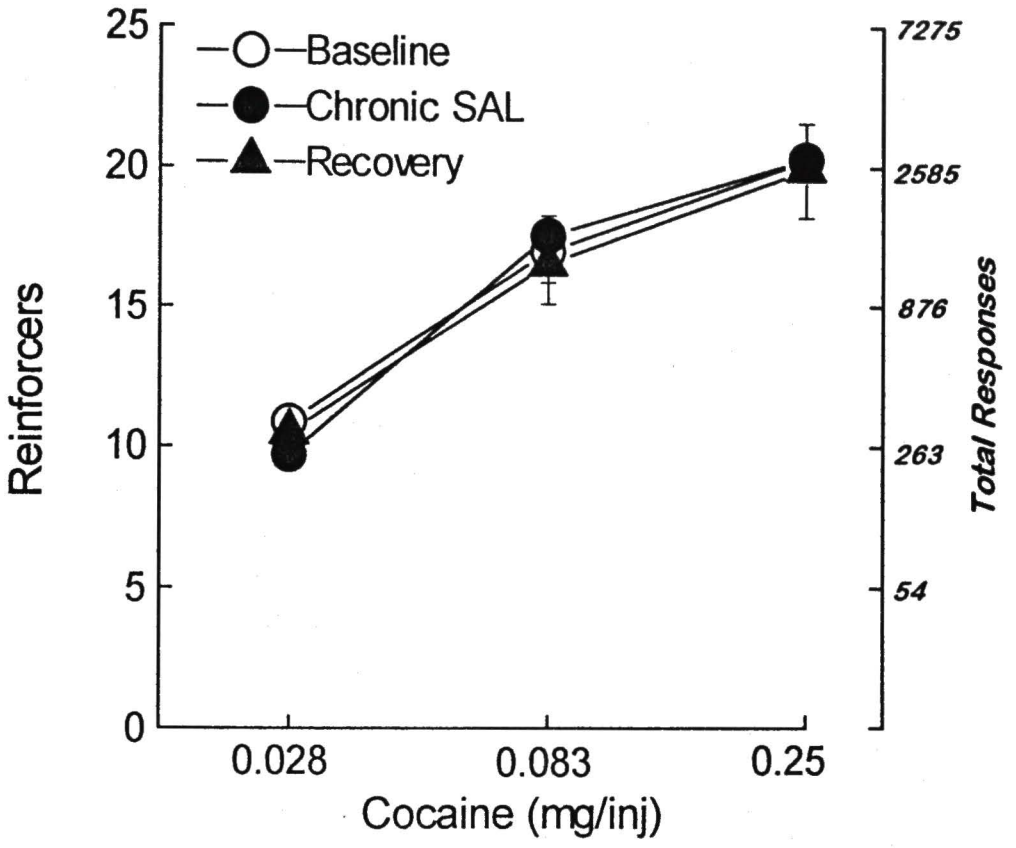


Fig. 2. Effect of chronic treatment with saline on the breaking point obtained for various doses of cocaine. See Fig 1 for description of abscissa and ordinate. All other information is identical to Fig 1, except that instead of chronic cocaine, subjects were treated with chronic saline, 20 infusions of 0.1 ml each, 1-min apart, every 8-hr for one week. N = 7.

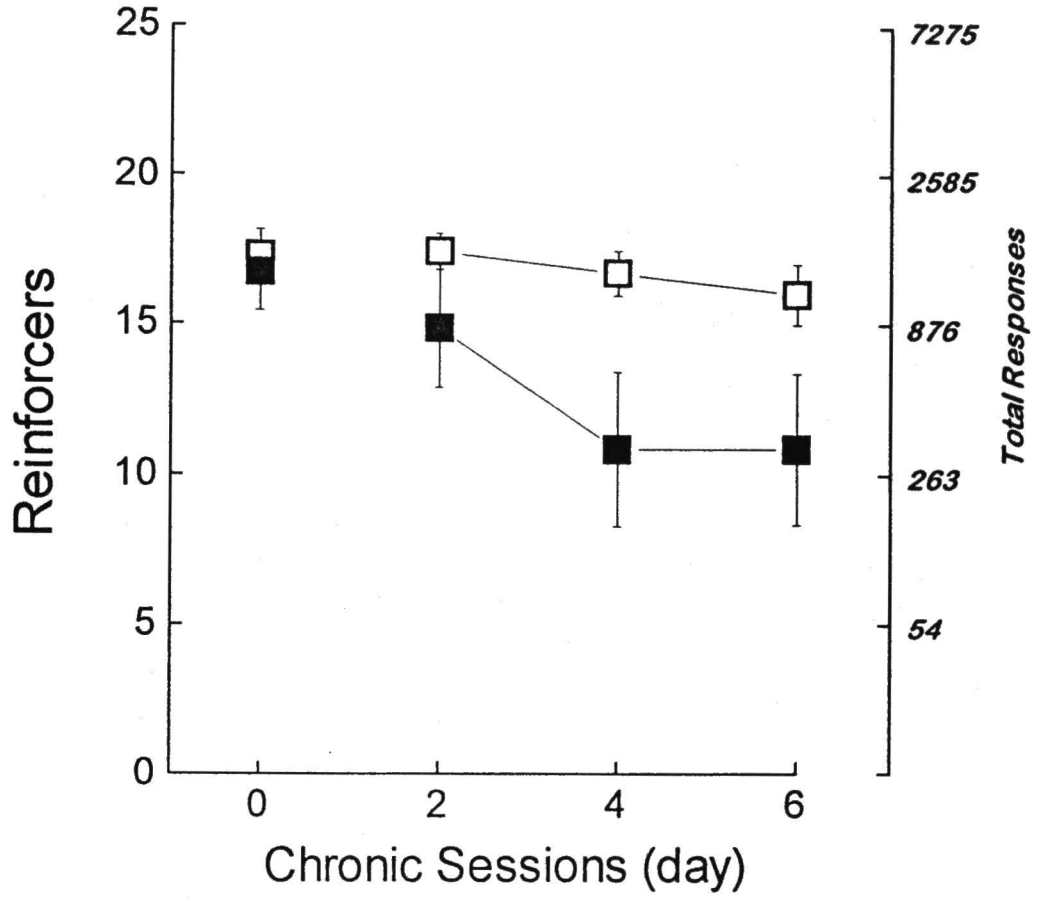


Fig. 3. Breaking point obtained with cocaine, 0.083 mg/inj, tested immediately prior to, and then on days 2, 4 and 6 of chronic administration. Abscissa: number of days of chronic administration; data above 0 are from the day prior to starting chronic administration. Ordinate (see Fig 1). Each symbol represents the average ± S.E.M. number of injections of cocaine, 0.083 mg/inj, obtained in a session. Chronic treatments are with saline (open square) or cocaine (closed square), approximately 18 mg/kg/8-h. N = 7 for each group.

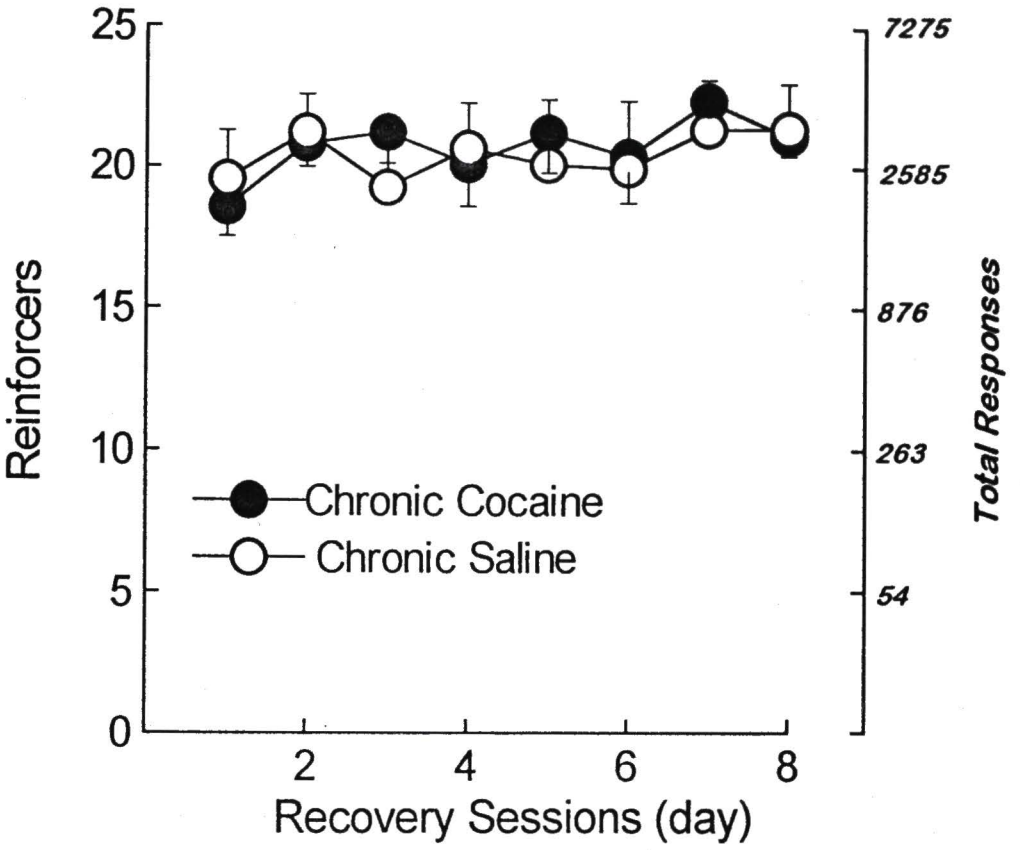
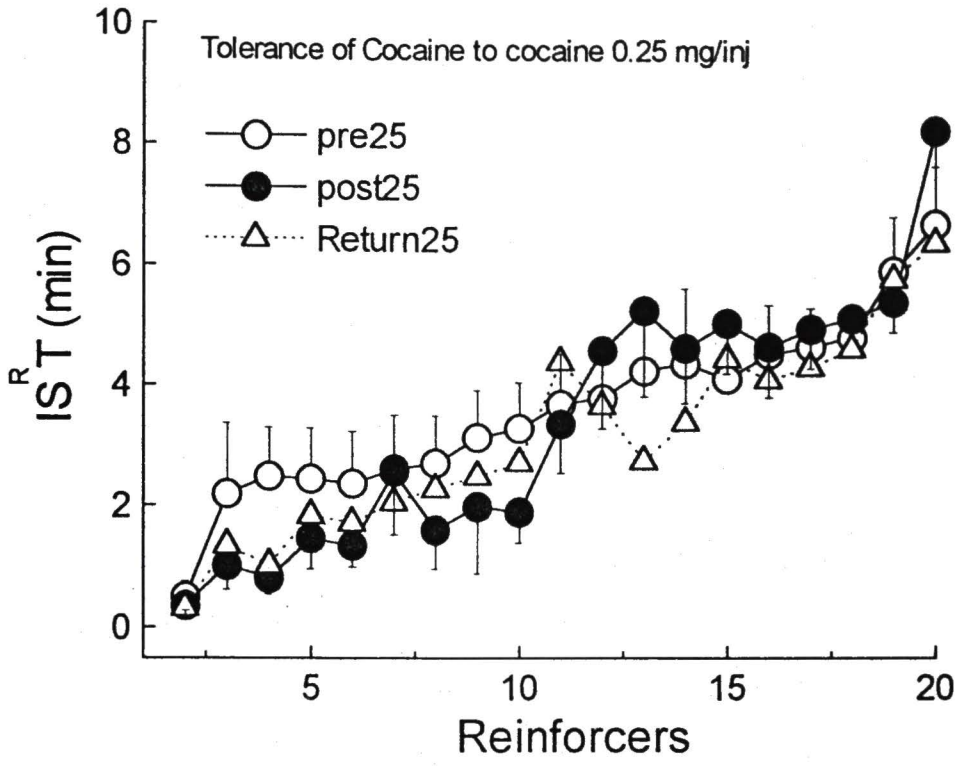
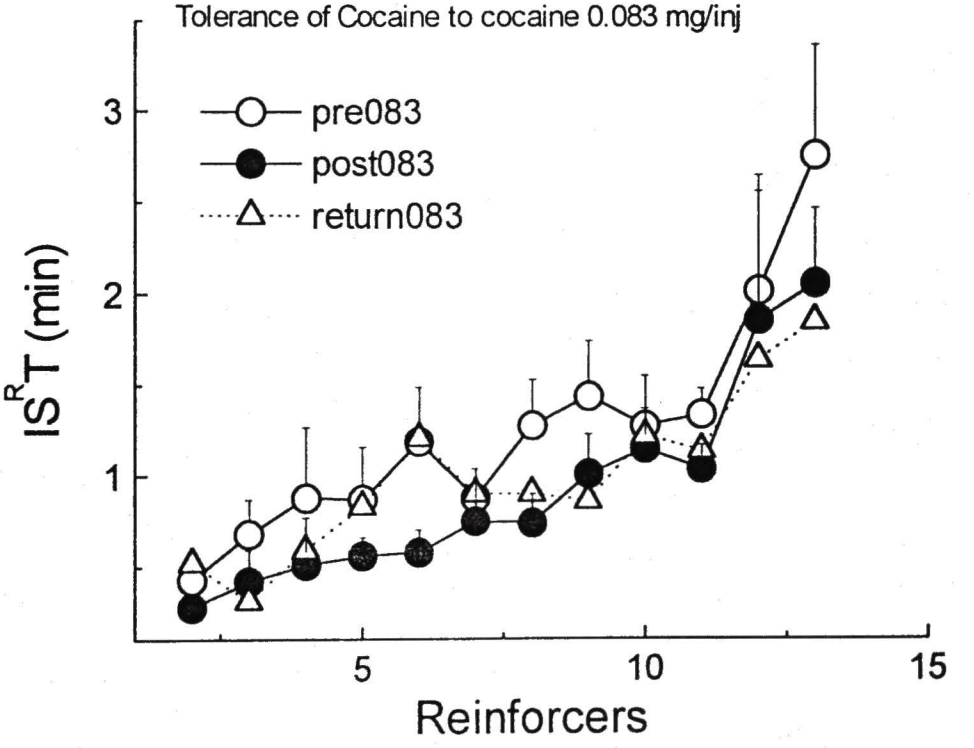


Fig. 4. Recovery to baseline following termination of chronic cocaine or saline. Abscissa: Data above Pre (pre-chronic) are from tests done prior to chronic administration; numbers refer to consecutive sessions of access to cocaine, 0.25 mg/inj, which began 8 days post termination of chronic injection. Ordinate (see Fig 1). Each symbol represents the average ± S.E.M. number of injections of cocaine, 0.25 mg/inj, averaged for two consecutive sessions. Chronic treatments were with saline (open square) or cocaine (closed square), approximately 18 mg/kg/8-h. N = 7 for each group.

Fig. 5. Top panel: Effects of chronic cocaine on the inter-reinforcer time of cocaine 0.25 mg/inj self-administration. Bottom panel: Effect of chronic cocaine on the inter-reinforcer time of cocaine 0.083 mg/inj self-administration. Abscissa: the number of reinforcers obtained. Ordinate: Inter-reinforcer time. Each symbol represents the mean IS^RT. For each dose of cocaine, the number of IS^RT plotted corresponds to the average number of reinforcers obtained rounded to the nearest integer. For clarity, some SEMs have been omitted. n = 7 for each graph.


Tolerance of Cocaine to cocaine 0.25 mg/inj
pre25
post25
Return25
IS R T (min)
Reinforcers
0
2
4
6
8
10
5
10
15
20


Tolerance of Cocaine to cocaine 0.083 mg/inj
pre083
post083
return083
IS R T (min)
Reinforcers
1
2
3
5
10
15

DISCUSSION

Chronic treatment with cocaine, 20 mg/kg/8-hr for a week, decreased the breaking point for *four of seven subjects* under this PR schedule of cocaine self-administration. This effect was reversible upon termination of chronic cocaine, and the time course of recovery from tolerance parallels data from previous studies using this chronic regimen (Wood *et al.*, 1984; Emmett-Oglesby *et al.*, 1993). In contrast to the results obtained with a chronic cocaine regimen, chronic treatment with saline produced essentially no effect on breaking points maintained by cocaine.

Tolerance to the self-administration of cocaine under PR schedules has not been reported previously, perhaps primarily because few studies have examined the effects of high-dose, chronic administration of cocaine on subsequent responding under this schedule. In the only previous study that addressed this question, Yanagita (1973) used a PR schedule to test the hypothesis that the intensity of drug-seeking behavior would increase following development of physical dependence on cocaine (Yanagita, 1973). Chronic treatment of two monkeys for one month with high-dose cocaine did not increase the breaking point, and Yanagita concluded that cocaine did not induce physical dependence. Interestingly, however, chronic treatment with cocaine resulted in a 50% reduction in total responses emitted in both subjects tested; thus, preliminary data from monkeys also supports the hypothesis that tolerance occurs to the reinforcing effects of cocaine self-administration under a PR schedule.

The dosing regimen of chronic cocaine used to produce tolerance in this experiment, 20 mg/kg/8-hr, is close to those that have previously been reported to produce tolerance in other experiments. For example, cocaine, 20 mg/kg/8-hr for 7 days produces an approximate two-fold shift to the right of the dose-effect curve for the detection of cocaine in a drug discrimination procedure (McKenna and Ho, 1977; Wood et al., 1984; Wood and Emmett-Oglesby, 1986; 1988). This same dosing regimen also shifts the dose-effect curve for cocaine self-administration under an FR2 schedule approximately two-fold to the right (Emmett-

Oglesby and Lane, 1992; Emmett-Oglesby et al., 1993). In the drug discrimination procedure, the degree of tolerance increases as a function of increasing dose: no tolerance occurred at 15 mg/kg/day, a small amount of tolerance occurred at 30 mg/kg/day, and two-fold tolerance occurred at 60 mg/kg/day (Wood et al., 1986). The effect of different dosing regimens on the degree of tolerance in self-administration procedures is an issue for further experimentation; however, under baseline conditions, subjects received a priming dose of cocaine, 1.8 mg/kg, and they then averaged approximately 22 injections of 0.25 mg/inj, totaling approximately 21.6 mg/kg/day. The total daily dose of cocaine that produced tolerance was less than three-fold higher than this (54 mg/kg/day). This difference is not large, and it may seem surprising that tolerance has not been seen more frequently in previous self-administration studies. However, as described above, the occurrence of tolerance in the drug discrimination procedure is obtained only with the higher doses used in this study; further, it may be the case that frequently alternating periods of drug and no-drug, such as were used here, are critical to the occurrence of tolerance.

In the present experiment, when chronic cocaine was discontinued, tolerance was seen in tests over the next three days, with one dose tested on each of these days. Over five subsequent days, rats were withheld from any additional testing, training or chronic injections, and when tested the following day with the baseline training dose, they once again showed baseline values for the breaking point. These findings parallel those from other studies using similar cocaine dosing regimens to study tolerance. For example, after terminating from a regimen of cocaine, 20 mg/kg/8-hr for one-week, tolerance to the discriminative stimulus effects of cocaine was maintained for three to four days, and a return to baseline sensitivity was seen within approximately a week (Emmett-Oglesby and Wood, 1984). Similarly, a comparable time course has been observed for loss of tolerance to cocaine self-administration under an FR2 schedule (Emmett-Oglesby *et al.*, 1993), where baseline rates of self-administration were resumed within one-week simply by letting the animal remain free of cocaine injections. Thus, the time-course of tolerance to cocaine in this PR paradigm appears

to parallel the time-course of tolerance that has been reported to occur with comparable dosing regimens in both discrimination and FR2 self-administration paradigms.

When we started to analyze the inter-reinforcer time (IS^RT) under the PR schedule, we realized that although there was a significant reduction in the IS^RTs tested at the low dose of cocaine (0.083 mg/inj), the dose-response curve of IS^RT failed to shift to the right at baseline dose of cocaine (0.25 mg/inj). This result was different from our previous result of IS^RT obtained from FR2 schedule (Emmett-Oglesby *et al.*, 1993). The possible reasons for this discrepancy may due to: 1) the variance of the IS^RT baselines caused by dramatic increase in the number of responses required to obtain each reinforcer; 2) The difference in function between FR2 and PR schedule. Under the FR 2 schedule, an animal is trained to emit only 2 responses to obtain each reinforcer, with a maximum of fifteen reinforcers per session. Therefore, the effect of the drug related with blood concentration on the animal will be a critical factor. If the response emitted by the animal is too high, the subject will be intoxicated (caused by over dose). Under this circumstance, the time pause to start to respond becomes very critical for the subject. Under the PR schedule, the animal is trained to emit the maximum responding to obtain each reinforcer. Under this circumstance, the time pause to start the lever pressing is less important. Once the system indicate the animal that drug is available, the animal has to continue to press the lever for the next reinforcer until the reinforcing effect of the drug is disappeared. Therefore, under the PR schedule the effort the animal has to emit, rather than the time spent in the box, will be a critical factor which influence the behavior of the animal. Based on above sugesstions, it may be the restriction of the condition rather than the supply of the reinforcement that controlled the time spend on lever pressing of an animal, which indicated the difference between both FR 2 and PR schedule.

We previously have discussed three possible mechanisms that might account for the development of tolerance to cocaine in a self-administration paradigm (Emmett-Oglesby *et al.*, 1993). These mechanisms are likely to be either pharmacokinetic (e.g., increased metabolism) or pharmacodynamic (e.g., decreased behavior-disrupting effects or decreased reinforcing

efficacy). The pharmacokinetic mechanism is plausible but unlikely because chronic administration is not known to increase metabolism of this drug in rats (Misra, 1976; Katz *et al.*, 1993).

The second mechanism, decreased behavior disrupting effects of cocaine, is also not likely to be the case. The hypothesis that behavioral disruption governs the rate of drug taking assumes that drug self-administration occurs in animals as rapidly as possible, such that under an *FR2 schedule*, the time between injections is primarily a function of behavioral intoxication; that is, subjects take as much drug as possible until they cannot emit an operant, and the next injection occurs when subjects once again recover from this disruption sufficiently to emit the operant (Katz, 1989). Thus, tolerance to the disruptive effects of cocaine would result in an increased rate of drug taking (subjects would take more drug per unit time simply because they were able to emit the operant sooner after a previous dose). Although such a hypothesis might account for some aspects of tolerance in FR schedules (Emmett-Oglesby and Lane, 1992; Emmett-Oglesby *et al.*, 1993), if this were the primary mechanism mediating tolerance to cocaine in these experiments, no tolerance should have been obtained in the PR schedule. It is the total amount of effort an animal emit rather than the time the animal spend in lever pressing that mediate the change of the breaking point. If anything, a reduction in the disruptive effects of cocaine should have resulted in an even greater intake of drug under this schedule such that higher breaking points should have been obtained.

The third mechanism, tolerance to the reinforcing effects of cocaine, is compatible with data from the present study as well as from our previous work. When Hodos (1961) first introduced the PR schedule, he suggested that this schedule could be employed to assess the relative reward strength of different quantities of food. The schedule has been used to study the relative reinforcing efficacy of different doses of various drugs (e.g., Griffiths *et al.*, 1975; Risner and Silcox, 1981; Roberts *et al.*, 1989; Roberts and Richardson, 1992), and over a wide range of doses, the breaking point is a direct function of the dose of drug tested. The interpretation that bigger doses have greater reinforcing efficacy than smaller doses is

strengthened by the observation that subjects given a choice between self-administration of two different doses of a drug of abuse typically choose the higher dose (for a review see Meisch and Lemaire, 1993).

The hypothesis that tolerance occurs to the reinforcing efficacy of cocaine is further supported by more careful examination of our previous data concerning self-administration of cocaine under an FR2 schedule. As noted above, chronic administration of cocaine shifted the cocaine dose-effect curve approximately two-fold to the right (Emmett-Oglesby and Lane, 1992; Emmett-Oglesby *et al.*, 1993). Importantly, however, this shift to the right occurred across the entire dose-effect curve, including the lowest dose that would maintain self-administration (Emmett-Oglesby and Lane, 1992). Because the lowest dose that maintained responding under pre-chronic conditions lost its efficacy as a reinforcer when tested in the post-chronic phase, those results are not compatible with the hypothesis that tolerance occurred to the disruptive effects of cocaine. Instead, they are compatible with the interpretation that tolerance occurs to the reinforcing effects of cocaine. The present observation of tolerance in the PR procedure also suggests that tolerance occurs to the reinforcing efficacy of cocaine. This conclusion is tempered by the observation that tolerance did not occur to the same extent in all subjects and, apparently, did not occur at all in three subjects. We can not specify the variables that predict which subjects will become tolerant and which will retain normal sensitivity to the drug, but variables such as different baseline dose-effect functions or different body weights do not account for the differences.

The failure of some of the subjects to show tolerance is consistent with other findings that chronic administration of CNS stimulants does not always produce tolerance; indeed, depending on the dosing regimen and the task studied, chronic administration of cocaine or amphetamines may produce either tolerance, sensitization, or have no effect on behavior. Variability in results across studies has frequently been attributed to an interaction between drug effects and environmental factors. That is, tolerance (or sensitization) is thought to be under the control of specific environmental stimuli that are associated with previous drug

injections (for a review of the role of such stimuli in tolerance and sensitization, see Wolgin, 1989; Siegel, 1989; Stewart and Badiani, 1993). Because subjects in the present study received chronic cocaine while in chambers similar to those in which they self-administered the drug (except levers were removed), it may be the case that environmental stimuli controlled the expression of tolerance. However, a similar degree of tolerance to the reinforcing effects of cocaine develops when subjects are injected with high doses of CNS stimulants in their home cages (e.g., Peltier et al., 1993), suggesting that environmental context is unlikely to account for differential development of tolerance. Rather, tolerance appears to occur solely as a function of chronic drug administration, per se.

There have been increasing numbers of reports that chronic administration of CNS stimulants produces sensitization rather than tolerance. Sensitization to the effects of CNS stimulants has been seen most frequently with measures of locomotor activity, where the locomotor activating effects become progressively greater when subjects are treated chronically with these drugs (for reviews, see Robinson and Berridge, 1993; Stewart and Badiani, 1993). Although sensitization has also been claimed for self-administration of CNS stimulants (Piazza et al., 1990; Horger et al., 1990; Schenk et al., 1991), those studies have only shown that during the acquisition phase of self-administration, and only when extremely low doses of CNS stimulants are made available for injection, subjects with a history of exposure to these drugs acquire the operant more rapidly. In contrast, when higher doses of drug are made available for injection, there is no significant effect of chronic pretreatment with CNS stimulants on the time to acquire self-administration behavior. Further, once self-administration has been established, groups do not differ in their dose-effect curves, regardless of chronic treatment history. Thus, the robust sensitization that is obtained in locomotor activity studies does not seem to hold for drug self-administration studies.

In trying to reconcile the results of sensitization studies with observations that tolerance can develop to subjective (Fischman et al., 1985); discriminative (Wood et al., 1986) and reinforcing effects of cocaine (Emmett-Oglesby and Lane, 1992), the variable of intermittent

versus continuous drug exposure has been suggested to play a critical role (Stewart and Badiani, 1993; Wise and Leeb, 1993). Although it may be the case that once every 24-hr administration is "intermittent" (a typical dosing regimen used in sensitization experiments), whereas once every 8-hr administration (the regimen of the present experiment) is "continuous", this hypothesis remains to be tested rigorously. Perhaps more importantly, no data are available to suggest that sensitization will be seen to the reinforcing effects of drugs once stable baseline behavior has been acquired. Consequently it is not surprising that the present study failed to obtain sensitization. In contrast to sensitization that occurs only during initial acquisition of CNS stimulant self-administration, when baseline behavior is well established, in either drug discrimination or self-administration paradigms, high-dose treatment with CNS stimulants produces tolerance to their effects.

REFERENCES

Cox BM (1990) Drug tolerance and physical dependence. In: Pratt WB, Taylor P (eds) Principles of drug action, the basis of pharmacology, 3rd edition. Churchill Livingstone, New York, pp 639-690

Depoortere RY, Li D-H, Lane JD, Emmett-Oglesby MW (1993) Parameters of self-administration of cocaine in rats under a progressive-ratio schedule. Pharmacol Biochem Behav 45: 539-548

Emmett-Oglesby MW, Peltier RL, Depoortere RY, Pickering CL, Hooper ML, Gong YH, Lane JD (1993) Tolerance to self-administration of cocaine in rats: time course and dose-response determination using a multi-dose method. Drug Alcohol Depend 32: 247-256

Emmett-Oglesby MW, Lane JD (1992) Tolerance to the reinforcing effects of cocaine. Behav Pharmacol 3: 193-200

Fischmen MW, Schuster CR, Javaid J, Hatano Y, Davis J (1985) Acute tolerance development to the cardiovascular and subjective effects of cocaine. J Pharmacol Exp Ther 235: 677-682

Griffiths RR, Bradford LD, Brady JV (1979) Progressive ratio and fixed ratio schedules of cocaine-maintained responding in baboons. Psychopharmacology 65: 125-136

Hodos W (1961) Progressive ratio as a measure of reward strength. Science 134: 943-944

Horger BA, Shelton K, Schenk S (1990) Preexposure sensitizes rats to the rewarding effects of cocaine. Pharmacol Biochem Behav 37: 707-711

Hubner CB, Moreton JE (1991) Effects of selective D1 and D2 dopamine antagonists on cocaine self-administration in the rat. Psychopharmacology 105: 151-156

Iwamoto ME, Martin WA (1988) A critique of drug self-administration as a method for predicting abuse potential of drugs. In: Harris LS (ed). Problems of drug dependence. Proceedings of the committee on problems of drug dependence. US Government Printing Office, Washington DC, pp 457-465

Johanson CE, Schuster CR (1981) Animal models of drug self-administration. In: Advances in substance abuse. Behavioral and biological research. Vol II. ed by Mello NK. JAI Press, Greenwich. Conn, pp219-297

Katz JL (1989) Drugs as reinforcers: pharmacological and behavioural factors. In: Liebman JM, Cooper SJ (eds), The neuropharmacological basis of reward. Oxford University Press, Oxford, UK, pp 164-213

Katz JL (1990) Models of relative reinforcing efficacy of drugs and their predictive utility. Behav Pharmacol 1: 283-301

Katz JL, Griffiths JW, Sharpe LG, De Souza EB Witkin JM (1993) Cocaine tolerance and cross-tolerance. J Pharmacol Exp Ther 264: 183-192

McKenna M, Ho BT (1977) Induced tolerance to the discriminative stimulus properties of cocaine. Pharmacol Biochem Behav 47: 153-155

Meisch RA, Lemaire GA (1993) Drug self-administration. In: van Haaren F (ed). Methods in Behavioral Pharmacology. Elsevier Science Publishers, NY, pp 257-300

Misra AL (1976) Cocaine: Chemical, Biological, Clinical, Social and Treatment Aspects. In: Mule, SJ (ed), CRC Press, Cleveland, pp 73-90

Peltier RL, Emmett-Oglesby MW, Lane JD (1993) Cross-tolerance between CNS stimulants in a self-administration paradigm in rats. Soc Neurosci Abstr 19 (3): 761.5

Piazza PV, Deminiere JM, LeMoal M , Simon H (1990) Stress- and pharmacologically-induced behavioral sensitization increases vulnerability to acquisition of amphetamine self-administration. Brain Research, 514: 22-26

Risner ME, Silcox DL (1981) Psychostimulant self-administration by beagle dogs in a progressive-ratio paradigm. Psychopharmacology 75: 25-30

Robinson TE, Berridge KC (1993) The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res Rev 18: 247-291

Roberts DCS, Richardson NR (1992) Self-administration of psychomotor stimulants using progressive ratio schedules of reinforcement. In: Boulton AA, Baker G B, Wu PH (eds)

(Neuromethods series: vol 24) Animal models of drug addiction, Humana Press, Totowa NJ, pp 233-269

Roberts DCS, Loh EA, Vickers G (1989) Self-administration of cocaine on a progressive ratio schedule in rats: dose-response relationship and effect of haloperidol pretreatment. Psychopharmacology 97: 535-538

Roberts DCS, Corcoran ME, Fibiger HC (1977) On the role of ascending catecholamine systems in intravenous self-administration of cocaine. Pharmacol Biochem Behav 6: 615-620

SAS System for personal computers, Release 6.03 (1987) SAS Institute Inc., SAS Circle, Cary, NC 27512

Schenk S, Snow S, Horger B (1991) Pre-exposure to amphetamine but not nicotine sensitizes rats to the motor activating effect of cocaine. Psychopharmacology 103: 62-66

Siegel S (1989) Pharmacological conditioning and drug effects. In: Goudie AJ, Emmett-Oglesby MW (eds) Psychoactive drugs: tolerance and sensitization. Humana Press, Totowa NJ, pp 115-180

Stewart J, Badiani A (1993) Tolerance and sensitization to the behavioral effects of drugs. Behav Pharmacol 4(4): 289-312

Wilson MC, Schuster CR (1973) Cholinergic influence on intravenous cocaine self-administration by rhesus monkeys. Pharmacol Biochem Behav 1: 643-649

Wise RA, Leeb K (1993) Psychomotor-stimulant sensitization: a unitary phenomenon? Behav Pharmacol 4(4): 339-349

Wise RA, Yokel RA, Hansson P and Gerber GJ (1977) Concurrent intracranial self-stimulation and amphetamine self-administration in rats. Pharmacol Biochem Behav 7: 459-461

Wolgin DL, Kinney GG (1989) The role of instrumental learning in behavioral tolerance to drugs. In: Goudie AJ, Emmett-Oglesby MW (eds) Psychoactive drugs: tolerance and sensitization. Humana Press, Totowa NJ, pp 17-114

Wood DM, Lal H, Emmett-Oglesby MW (1984) Acquisition and recovery of tolerance to the discriminative stimulus properties of cocaine. Neuropharmacology 23: 1419-1423

Wood DM, Emmett-Oglesby MW (1986) Characteristics of tolerance, recovery from tolerance, and cross-tolerance to cocaine used as a discriminative stimulus. J Pharmacol Exp Ther 237: 120-125

Wood DM, Emmett-Oglesby (1988) Substitution and cross-tolerance profiles of anorectic drugs in rats trained to detect the discriminative stimulus properties of cocaine. Psychopharmacology 95: 364-368

Wood DM, Emmett-Oglesby MW (1989) Mediation in the nucleus accumbens of the discriminative stimulus produced by cocaine. Pharmacol Biochem Behav 33: 453-457

Yanagita T (1973) An experimental framework for evaluation of dependence liability of various types of drugs in Monkeys. Bulletin on Narcotics 25: 57- 64

Chapter 4

If the reinforcing effect of cocaine is mediated by a common dopaminergic reward pathway, cross-tolerance is expected when the reward system is repeatedly exposed to a dopamine agonist, e.g., amphetamine. The experiments in this chapter were performed to determine if the chronic administration of indirect DA agonists, amphetamines, will result in cross-tolerance to the reinforcing effect of cocaine. The hypothesis was that chronic administration of *d*-amphetamine (either 3.2 mg/kg/8hr or 3.2 mg/kg/12 hr) and methamphetamine (3.2 mg/kg/12 hr) will result in a rightward shift of the dose-response curve of the breaking points for cocaine self-administration. This work was done as a portion of a larger study in which FR2 and PR data were compared for their ability to detect cross-tolerance to cocaine.

The original version of this manuscript was submitted and will be published as

Peltier RL, Li D-H, Lytle D,Taylor CM, Emmett-Oglesby MW (1996) Chronic d-amphetamine or methamphetamine produces cross-tolerance to the discriminative and reinforcing stimulus effects of cocaine. J Pharmacol Exp Ther 27, 1996.

I initiated the amphetamine cross-tolerance study under the PR schedule. Later, based on my results, Dr. Emmett-Oglesby coordinated and the other authors performed the drug discrimination and self-administration (FR2) studies. Since the FR2 schedule is more rapid than PR, it allowed the experimenter to test more variations of treatment and more numerous results were obtained from studies under the FR2 schedule. As a result Rachel Peltier was the first author on the paper. To insure acceptance by the J Pharmacol Exp Ther, my previous advisor decided to combine the results from three individual subdivisions (FR2, PR, and S^D) to make the paper stronger. Independently, I finished the sections of the methods and results for data produced under the PR schedule. In the extracted manuscript, I included the experiments which I designed and performed for the original manuscript and re-organized these parts. Additional material was added to the original data as follows: 1) the results from the study of chronic treatment with *d*-AMPH at 8-hr intervals now includes a chronic saline-injected control; 2) analysis of the IS^RT of

cocaine self-administration under the PR schedule after chronic treatment with *d*-AMPH. Appropriate changes were made to the methods section and additions were made to the discussion as necessary to include the conclusions from this additional data. Major changes to text from previous version are indicated in italics.

Chronic *d*-amphetamine or methamphetamine produces cross-tolerance to the reinforcing effects of cocaine under the progressive-ratio schedule.[1]

Li, D-H. and Emmett-Oglesby, M.W.

Department of Pharmacology

University of North Texas HSC at Fort Worth

Fort Worth, TX 76107-2699 USA

Running Title: Cross-Tolerance to Cocaine.

1 Supported by grants RO1-4137 and Texas Advanced Technology Award 3711

2 This project is taken from a dissertation submitted to the University of North Texas Health Science Center at Fort Worth in partial fulfillment of the requirements for the degree Doctor of Philosophy

Abbreviations:

d-amphetamine (*d*-A)

Methamphetamine (METH)

Discriminative stimulus (S^D)

Reinforcing stimulus (S^R)

Inter-reinforcer time (IS^RT)

ABSTRACT

These experiments tested the hypothesis that chronic administration of *d*-amphetamine (*d*-A) or methamphetamine (METH) would produce cross-tolerance to the reinforcing (S^R) effects of cocaine. Fifteen rats were implanted with indwelling jugular catheters and trained to self-administer cocaine under a progressive-ratio (PR) schedule of reinforcement. Chronic administration of *d*-AMPH(3.2 mg/kg/8-hr and 3.2 mg/kg/12-hr for 7 days) and METH (3.2 mg/kg/12-hr for 7 days) resulted in cross-tolerance to the self-administration of cocaine under this PR schedule. The data obtained from these experiments demonstrate that chronic treatment with CNS stimulants of the amphetamine type (*d-AMPH* or METH) produces cross-tolerance to reinforcing effects of cocaine.

Keywords: cocaine, *d*-amphetamine, methamphetamine, progressive-ratio schedule, rats, self-administration, cross-tolerance, tolerance

INTRODUCTION

Tolerance develops to CNS stimulants in both drug discrimination (Barrett and Leith, 1981; Steigerwald *et al.*, 1994; Wood and Emmett-Oglesby, 1986, 1987, 1988, 1989; Young and Sannerud, 1989) and drug self-administration paradigms in rats (Emmett-Oglesby and Lane, 1992; Emmett-Oglesby *et al.*, 1993; Li et al., 1994; McCown and Barrett, 1980). For example, Wood and Emmett-Oglesby (1986, 1988) showed that in rats trained to discriminate cocaine, 10 mg/kg, from saline, a two-fold shift to the right of the cocaine dose-effect curve occurred after 7 days of chronic treatment with cocaine, either 10 or 20 mg/kg/8 hr. Using *d*-AMPH as a training drug, Barrett and Leith (1981) showed that rats treated chronically with a total of 78 mg/kg of *d*-AMPH over three days were tolerant to the training dose of *d*-A. Similarly, Steigerwald *et al.* (1994) showed that rats trained to discriminate *d*-A, 0.80 mg/kg, from saline demonstrated a three-fold shift to the right of the *d*-AMPH dose-effect curve after chronic treatment with *d*-A, 3.2 mg/kg/12 hr for 7 days, and this tolerance increased to a four-fold shift after 14 days of treatment.

Tolerance to the reinforcing (S^R) effects of cocaine has also been demonstrated in rats trained to self-administer cocaine under both low value FR (Emmett-Oglesby and Lane, 1992; Emmett-Oglesby *et al.*, 1993) and PR schedules (Li *et al.*, 1994). In parallel to the data obtained in the cocaine discrimination studies, rats trained to self-administer 15 injections of 1.0 mg/kg/inj of cocaine under an FR2 schedule of reinforcement demonstrated a two-fold shift to the right of the cocaine self-administration dose-response curve after receiving intravenous cocaine (20 mg/kg/8 hr for 7 days) (Emmett-Oglesby and Lane, 1992; Emmett-Oglesby *et al.*, 1993). Similarly, rats trained to self-administer cocaine (0.9 mg/kg/injection) under a PR schedule, where an increasing number of responses is required to complete the ratio for each subsequent reinforcer, showed approximately two-fold tolerance to the reinforcing effects of cocaine after receiving intravenous cocaine (20 infusions of 0.9 mg/kg/8 hr for 7 days; Li *et al.*, 1994). In addition, tolerance to the S^R effects of *d*-AMPH has also been demonstrated in rats trained to self-administer *d*-AMPH under an FR1 schedule of reinforcement (McCown and Barrett, 1980). In that study,

rats were trained to self-administer *d*-A, either 0.125 or 0.25 mg/kg/inj. Following chronic treatment with 78 mg/kg of *d*-AMPH over three days, all subjects showed an increase in the amount of *d*-AMPH self-administered by at least 45% over baseline levels.

In summary, this laboratory has previously demonstrated that chronic high doses of cocaine produce tolerance to the reinforcing S^R effects of cocaine. In drug discriminative study, chronic administration of *d*-AMPH was demonstrated to produce cross-tolerance to the S^D effects of cocaine; similarly, chronic administration of cocaine produces cross-tolerance to the S^D effects of a wide variety of amphetamine-type compounds (Wood and Emmett-Oglesby, 1988). The present experiment was designed to extend these results by comparing the cross-tolerance effects of *d*-AMPH and METH in rats trained to self-administer cocaine under a PR schedule of reinforcement. No previous studies have investigated cross-tolerance profiles for CNS stimulants under a PR schedule in a self-administration paradigm. The study on this project will supply some evidence to support the hypothesis that there is a common mechanism in mediate tolerance and dependence of psychostimulants which is related with the reinforcing effect of the drug.

METHODS

Subjects Fifteen male Fisher F-344 rats were housed singly and maintained at 270 $\pm$ 10 g by restricting their access to food. For all subjects, water was available *ad libitum* outside of training and testing periods. Subjects were housed in a temperature-controlled room under a 12-hour on/off light cycle.

Training For a detailed description of the apparatus as well as the training and testing procedures see Emmett-Oglesby *et al.* (1993). Briefly, rats were implanted with an indwelling catheter inserted into the right external jugular, the free end of which was fixed to the skull. After five days of recovery, subjects were given the opportunity to self-administer cocaine on an FR1

schedule with a maximum of 25 cocaine infusions. At the beginning of each training session, a priming injection of 0.3 mg of cocaine was given, with each subsequent injection consisting of 0.25 mg of cocaine in 0.1 ml. Once rats self-administered all 25 infusions within 3 hr during two consecutive training sessions, they were then switched to PR training schedule. Before each self-administration session, patency of the catheter was assessed by drawing blood into the catheter and then by flushing 0.1 ml of heparinized saline back into the catheter.

Under the PR schedule, to obtain each subsequent injection of the training dose (0.25 mg/inj), rats had to complete each of the following ascending number of bar presses to receive drug injection (reinforcer) in the following sequence: 3, 6, 10, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 603, 737, 901, 1102, and 1347. There was a 1 hr time limit to obtain each reinforcer, and failure to do so terminated the session. The last reinforcer that was obtained for each session was termed the breaking point. Rats were tested once they met a criterion of the total number of reinforcers obtained in one session (breaking point) not varying by more than three reinforcers across seven consecutive training sessions.

PR self-administration testing After the stability criterion was met, rats (n=15) were tested by substituting different doses of cocaine (0.028 and 0.083 mg/inj) for the training dose (0.25 mg/inj) as well as the training dose itself (0.25 mg/inj). These substitutions took place several times until reproducible dose-response data were obtained. Subsequently, for the pre-chronic phase of these experiments these same doses of cocaine were tested, one dose per day, on the three days immediately prior to the start of the chronic injections. The order of dose presentation was random across the group; however, the same order was used for each rat throughout all tests. In all tests, subjects were first infused with the dose of cocaine to be tested on that day. Once the pre-chronic dose-response curves were obtained, rats were randomly assigned to one of two test groups; *d*-AMPH(n=7) and METH (n=8). The post-chronic dose-response curve was obtained by making the same dose regimen within three days immediately following the chronic injections, with the first dose occurring 24 hr after the last chronic injection of *d*-AMPH or METH.

After the post-chronic dose-response curve was obtained, the rats were allowed one week without testing or training, after which time, a dose-response curve was reobtained (this was termed the recovery curve).

Chronic Treatment with d-AMPH or METH After baseline dose-response data were obtained in the cocaine self-administration experiment, all training and testing were suspended. During this time, animals received either saline (every 8 hr) or d-AMPH (3.2 mg/kg, s.c., every 8 hr or 12 hr), or METH (3.2 mg/kg, s.c., every 12 hr) for seven days. Tests were limited to this dose for two reasons: it was the only effective dose in the previous paradigms in either discriminative and FR2 testing (Peltier et al, in press), and the duration required to train and stabilize dose-response testing in the PR paradigm was lengthy enough that it was unlikely that subjects would maintain viable catheters if more than one dose was tested chronically. Twenty-four hours following the last chronic injection, cocaine dose-response curves were reobtained. Following the post-chronic dose-response curve tests, rats were allowed one week without testing or training to recover from the chronic experiment, after which time, another dose-response curve was obtained. This curve was termed the recovery curve. Rats were then trained until their baseline breaking points were within 20% of pre-chronic values, which took approximately one week. When the baseline breaking points were stable, they were then included in another chronic regimen using a different dose of *d*-AMPH or METH. Subjects were assigned to receive either *d*-AMPH or METH; within this assignment, all subjects received all doses in a random block design. Thus, at each round of chronic administration, all three doses were assigned across subjects, and across repetitions of the experiment, these doses were shifted such that all subjects received all the treatments.

Drugs. Cocaine HCl (National Institute of Drug Abuse, Research Triangle Park, NC) was dissolved in 0.9% saline for drug discrimination experiments and injected i.p. For self-administration experiments, cocaine HCl was dissolved in heparinized saline (0.5 U/ml) and filtered through 0.22 µm filters (Millipore, Bedford, MA) into sterile 10 ml syringes immediately

before use. *d*-AMPH sulfate (Sigma, St. Louis, MO) and METH HCl (Sigma, St. Louis, MO) were dissolved in 0.9% saline and injected s.c.

Data Analysis

The number of reinforcers obtained was used as the dependent measure, which is termed the breaking point. This breaking point measure was used rather than the final ratio completed or the total number of responses emitted because these latter variables are not amenable to parametric analysis (for a discussion of this problem in analysis of response and reinforcer data from PR procedures, see Depoortere *et. al.*, 1993; and Roberts and Richardson, 1992). Breaking points were analyzed using a two-way repeated measures ANOVA with treatment condition and dose of cocaine as within subject factors. SYSTAT software (Wilkinson *et. al*, 1992) was used for data analysis.

Effect of chronic treatment on the Inter-Reinforcer Time (IS^RTs) between each reinforcer was introduced as another dependent measure, which was used to determine the response rate related to response pattern. The first step to calculate this variable was to exclude each subjects' first and last IS^RT. These two IS^RTs were excluded because they were not representative of the general pattern of IS^RTs: the first IS^RT was variable because some rats took much longer than others to start self-administering following the infusion of the priming dose; the last IS^RT also showed greater variability because at this final ratio value, some rats occasionally displayed a pattern of lever pressing in which responding was interrupted by periods of non-responding. The second step to analyze the IS^RT s was to logit transformation the data according to the formula 2 + log (IS^RT). The purpose of this treatment is to minimize the error introduced by dramatically increased number of responding as the number of reinforcers increases, which will cause the value of the mean IS^RT less dependent upon lower ratio component than the higher one. After this treatment, each component (from 1st to last reinforcer) will have similar contribution to the mean IS^RTs.

After chronic treatment, the breaking points of some animals at the lowest dose of cocaine (0.028 mg/kg/inj) were very low (sometimes, the animal did not self-administer at this dose), and therefore, the sample size of the experiment will be significantly reduced. To avoid the data analysis being biased by the low dose cocaine self-administration, data analysis of IS^RT was limited to the results obtained using on 0.083 and 0.25 mg/inj cocaine self-administration dosage. Based on the transformed data, IS^RTs of different pre-treatments on either 0.083 or 0.25 mg/inj cocaine were further subjected to fully factorial ANOVA (MANOVA) for repeated measure with the value of IS^RT as dependent variable and pretreatments as factors.

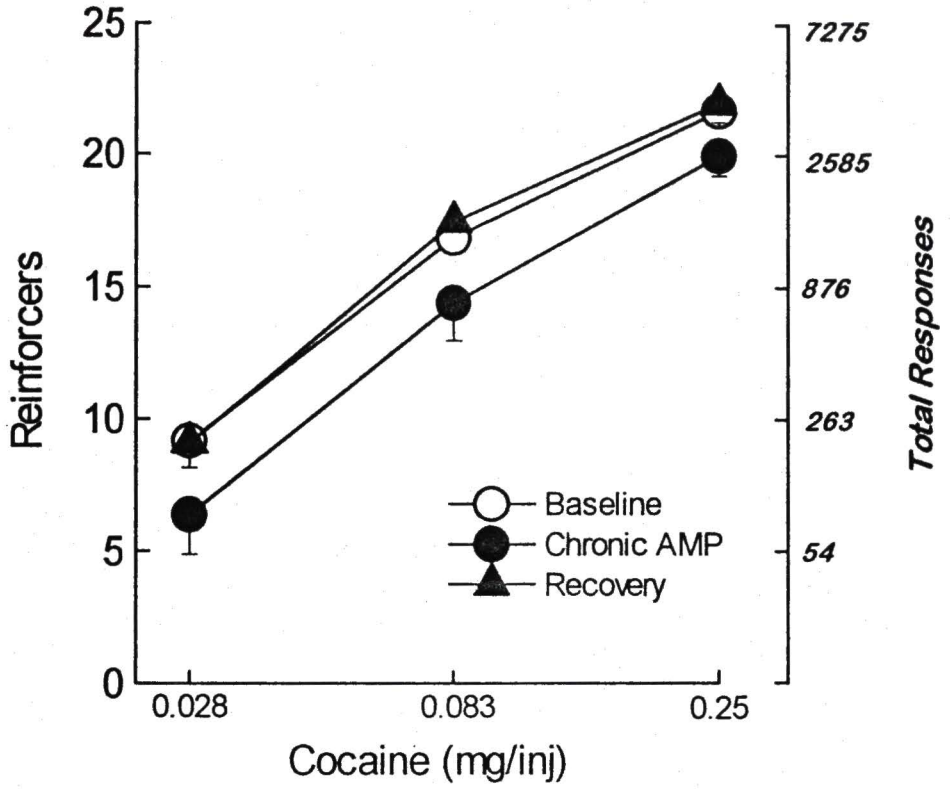
All MANOVA were performed with SYSTAT software (Wilkinson, 1990), and all ANOVAs were performed with Statistic Analysis Systems (SAS, 1987) software.

RESULTS

Effect of chronic d-AMPH (3.2 mg/kg/8hr for 7 days) and saline on cocaine self-administration under a PR paradigm. Under baseline conditions, increasing the dose of cocaine resulted in an orderly increase in the breaking point ([$F_{2,30}$ = 67.86; p< 0.0001]; (Figure 1). Chronic treatment with d-AMPH(3.2 mg/kg/8 hr for 7 days, s.c.) shifted the dose-response curve for breaking points significantly to the right ($F_{1,30}$ = 9.32; p< 0.05; Fig 1 top) compared with the chronic treatment with saline ($F_{1,30}$ = 1.00, p> 0.1; Fig 1 bottom). In addition, following one week of recovery from chronic d-AMPH (or saline injections), the dose-response curve for cocaine self-administration spontaneously returned to pre-chronic levels ($F_{1,27}$ = 0.09, p>0.5 for AMPH; $F_{1,27}$ = 1.29, p>0.1 for saline; Fig 1).

Effect of chronic d-AMPH(3.2 mg/kg/12hr for 7 days) on cocaine self-administration under a PR paradigm. Under baseline conditions, increasing the dose of cocaine resulted in an orderly increase in the breaking point ([$F_{2,18}$ = 15.6; p< 0.0001]; (Fig 2 top). Chronic treatment with *d-*

Fig. 1. Effect of chronic *d-AMPH* (3.2 mg/kg/8hr) or saline on cocaine self-administration under a PR paradigm. Abscissa: Dose of cocaine made available for self-administration. Ordinate: the breaking point. Open circles (O) indicate self-administration of cocaine prior to chronic treatment. Inverted triangles (▼) indicate chronic treatment with *d-AMPH* (top panel; 3.2 mg/kg/8hr/7 days) or saline (bottom panel); closed circles (●) indicate dose-response data obtained 10 days following the last chronic injection of *d-AMPH* or saline. Data are shown as mean ± S.E.M. N=11


25
20
15
10
5
0
Reinforcers
7275
2585
876
263
54
Total Responses
Baseline
Chronic AMP
Recovery
0.028
0.083
0.25
Cocaine (mg/inj)

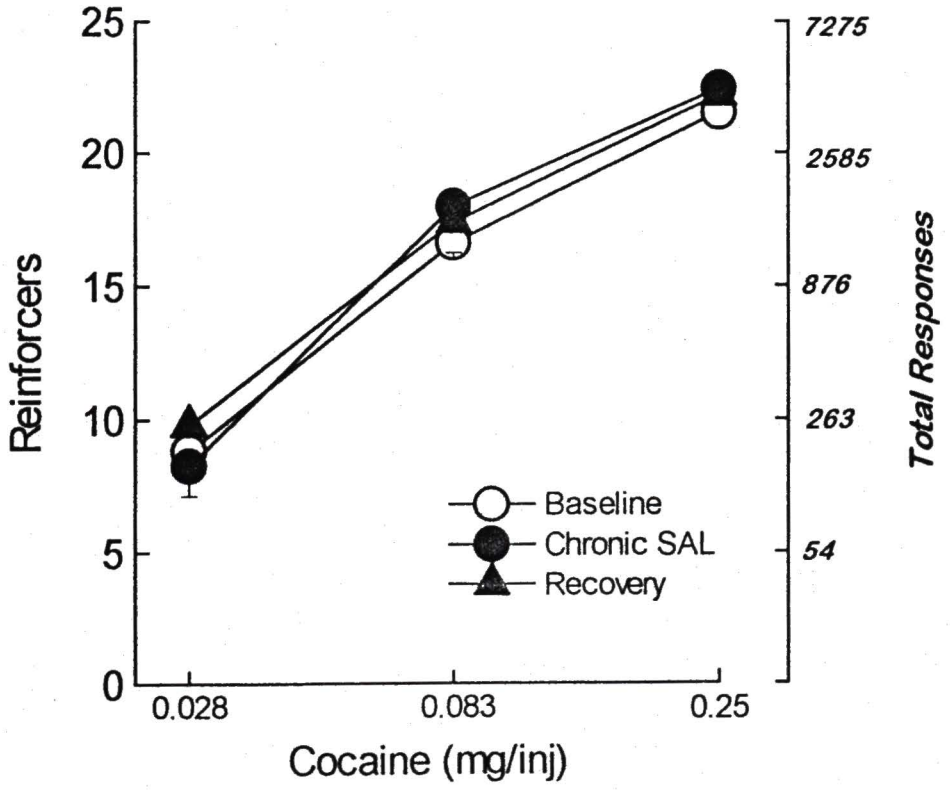
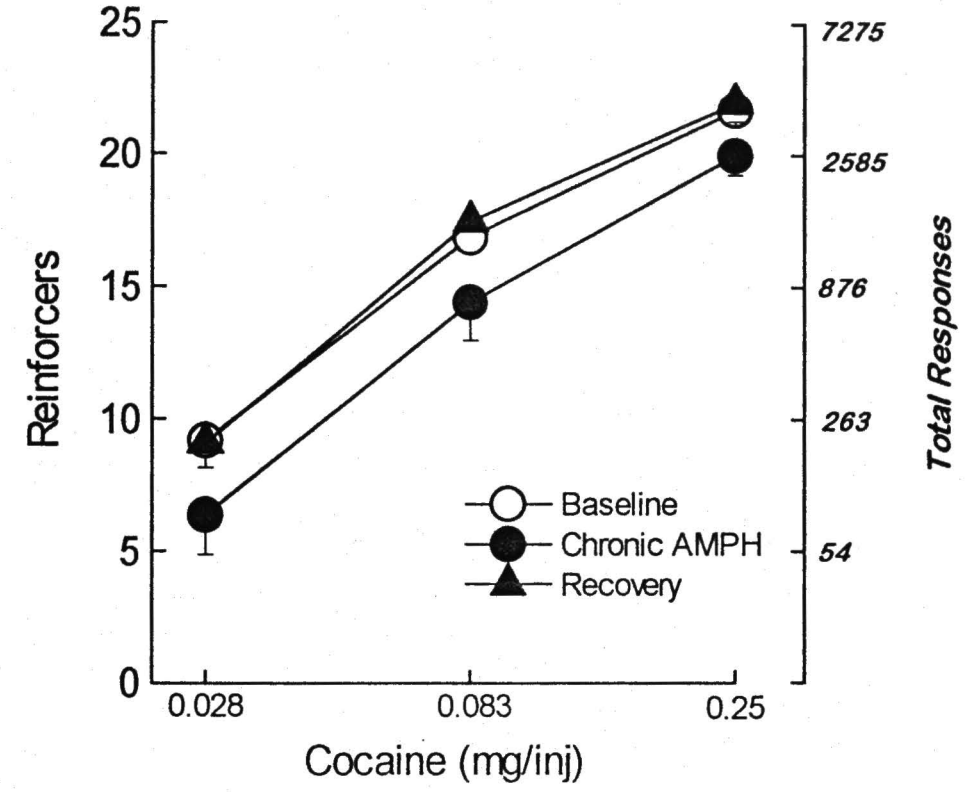
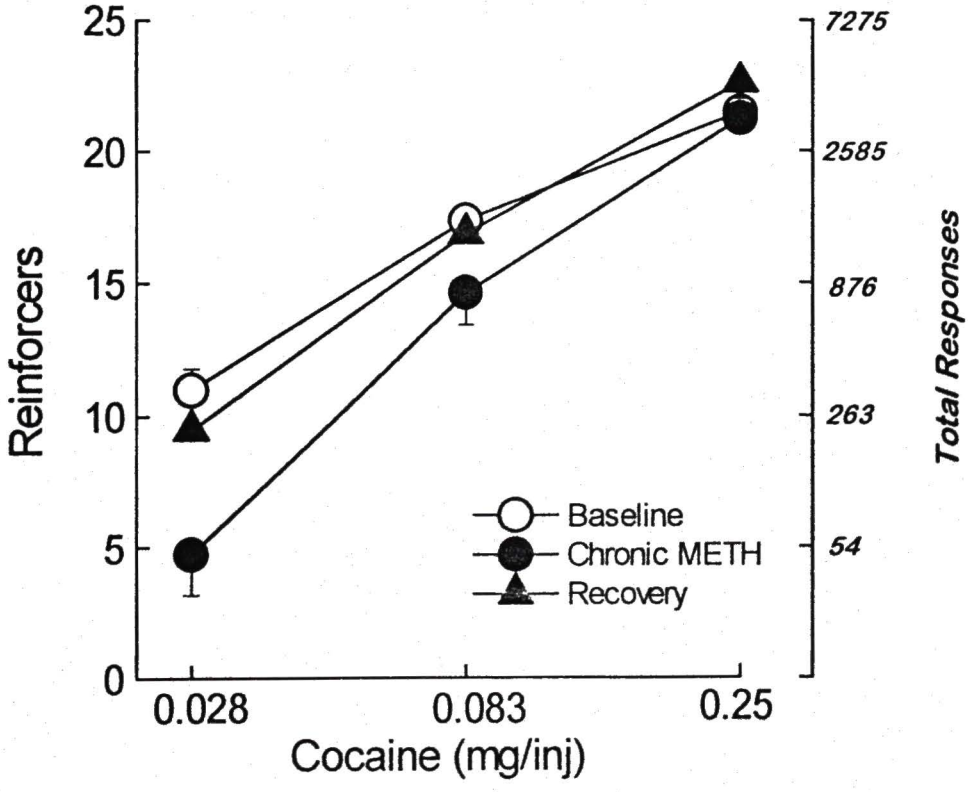

25
20
15
10
5
0
Reinforcers
7275
2585
876
263
54
Total Responses
Baseline
Chronic SAL
Recovery
0.028
0.083
0.25
Cocaine (mg/inj)

Fig. 2. Effect of chronic *d-AMPH* or METH on cocaine self-administration under a PR paradigm. Abscissa: Dose of cocaine made available for self-administration. Ordinate: breaking point. Open circles (O) indicate self-administration of cocaine prior to chronic treatment. Inverted triangles (▼) indicate chronic treatment with *d-AMPH* (top panel) or METH (bottom panel) (3.2 mg/kg/12 hr/7 days); closed circles (●) indicate dose-response data obtained 10 days following the last chronic injection of *d-AMPH* or METH. Data are shown as mean ± S.E.M. N=7 (top); N=11 (bottom).


25
20
15
10
5
0
Reinforcers
7275
2585
876
263
54
Total Responses
Baseline
Chronic AMPH
Recovery
0.028
0.083
0.25
Cocaine (mg/inj)


25
20
15
10
5
0
Reinforcers
7275
2585
876
263
54
Total Responses
Baseline
Chronic METH
Recovery
0.028
0.083
0.25
Cocaine (mg/inj)

AMPH (3.2 mg/kg/12 hr for 7 days, s.c.) shifted the dose-response curve for breaking points significantly to the right ($F_{1,18}$ = 24.0; p< 0.001). In addition, following one week of recovery from chronic *d*-AMPH injections, the dose-response curve for cocaine self-administration spontaneously returned to pre-chronic levels ($F_{1,18}$ =2.19, p>0.1; Fig 2 top).

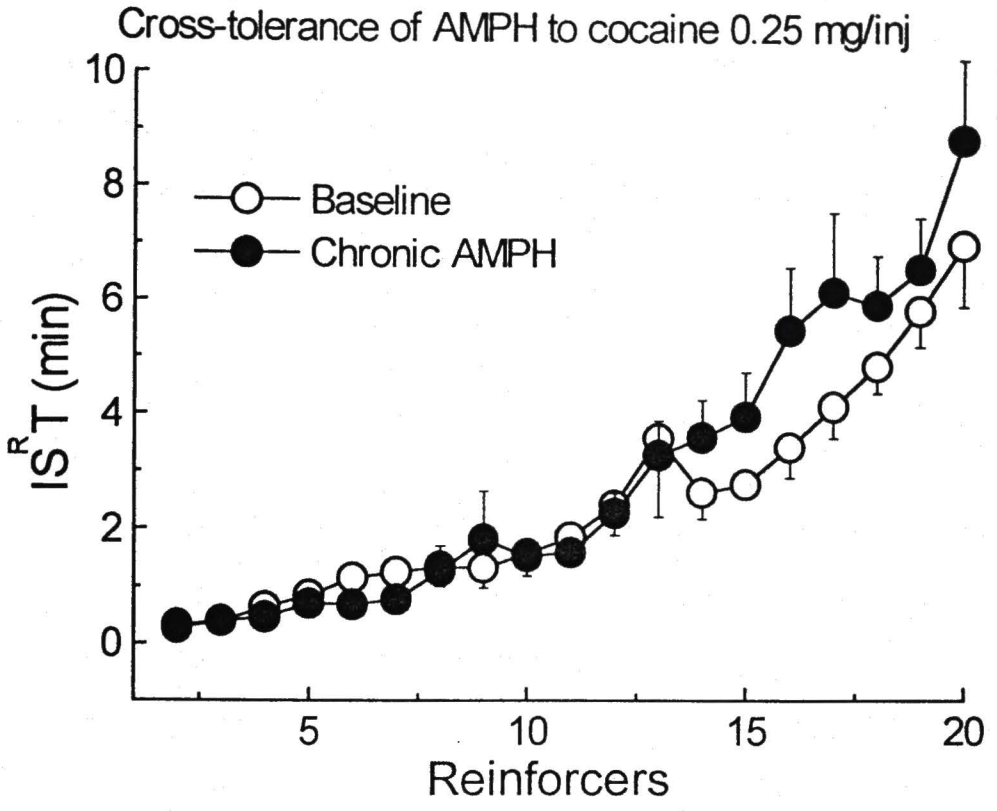
Effect of chronic METH (3.2 mg/kg/12hr for 7 days) on cocaine self-administration under a PR paradigm Chronic METH treatment (3.2 mg/kg/12 hr for 7 days, s.c.; Figure 2 bottom) also shifted the dose-response curve for cocaine self-administration significantly to the right ($F_{1,30}$ = 25.1; p< 0.0001). Similar to the *d*-AMPH group, one week of recovery from chronic METH injections produced a spontaneous return to pre-chronic levels of cocaine self-administration ($F_{1,30}$ = 2.64; p> 0.1; Fig 2 bottom).

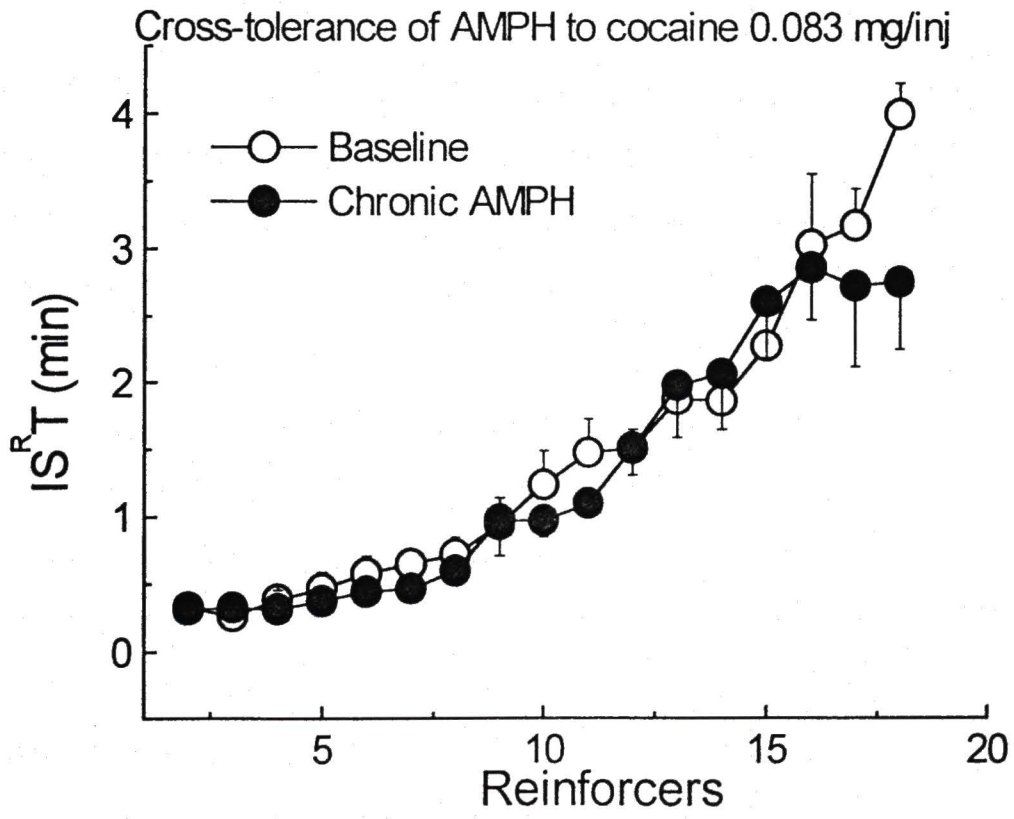
Effect of chronic d-AMPH (3.2 mg/kg/8hr for 7 days) and saline on IS^RT of cocaine self-administration under a PR paradigm. Chronic treatment with d-AMPH (3.2 mg/kg/8 hr for 7 days, s.c.) failed to change the IS^RT of either cocaine tested at 0.25 mg/inj ($F_{1,139}$ = 0.29; p> 0.5; Fig 3 top) or cocaine tested at 0.083 mg/inj ($F_{1,87}$ = 1.48; p> 0.1; Fig 3 bottom), although there is a significant difference in IS^RT between cocaine 0.25 and 0.083 mg/inj groups ($F_{3, 261}$ = 3.83; p<0.01)

DISCUSSION

Based on the results of the FR2 self-administration experiments (Peltier et al, in press), a high dose of *d*-AMPH (3.2 mg/kg) and METH (3.2 mg/kg) were examined using a PR self-administration paradigm. Chronic treatment with this dose produced cross-tolerance to cocaine under a PR schedule of cocaine self-administration. Chronic treatment of *d*-AMPH caused cross-tolerance to cocaine at both time intervals (either 8 hrs or 12 hrs), which indicated that cross-tolerance to cocaine can be introduced even under the less frequency of the administration of the *d*-AMPH. Chronic treatment with either *d*-AMPH or METH resulted in an approximate

Fig. 3. Effect of *d-AMPH* on the IS^RTs of cocaine self-administration under a PR paradigm. Abscissa: number of reinforcer obtained during the self-administration session. Ordinate: IS^RT. Open circles (O) indicate self-administration of cocaine prior to chronic treatment. Inverted triangles (▼) indicate cocaine 0.25 mg/inj (top panel) or 0.083 mg/inj (bottom panel) self-administered; Data are shown as mean ± S.E.M.


Cross-tolerance of AMPH to cocaine 0.25 mg/inj
Baseline
Chronic AMPH
IS^R T (min)
Reinforcers
0
2
4
6
8
10
5
10
15
20


Cross-tolerance of AMPH to cocaine 0.083 mg/inj
Baseline
Chronic AMPH
IS^R T (min)
Reinforcers
0
1
2
3
4
5
10
15
20

two-fold shift of the cocaine dose-response curve to the right. Although there is a significant decrease in breaking points, it is only a decrease of 1 to 6 reinforcers. However, the decrease in the real number of lever presses ranged from approximately 300 to 800 responses. These data were similar to data obtained when rats received intravenous cocaine (20 infusions of 0.9 mg/kg/8 hr for 7 days) (Li *et al.*, 1994). In that study, chronic treatment with cocaine produced tolerance to the reinforcing effect of cocaine as demonstrated by a two-fold shift to the right of the cocaine self-administration dose-response curve.

Although chronic treatment with either *d*-AMPH or METH produced an increase in the number of responses in the FR2 paradigm (Peltier *et al*, in press) and a decrease in the number of responses in the PR paradigm, both results are indicative of tolerance. If a rat is tolerant to the S^R effects of cocaine in an FR2 or PR paradigm, a given dose of cocaine will now be self-administered as if it were a lower dose. In these experiments, doses of cocaine were used that were on the descending limb of the dose-effect curve in the FR2 paradigm. On the descending limb of the dose-response curve, there is an inverse relationship between the dose of cocaine available for self-administration and the number of reinforcers obtained. In other words, as the dose of cocaine decreases, rats will respond faster. In contrast, in the PR paradigm there is a direct relationship between the dose of cocaine available for self-administration and the number of responses that a subject emits. If a rat is tolerant to the S^R effects of cocaine, then fewer reinforcers should be obtained under the PR schedule.

When we analyzed the inter-reinforcer time (IS^RT) under the PR schedule, we observed that there was no significant reductions in the IS^RTs at either dose of cocaine 0.25 and 0.083 mg/inj compared with pre-chronic data.. This result was different from our results for IS^RT obtained from FR2 schedule (Peltier et al, in press). The possible reasons for this discrepancy may be due to the difference in drug access between FR2 and PR schedule. Under the FR2 schedule, an animal is trained to emit only 2 responses to obtain each reinforcer, with a maximum of fifteen reinforcers per session. Therefore, the time an animal spends in lever pressing will be directly related

to drug concentration in the blood of animal. If the response emitted by the animal is too fast, then the subject will be intoxicated by the overdose of cocaine. It is hypothesized that to avoid the negative effects of an overdose of cocaine, the animal maintains very constant rates of drug intake. Since under a PR schedule, the animal was trained to emit maximum responding to obtain more reinforcers, the time pause to start the lever pressing is less important under this schedule. It is hypothesized that once the animal knows the drug is available, he begins to press the lever for more reinforcers until the rewarding effect of the drug has disappeared. Therefore, under the PR schedule, the effort the animal has to emit, rather than the time to start to work, will be a critical factor which influences the results of the animal. Based on this suggestion, it is hypothesized that the restriction of the condition rather than the supply of the reinforcement that controlled the time an animal spends on lever pressing, resulting in differences in the IS^RT parameter between both the FR 2 and PR schedules.

The reinforcing effects of cocaine are mediated at least in part by mesolimbic dopamine systems (Koob and Bloom, 1988; Di Chiara, 1995; Wood and Emmett-Oglesby, 1989). Direct electrical stimulation of these dopamine neurons will serve as a reinforcer, and CNS stimulants are known to potentiate the reinforcing effects of electrical brain stimulation. Chronic treatment with d-AMPH (Leith and Barrett, 1981; Wise and Munn, 1985) or cocaine (Markou and Koob, 1991) produces an increase in brain self-stimulation reward thresholds. These data have been interpreted as providing evidence for cocaine-withdrawal-induced "anhedonia" (Markou and Koob, 1991). However, the data from self-stimulation experiments can also be interpreted as showing tolerance to the reinforcing effects of the stimulating current (larger current intensities are required to produce a reinforcing effect). The data from our experiments supported the hypothesis that the reinforcing effect of psychostimulants is mediated mainly through a common DA mechanism, since a cross-tolerance to cocaine was observed after chronically exposure a DA releaser (amphetamine) to the animal.

Tolerance in these experiments was seen as rightward shifts in dose-effect curves. This phenomenon has been seen in other tolerance experiments (e.g., Blasig *et al.*, 1979), where increasing opioid tolerance first resulted in a parallel shift to the right of the dose-response curve, and as even more tolerance developed, further rightward shifts were seen. This effect can be explained by phenomena such as receptor down-regulation or receptor desensitization (for a review see Cox, 1990). Whether such phenomena in the dopamine system account for the present observation is unknown, and biochemical studies using appropriate chronic dosing regimens are necessary to confirm or deny their role.

REFERENCES

Ambre, J.J., Belknap, S.M., Nelson, J., Ruo, T.I., Shin, S.G. and Atkinson A.J. Jr.: Acute tolerance to cocaine in humans. *Clin. Pharmacol. Ther.* 44: 1-8, 1988.

Barrett, R.J. and Leith, N.J.: Tolerance to the discriminative stimulus properties of *d*-amphetamine. *Neuropharmacol.* 20: 251-255, 1981.

Blasig, J. Meyer, G. Hollt, V., Hengstenberg, J., Dum J. and Herz, A: Non-competitive nature of the antagonistic mechanism responsible for the tolerance development to opiate-induced analgesia, *Neuropharmacol.* 18: 473-481, 1979).

Cox B.M.: Drug tolerance and physical dependence. In: Pratt, W.B. and Taylor, P. (eds.) *Principles of Drug Action. The Basis of Pharmacology*, pp. 639-690. Churchill Livingstone, NY, 1990.

Depoortere, R.Y., Li, D.-H., Lane J.D. and Emmett-Oglesby, M.W.: Parameters of self-administration of cocaine in rats under a progressive-ratio schedule. *Pharmacol. Biochem. Behav.* 45: 539-548, 1993.

Di Chiara, G.: The role of dopamine in drug abuse viewed from the perspective of its role in motivation. *Drug Alcohol Dep.* 38: 95-137, 1995.

Emmett-Oglesby, M.W.: Tolerance to the discriminative effects of ethanol. *Behav. Pharmacol.* 1: 667-670, 1990.

Emmett-Oglesby, M.W. and Lane, J.D.: Tolerance to the reinforcing effects of cocaine. *Behav. Pharmacol.* 3: 193-200, 1992.

Emmett-Oglesby, M.W., Peltier, R.L., Pickering, C.L., Hooper, M.L., Gong, Y.H. and Lane, J.D.: Tolerance to self-administration of cocaine in rats: time course and dose-response determination using a multi-dose method. *Drug Alcohol Dep.* 32: 247-256, 1993.

Fischman, M.W., Schuster, C.R., Javaid, J., Hatano, Y. and Davis, J.: Acute tolerance development to the cardiovascular and subjective effects of cocaine. *J. Pharmacol. Exp. Ther.* 235: 677-82, 1985.

Johanson, C-E., and Fischman, M.W.: The pharmacology of cocaine related to its abuse. *Pharmacol. Rev.* 89: 3-52, 1989.

Koob, G.F., Bloom, F.E.: Cellular and molecular mechanisms of drug dependence. *Science.* 242: 715-723, 1988.

Lane, J.D., Pickering, C.L., Hooper, M.L., Fagan, K., Tyers, M.B. and Emmett-Oglesby, M.W.: Failure of ondansetron to block the discriminative or reinforcing effects of cocaine in the rat. *Drug Alcohol Dep.* 30: 151-162, 1992.

Leith, N.J. and Barrett, R.J.: Self-stimulation and amphetamine: tolerance to d and l isomers and cross tolerance to cocaine and methylphenidate, *Psychopharmacology.* 74: 23-28, 1981.

Li, D.-H., Depoortere, R.Y. and Emmett-Oglesby, M.W.: Tolerance to the reinforcing effects of cocaine in a progressive ratio paradigm. *Psychopharmacology.* 116: 326-332, 1994.

Markou, A. and Koob, G.F.: Postcocaine anhedonia: An animal model of cocaine withdrawal. *Neuropsychopharmacol.* 4: 17-26, 1991.

McCown, T.J. and Barrett, R.J.: Development of tolerance to the rewarding effects of self-administered S(+)-amphetamine. *Pharmacol. Biochem. Behav.* 12: 137-141, 1980.

Nayak, P.K., Misra, A.L. and Mule, S.J.: Physiological disposition and biotransformation of [^{3}H] cocaine in acutely and chronically treated rats. *J. Pharmacol. Exp. Ther.* 196: 556-569, 1976.

Overton, D.A.: Application and limitations of the drug discrimination method for the study of drug abuse. In: Bozarth, M.A., (ed.) *Methods of Assessing the Reinforcing Properties of Abused Drugs*, pp. 291-340. Springer-Verlag, NY, 1987.

Peltier RL, Li D-H, Lytle D, Taylor CM, and Emmett-Oglesby MW (1996) Chronic *d*-amphetamine or methamphetamine produces cross-tolerance to the discriminative and reinforcing stimulus effects of cocaine. in press in J Pharmacol Exp Ther

Preston, K.L. and Bigelow, G.E.: Subjective and discriminative effects of drugs. *Behav. Pharmacol.* 2: 293-314, 1991.

Roberts, D.C.S. and Richardson, N.R.: Self-administration of psychomotor stimulants using progressive ratio schedules of reinforcement. In: Boulton AA, Baker GB, Wu PH (eds) (Neuromethods series: Vol 24) *Animal models of drug addiction*, Humana Press, Totowa NJ, pp 233-269, 1992.

Steigerwald, E.S., Anderson, D.W. and Young, A.M.: Tolerance to the discriminative stimulus effects of *d*-amphetamine. *Exp. Clin. Psychopharm.* 2: 13-24, 1994.

Wenger, G.R.: Cumulative dose-response curves in behavioral pharmacology. *Pharmacol. Biochem. Behav.* 13: 647-651, 1980

Wilkinson, L.; Hill, M., Welna, J.P. and Birkenbeuel, G.K.: SYSTAT for windows: Version for statistics, Version 5 Edition, Evanston, IL: SYSTAT, Inc. 1992.

Wise, R.A. and Munn, E.: Withdrawal from chronic amphetamine elevates baseline intracranial self-stimulation thresholds. *Psychopharmacology*. 117: 130-136, 1985.

Wood, D.M. and Emmett-Oglesby, M.W.: Characteristics of tolerance, recovery from tolerance, and cross-tolerance to cocaine used as a discriminative stimulus. *J. Pharmacol. Exp. Ther.* 237: 120-125, 1986.

Wood, D.M. and Emmett-Oglesby, M.W.: Role of dopaminergic mechanisms in the development of tolerance to the discriminative stimulus properties of cocaine. *Eur. J. Pharmacol.* 138: 155-157, 1987.

Wood, D.M. and Emmett-Oglesby, M.W.: Substitution and cross-tolerance profiles of anorectic drugs in rats trained to detect the discriminative stimulus properties of cocaine. *Psychopharmacology*. 95: 364-369, 1988.

Wood, D.M. and Emmett-Oglesby, M.W.: Mediation in the nucleus accumbens of the discriminative stimulus produced by cocaine. *Pharmacol. Biochem. Behav.* 33: 453-457, 1989.

Young, A.M. and Sannerud, C.A.: Tolerance to drug discriminative stimuli. In: *Psychoactive Drugs: Tolerance and Sensitization* (Eds A.J. Goudie and M.W. Emmett-Oglesby), pp. 221-270, Humana Press, Clifton, NJ, 1989.

The results from Chapter 1, 3 and 4 partially support the hypothesis that the reinforcing effects of cocaine are mediated by dopamine. Chronic treatment with cocaine was demonstrated to produce tolerance (Chapter 3), and chronic treatment with amphetamines produce cross-tolerance (Chapter 4) to the reinforcing effect of cocaine. The failure of opioids to potentiate the reinforcing effect of cocaine (Chapter 2) suggests that opioid and cocaine may not be synergistic at the dopaminergic site of reward, but morphine did prolong the IS^RT under the PR schedule suggesting that morphine could either prolong the reinforcing effect of cocaine or produce motor incoordination. We then expanded our study to the involvement of the NMDA-type glutamate receptor system in mediating the acute and chronic effect of cocaine self-administration. Since glutamate stimulates dopamine release in the NAc, the NMDA-type glutamate receptor system could also be part of the DA reward pathway. The experiments in this chapter were designed to study the effect of both acute and chronic administration of ketamine on the reinforcing effect of cocaine. Ketamine was chosen because it is an non-competitive NMDA antagonist and is abused among human abusers. These experiments tested the hypothesis that acute administration of the NMDA antagonist, ketamine, will reduce both IS^RT (FR2) and the breaking point (PR) of cocaine self-administration. These studies also focused on the hypothesis that chronic blockade of NMDA receptor (by ketamine) will cause an increase in either the IS^RT or the breaking point. This effect would be induced by a potentiation effect of cocaine's reinforcing properties by long term blockade of NMDA receptor. The experiment also tested the hypothesis that chronic administration of ketamine will prevent the shift to the right of cocaine dose-response curve induced by chronic treatment of cocaine. A final form of this manuscript will be submitted to Psychopharmacology.

Effect of ketamine on the reinforcing effects of cocaine [1]

D.-H. Li[2], M.W. Emmett-Oglesby, C Wallis

Department of Pharmacology

University of North Texas HSC

3500 Camp Bowie Blvd

Fort Worth TX 76107-2699

USA

Running Title: NMDA and tolerance

1 Supported by grants RO1-4137 and Texas Advanced Technology Award 9768031

2 This project is taken from a dissertation submitted to the University of North Texas Health Science Center at Fort Worth in partial fulfillment of the requirements for the degree Doctor of Philosophy

Corresponding Author:

C Wallis

Department of Pharmacology

University of North Texas HSC at Fort Worth

3500 Camp Bowie Blvd.

Fort Worth TX 76107-2699 USA

Phone: (817) 735-2056

FAX: (817) 735-2091

Abbreviations:

Ketamine (KET)

Progressive-ratio (PR)

Injection (inj)

Inter-reinforcer time (IS^RT)

Ventral tegmental area (VTA)

Excitatory amino acid (EAA)

Reinforcing stimulus (S^R)

ABSTRACT

This experiment tested the hypothesis that ketamine (a non-competitive NMDA antagonist) can be used to reduce the reinforcing effect of cocaine. If the reduction occurs as a result of an interaction of NMDA antagonist and dopamine at the dopamine receptor, ketamine is also expected to prevent the occurrence of tolerance to the reinforcing effects of cocaine. The effect of ketamine on cocaine self-administration was determined under both a fixed-ratio schedule (FR 2) and a progressive-ratio schedule (PR) of cocaine self-administration. Following the implantation of indwelling jugular catheters, rats were trained to self-administer cocaine, 0.25 mg/infusion until each subject showed a stable response for at least three consecutive days. Subjects in both groups were tested for their pretreatment dose-response to cocaine or the combination of cocaine and ketamine. Subsequently, the animals were assigned to four subgroups. Two subgroups were treated chronically with either saline or ketamine (25mg/kg/8hr) for 7 days; the other groups received either cocaine (20mg/kg/8hr) or combined cocaine and ketamine (25 mg/kg/8hr; i.e) for 7 days. Results from the groups treated with either saline or ketamine alone failed to change the cocaine dose-response curve. Animals treated chronically with cocaine alone showed a significant increase in the rate of cocaine self-administration under the FR 2 schedule, and a significant decrease in breaking points under the PR schedule. In addition, chronic treatment with the cocaine/ketamine combination also showed the same pattern of change in both schedules as cocaine treatment alone. These data support the hypothesis that ketamine blocks the reinforcing properties of cocaine but it does not block the adaptive process which produces tolerance to the reinforcing properties of cocaine.

Keywords: Cocaine, Ketamine, Rats, Self-administration, Cross-tolerance, Tolerance

INTRODUCTION

Within the ventral tegmental area (VTA), dopamine-containing neurons are hyperpolarized by either dopamine acting at D_2 receptors or GABA acting at $GABA_B$ receptors (Koob 1992). Excitatory input, due to activation of N-methyl-D-aspartate (NMDA) receptors, results in increased dopamine release in the VTA and striatum (Karler and Calder, 1992). Although the physiological factors that affect either the release or the re-uptake of dopamine from nerve terminals are not clearly defined, there is evidence from a wide variety of experimental approaches to suggest that excitatory amino acids (EAA) participate in the mechanism of the release of dopamine in the striatum (Freed and Cannon-Spoor, 1990; Rao et al., 1991). There are also several lines of evidence to demonstrate that EAA have some interaction with CNS stimulants. Experiments demonstrate that the inhibition of dopamine reuptake by cocaine is inhibited by the removal of Ca^{2+} from the dialysis probe (Hurd et al, 1989). These experiments suggested that cocaine may act to increase synaptic dopamine by inhibiting the reuptake of dopamine released by Ca^{2+} -dependent mechanisms (Nielsen et al, 1983), which can be mediated by the NMDA-type glutamate-gated calcium ion channel.

Following a chronic regimen with cocaine (20 mg/kg/8 hr for 7 days), there is an increase in the rate of cocaine self-administration in rats trained to self-administer cocaine under a fixed-ratio (FR) schedule of reinforcement (Emmett-Oglesby and Lane, 1992; Emmett-Oglesby *et al.*, 1993). Similarly, rats trained to self-administer cocaine (0.25 mg/injection) under a PR schedule, where an increasing number of responses is required to complete the requirement of response within each ratio for each subsequent reinforcer, showed approximately two-fold tolerance to the reinforcing effects of cocaine after receiving intravenous cocaine (20 infusions of 0.9 mg/kg/8 hr for 7 days; Li *et al.*, 1994). In addition, tolerance to the reinforcing (S^R) effects of *d*-A has also been demonstrated in rats trained to self-administer *d*-A under both FR2 schedule and PR schedule of reinforcement (Peltier *et al*, 1996; Chapter IV of the dissertation). If there is an interaction between the NMDA system and the cocaine reward system, then NMDA antagonists

will be expected to influence the reinforcing effect of cocaine after either acute or chronic cocaine treatment.

To demonstrate the effect of NMDA antagonists on the development of sensitization to cocaine, Karler and co-workers indicated that pretreatment with non-competitive antagonists of the NMDA receptor subtype prevents the initiation of behavioral sensitization to cocaine or amphetamine (Karler et al, 1989). This finding has been generally replicated (Wolf and Khansa, 1991; Kalivas and Alesdatter, 1993; Stewart and Druhan, 1993) and non-NMDA antagonists have been found to prevent the initiation and expression of behavioral sensitization (Karler et al 1991). In addition, antagonists of NMDA type of EAA receptors blocked cocaine-induced stereotypy, locomotor stimulation and convulsions (Karler and Calder, 1992). In De Montis' experiment (De Montis et al, 1992), MK-801 0.25 mg/kg i.p. successfully prevented the development of both tolerance and sensitization to the stimulation of locomotor activity induced by cocaine and by the dopamine D_2 agonist quinpirole. Their interpretation of the results was that both tolerance and sensitization phenomena are different aspects of a common neuronal response in which NMDA transmission plays a crucial role. If NMDA receptors play a role in the reinforcing effect of cocaine, then NMDA antagonists should reduce the tolerance to the self-administration of cocaine.

Our current experiment used rats trained on either a FR2 schedule or a PR schedule to determine whether the NMDA antagonist, ketamine, reduces the reinforcing effect of cocaine. If ketamine reduces the release of dopamine then the reinforcing properties of cocaine should be reduced as indicated by a reduction in both the IS^RT and the breaking point in cocaine self-administration.

METHODS

Subjects and Apparatus. Adult male Fisher F344 rats (Harlan, Indianapolis IN) were housed individually and maintained at 260-290g by daily feeding of measured quantities of food.

Initially, rats were trained to press a lever under a FR1 schedule using food pellets as a reinforcer; after responding was stable, each subject was implanted with a permanently indwelling jugular catheter (for details, see Depoortere *et al.*, 1993). After surgery the catheter was flushed with a combination of heparin, streptokinase, and Timentin (ticarcillin plus clavulinic acid) every 12 h for 5 days. On the 6th day, Timentin was removed from the regimen. Subjects were trained and tested in locally constructed operant chambers that have been described previously (Depoortere et al., 1993).

Preliminary Training Phase. Five days following surgery, animals began training under a fixed-ratio (FR) one schedule of reinforcement, with cocaine, 0.25mg/0.1ml injection serving as the reinforcer. Sessions lasted either until 25 injections were self-administered or until approximately 12-hr elapsed. Each session started with a priming injection of cocaine (2.70 mg/kg) delivered by the experimenter. Priming and subsequent injections were accompanied by a flashing of two stimulus lights in the chamber; each injection was followed by a 30-s time-out in the dark, during which responding had no programmed consequences. Once rats self-administered all 25 infusions within 3 hr during two consecutive training sessions, they were then switched to either an FR2 or PR training schedule. Before each self-administration session, patency of the catheter was assessed by drawing blood into the catheter and then by flushing 0.1 ml of heparinized saline back into the catheter.

The FR2 training schedule had a maximum of 15 reinforcers (0.25 mg of cocaine, 0.1 ml) and was limited to 3 hr. Rats were tested once a stability criterion was met. This criterion was defined as the average time occurring between reinforcers (the inter-reinforcer time; IS^RT, in min.) not varying by more than 20% across three consecutive training sessions. The PR training schedule began with a 0.30 mg/inj priming infusion of cocaine. To obtain each subsequent injection of the training dose (0.25 mg/inj), rats had to complete each of the ratios in the following sequence: 3, 6, 10, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 603, 737, 901, 1102, and 1347. There was a 1 hr time limit to obtain each reinforcer, and failure to do so terminated the session. The last reinforcer that was obtained for each session was

termed the breaking point. Rats were tested once they met a criterion of the total number of reinforcers obtained in one session (breaking point) not varying by more than three injections across seven consecutive training sessions.

FR2 self-administration testing. Rats (n=8) were tested using a multi-dose procedure (Emmett-Oglesby *et al.*, 1993). With this procedure, three doses of cocaine (0.125, 0.25 and 0.5 mg/infusion) were available for self-administration during a single test session. This test session consisted of a priming infusion (0.3 mg) followed by 24 infusions. These 24 infusions were divided into three blocks of 8 infusions each, with the first block of eight reinforcers containing 0.5 mg/infusion of cocaine, the second containing 0.25 mg/infusion and the third containing 0.125 mg/infusion. Cocaine self-administration tests were conducted in a descending order of cocaine doses (0.5, 0.25, 0.125 mg/kg/infusion); testing was initiated with the high dose in order to increase the probability that the rats would begin self-administration.

Acute administration of ketamine, i.v., against intravenous cocaine self-administration After initial dose-response curve determination (saline as a control) with a cocaine multi-dose procedure and a stabilization of the baselines was demonstrated, each of the multi-dose syringes was replaced with a solution with the same concentration of cocaine, but each syringe also contained one of concentrations of ketamine 1.0, 3.2, or 10 mg/ml (which equal to 0.1, 0.32 or 1.0 mg/inj dose of ketamine) for the self-administration testing. Then, the effect of ketamine on cocaine self-administration was determined using this combination, and ketamine 0.32 mg/inj was chosen for further chronic tests since this dose was close to the ED50 of ketamine on cocaine self-administration.

PR self-administration testing. After the stability criterion was met, rats (n=15) were tested by substituting different doses of cocaine (0.028 and 0.083 mg/inj) for the training dose (0.25 mg/inj). These substitutions took place several times until reproducible dose-response data were obtained. After initial dose-response curve determination (ketamine = 0 mg/inj) and a stabilization of the baselines was demonstrated, animals were tested with a combination of cocaine 0.25 mg/inj baseline dose with one of the concentrations of ketamine 1.0, 3.2, 10, or 32

mg/ml (which equal to 0.1, 0.32, 1.0 or 3.2 mg/inj dose of ketamine) for self-administration. The effect of ketamine on cocaine 0.25 mg/inj self-administration was actually determined under this design, and then ketamine 0.32 mg/inj was chosen to test the effect of ketamine on the dose-response curve of breaking point in cocaine self-administration at doses of cocaine of 0.028 and 0.083 mg/inj.

Chronic administration of ketamine Seven rats in each group were trained to self-administer cocaine (0.25 mg/inj) under either FR2 or the PR schedule. After stabilization of the baseline and initial dose-response determination (pre-chronic dose-response curve), rats were randomly assigned into two groups and the chronic treatments were started as follows: 1) (Ketamine) received 20 infusions of Ketamine, (0.32 mg/0.1ml/infusion, each infusion at 2-min intervals) at 7-hr intervals for one week; 2) (Saline) received saline as a control. The 20th infusion was followed by a heparinized-saline flush (0.4U in 0.1ml), and this flush was repeated 3 and 6 hr later. The ketamine dose was then approximately 75 mg/kg/day for seven days.

Combined chronic treatment with ketamine and a baseline dose of cocaine Nine rats were trained to self-administer cocaine (0.25 mg/inj) under either FR2 or the PR schedule. After stabilization of the baseline and initial dose-response determination (pre-chronic dose-response curve), rats were randomly assigned into two groups and the chronic treatment was started: 1) (Cocaine) received 20 infusions of cocaine, (0.25 mg/0.1ml/infusion, each infusion is given over a 2-min interval) at 7-hr intervals for one week; 2) (Cocaine Plus Ketamine) received this same cocaine regimen, but each injection was accompanied by an infusion of ketamine (0.32 mg/infusion) at 7-hr intervals for one week. The treatment was applied inside self-administration chambers, with the chambers modified such that no levers were present.

Determination of cocaine dose-response data immediately upon termination of chronic treatment (post-chronic tests). After the final infusion in the chronic treatment regimens, subjects were returned to their home cages for 24 hours. The post-chronic dose-response curve was then obtained by multi-dose testing within one session under the FR2 schedule. Under the PR schedule, the post-chronic dose-response curve (0.028, 0.083 and 0.25 mg/inj) was obtained

in the same order of dose in the pre-chronic baseline test. The dose-response curve data were obtained by testing the dose substitutions within the three days following the chronic infusions, with the first substitution occurring 24 hr after the last chronic infusion.

Recovery. After the post-chronic dose-response curve were tested, subjects were left without training or testing for 5 days, and then training was resumed using the baseline cocaine training dose (0.25 mg/inj). Recovery from chronic administration was defined as three consecutive sessions in which either the IS^RTs or the breaking points of each rats were within 20% of the pre-chronic values, which took approximately one week. When each animal responded within the criterion, dose-response curves were again obtained with doses of cocaine presented in the same order as for pre-chronic tests to determine recovery from tolerance, which were termed as recovery curves.

When either the baseline IS^RT's or the breaking points were stable, they were then included in another chronic regimen using a shifted chronic treatment. Subjects were assigned to receive the opposite chronic treatment (i.e., if the animal have received chronic cocaine treatment previously, then he receives chronic cocaine plus ketamine currently); within this assignment, all subjects received all doses in a random block design. Thus, at each round of chronic administration, all three doses were assigned across subjects, and across repetitions of the experiment, these doses were shifted such that all subjects received all treatments.

Data Analysis In FR2 self-administration experiment, data were scored as the average time between the administration of consecutive injections of cocaine (inter-reinforcer interval, IS^RT) without including the 30s time-out that followed the delivery of each reinforcer. This measure is the reciprocal of reinforcers per unit time. For training sessions, the time between the start of the session and the time of acquisition of the first reinforcer was not included in the data analysis because this time was more variable than subsequent IS^RTs. Thus, for the multi-dose test procedure, only the last 7 IS^RTs for each dose of cocaine were used for data analysis. A subject was required to take all available reinforcers during the training or testing sessions to be included in the analysis. Data were analyzed using a 2 x 3 x 3 way repeated measures

ANOVA with treatment condition, chronic treatment of either cocaine or cocaine plus ketamine, and dose of cocaine as within subject factors.

In PR self-administration experiments, the number of reinforcers obtained was used as the dependent measure, which is termed the breaking point. This breaking point measure was used rather than the final ratio completed or the total number of responses emitted because these latter variables are not amenable to parametric analysis (for a discussion of this problem in analysis of response and reinforcer data from PR procedures, see Depoortere *et. al.*, 1993; and Roberts and Richardson, 1992). The breaking points were analyzed using a two-way repeated measures ANOVA with treatment condition and dose of cocaine as within subject factors. SYSTAT software (Wilkinson *et. al*, 1992) was used for data analysis.

Drugs. Cocaine HCl was obtained from the National Institute on Drug Abuse (Research Triangle Park, NC). Ketamine HCl injection (100 mg/ml, 5 ml/vial) was obtained from Boehringer Ingelheim Animal Health, Inc. All doses refer to the weight of the salt.

RESULTS

Effect of ketamine on acute cocaine self-administration

FR2 paradigm Under baseline conditions, increasing the dose of cocaine resulted in an orderly increase in the time between injections ($F_{2,10} = 87.76$; $p < 0.001$; Fig. 1). The combination with ketamine (0.1, 0.32 or 1.0 mg/inj) shifted the acute cocaine dose-response curve significantly to the left ($F_{3,15} = 18.3$; $p < 0.001$). When each dose was analyzed independently, only the highest dose of ketamine (1.0 mg/inj) resulted in a significant shift of the cocaine dose-response curve ($F_{1,5} = 19.91$, $p<0.01$). The treatment with ketamine 0.1 and 0.32 mg/inj failed to show significant reduction ($F_{1,5} = 1.55$, $p>0.1$; $F_{1,5} = 0.86$, $p>0.1$ separately).

PR paradigm Ketamine (0.1, 0.32, 1.0, and 3.2 mg/inj) caused a dose related decrease in the breaking point of cocaine self-administration at the baseline dose (0.25 mg/inj) (Fig. 2). The combination of ketamine (0.32 mg/inj) also significantly reduced the breaking point for

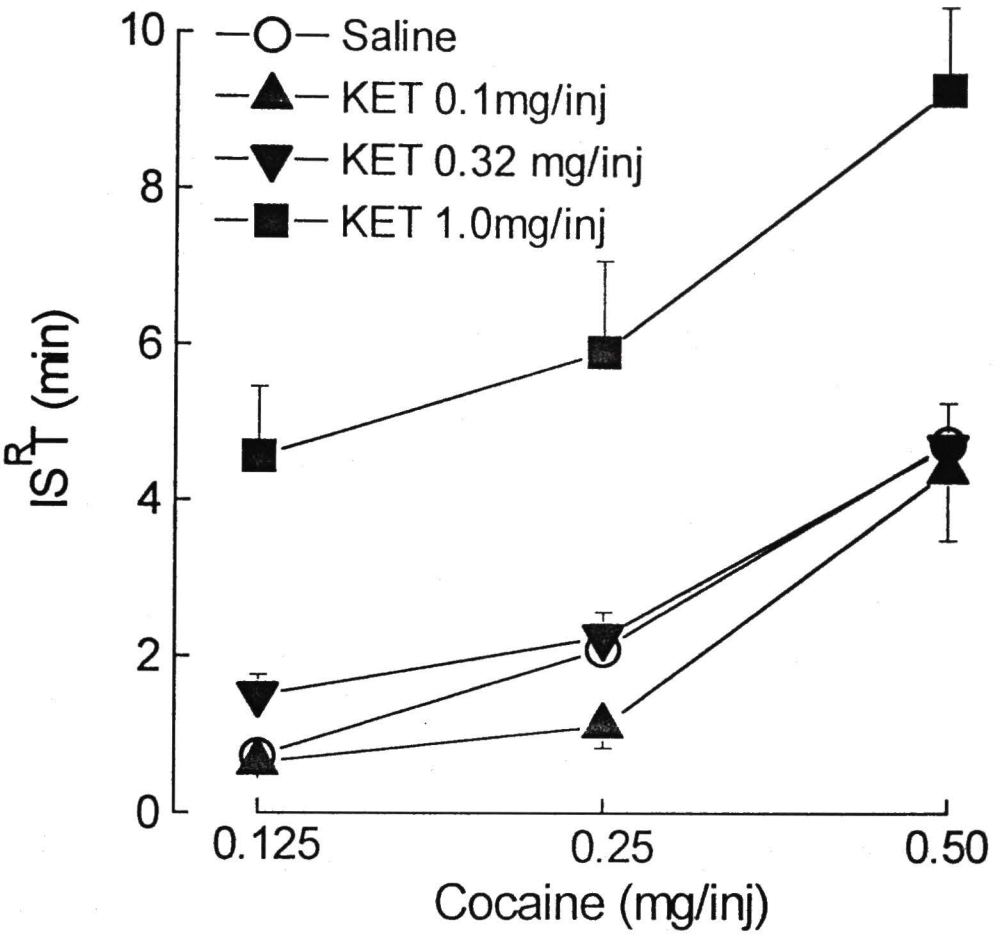


Fig. 1. Effect of ketamine 0.1, 0.32 and 1.0 mg/inj on cocaine self-administration under a FR2 paradigm. Abscissa: Dose of cocaine made available for self-administration. Ordinate: inter-reinforcer time (min). Each symbol represents the inter-reinforcer time in a session, and vertical bars represent S.E.M. Open circles indicate self-administration of cocaine without ketamine; closed up or down triangles indicate self-administration of cocaine with co-administration of low dose ketamine (either 0.1 or 0.32 mg/inj separately); closed squares indicate high dose ketamine (1.0 mg/inj) was co-administered. The same subjects received all treatments in a randomized order. N=7

cocaine self-administration at all three doses of the cocaine (0.028, 0.083, and 0.25 mg/inj) ($F_{1,6}$ = 36.73, p=0.005; Fig. 3).

Effect of chronic ketamine on cocaine self-administration

FR2 paradigm Under baseline conditions, increasing the dose of cocaine resulted in an orderly increase in the time between injections ($F_{2,12}$ = 22.8, p< 0.001; Fig. 4). Chronic treatment with ketamine (0.32 mg/inj) failed to shift the cocaine dose response curve to the left ($F_{1,6}$ = 2.39, p>0.1). However, chronic administration of saline shifted the cocaine dose-response curve to the left ($F_{1,6}$ = 7.76, p<0.05; Fig. 4) which indicated that after suspension from cocaine administration, animals showed a slight sensitization to the reinforcing effect of cocaine. There was no significant difference between the cocaine dose-response curves of the ketamine and saline treated animals either before chronic treatment ($F_{1,6}$ = 0.03, p>0.5) or after chronic treatment ($F_{1,6}$ = 0.13, p>0.5).

PR paradigm In all tests, the breaking point increased as a function of the dose of cocaine ($F_{2,12}$=18.0, p<0.001; Fig 5). Chronic treatment with either ketamine (0.32 mg/inj) or saline failed to shift the cocaine self-administration dose-response curve ($F_{1,6}$ = 0.10, p>0.5; $F_{1,6}$ = 1.12, p>0.1; Fig. 5).

Effect of ketamine on tolerance to cocaine self-administration

FR2 paradigm Under baseline conditions, increasing the dose of cocaine resulted in an orderly increase in the time between injections ($F_{2,16}$ = 38.4, p< 0.001; Fig. 6). Chronic treatment with cocaine (20 mg/kg/8 hr) caused a significant shift to the right of cocaine dose-response curve ($F_{1,8}$ = 15.32, p< 0.001) which indicated tolerance to the reinforcing effect of cocaine as previously reported (Emmett-Oglesby and Lane, 1992; Emmett-Oglesby et al, 1993). However, chronic treatment with a combination of ketamine (0.32 mg/inj) with cocaine (20 mg/kg/8hr) also induced a significant ($F_{1,8}$ = 5.06, p= 0.05) shift of the cocaine dose-response curve to the right. There was no significant difference between the post-chronic dose-response curves of cocaine

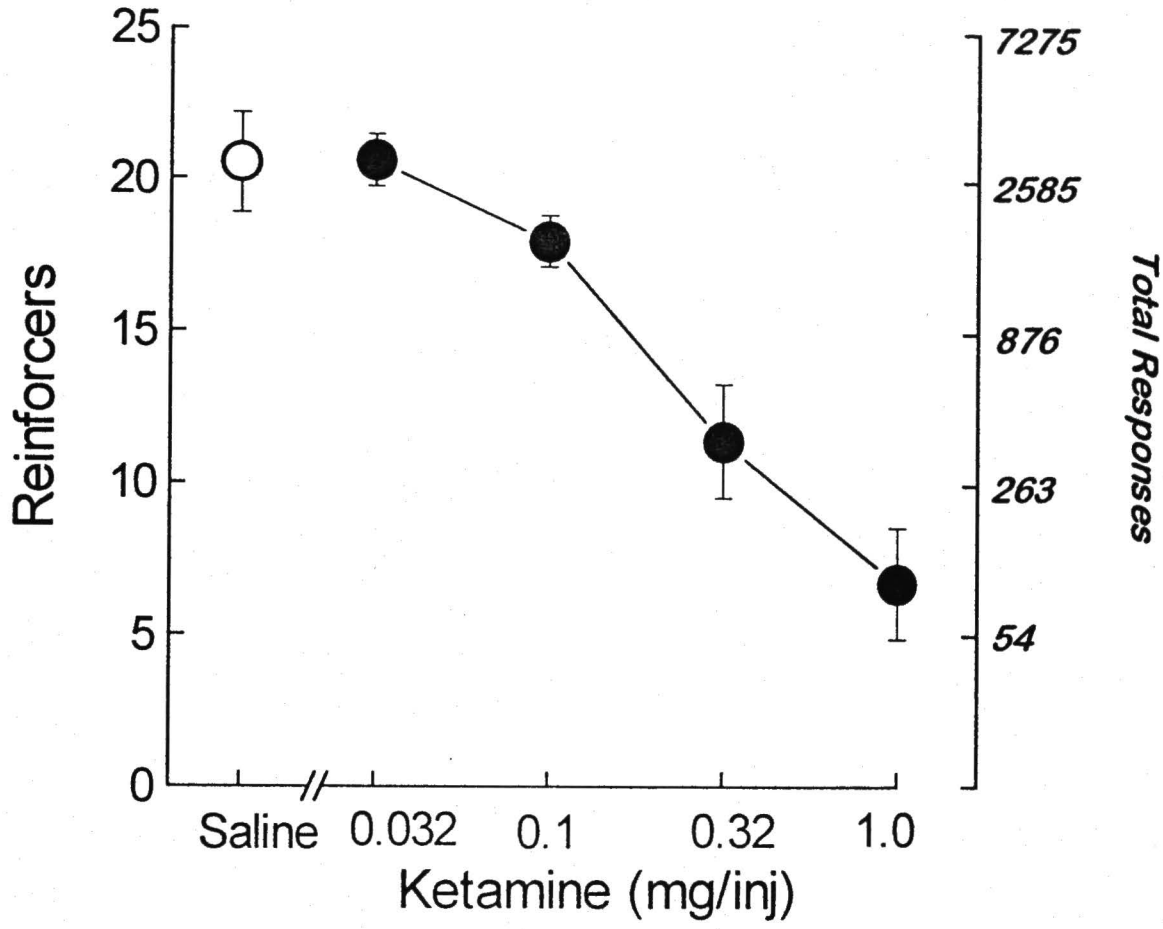


Fig. 2. Effect of ketamine on the breaking point obtained for baseline dose (0.25 mg/inj) of cocaine. Abscissa: dose of ketamine co-administered for self-administration of cocaine. Ordinate: the number of reinforcers obtained. Each symbol represents the average number of reinforcers obtained in a session, and vertical bars represent S.E.M. Open circle indicates cocaine self-administration without ketamine; closed circles indicate different dose of ketamine co-administered with cocaine. The same subjects received all treatments in a randomized order. N = 9.

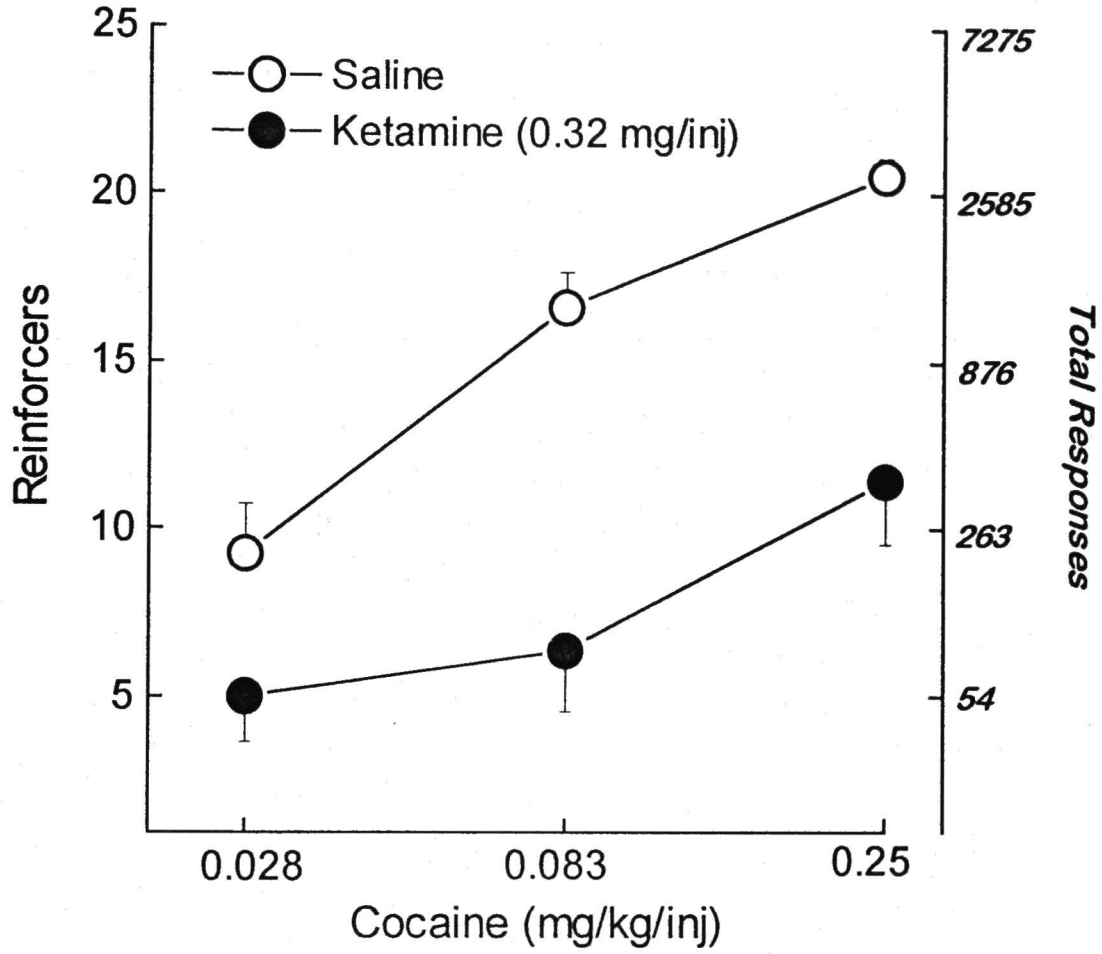


Fig. 3. Effect of ketamine (0.32 mg/inj) on the breaking point obtained for various doses of cocaine. Abscissa: dose of cocaine available for self-administration. Ordinate: the number of reinforcers obtained. Each symbol represents the average number of reinforcers obtained in a session, and vertical bars represent S.E.M. Open circles indicate cocaine self-administration without ketamine (control); closed circles indicate ketamine was co-administered with various dose of cocaine. The same subjects received all treatments in a randomized order. N = 9.

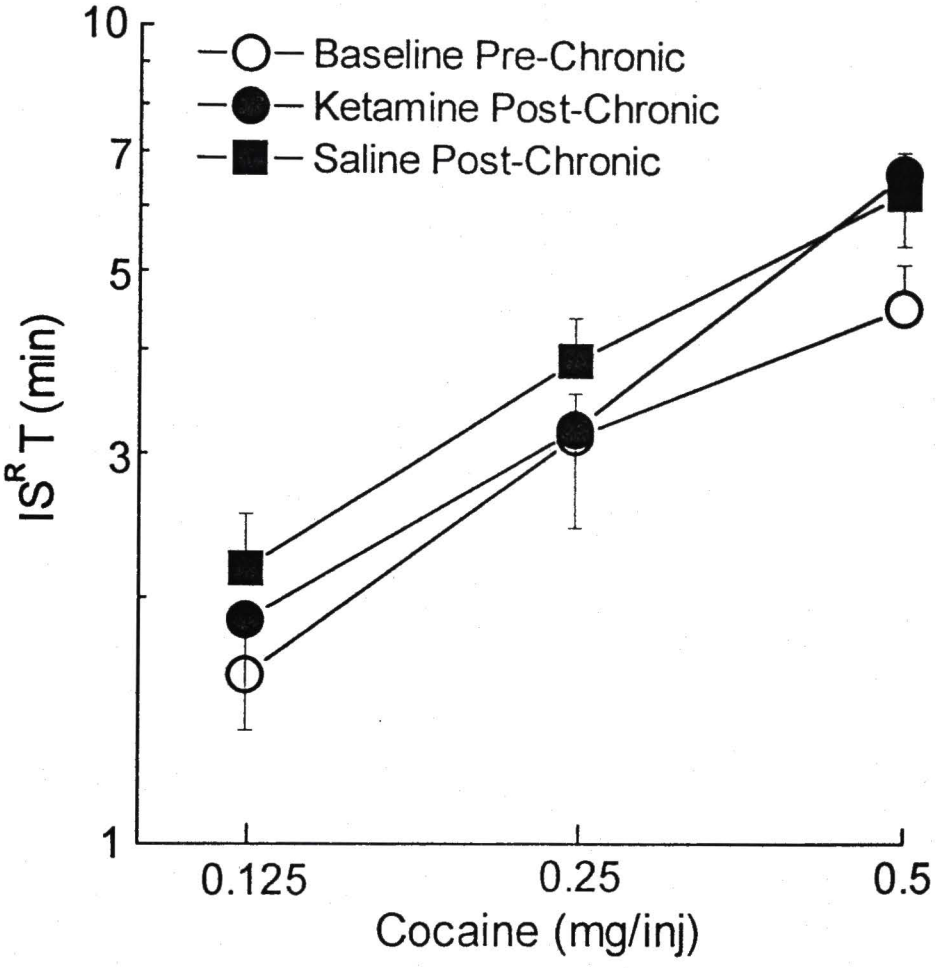


Fig. 4. Effect of chronic ketamine on cocaine self-administration under a FR2 paradigm. Abscissa: Dose of cocaine made available for self-administration. Ordinate: inter-reinforcer time (min). Each symbol represents the inter-reinforcer time in a session, and vertical bars represent S.E.M. Open circles indicate self-administration of cocaine prior to chronic treatment; closed circles indicate self-administration of cocaine 24 hr following the last chronic injection of ketamine; closed squares indicate self-administration of cocaine 24 hr following the last chronic injection of saline. The same subjects received all treatments in a randomized order. N=7.

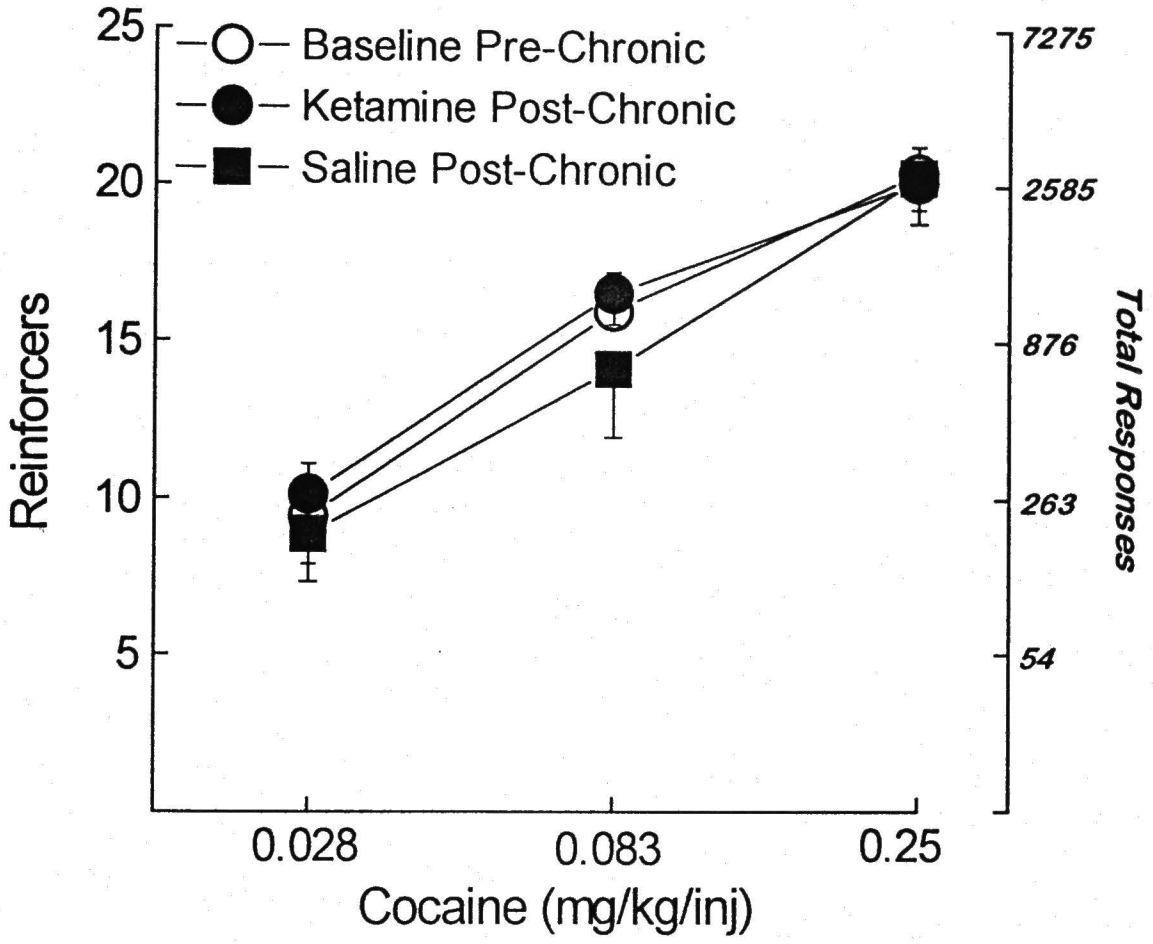


Fig. 5. Effect of chronic treatment with ketamine on the breaking point obtained for various doses of cocaine. Abscissa: dose of cocaine available for self-administration. Ordinate: the number of reinforcers obtained. Each symbol represents the average number of reinforcers obtained in a session, and vertical bars represent S.E.M. Dose-response data were obtained over a three day period: prior to chronic ketamine (open circle), immediately after chronic ketamine (closed circle) and immediately after chronic saline (closed squares). N = 9.

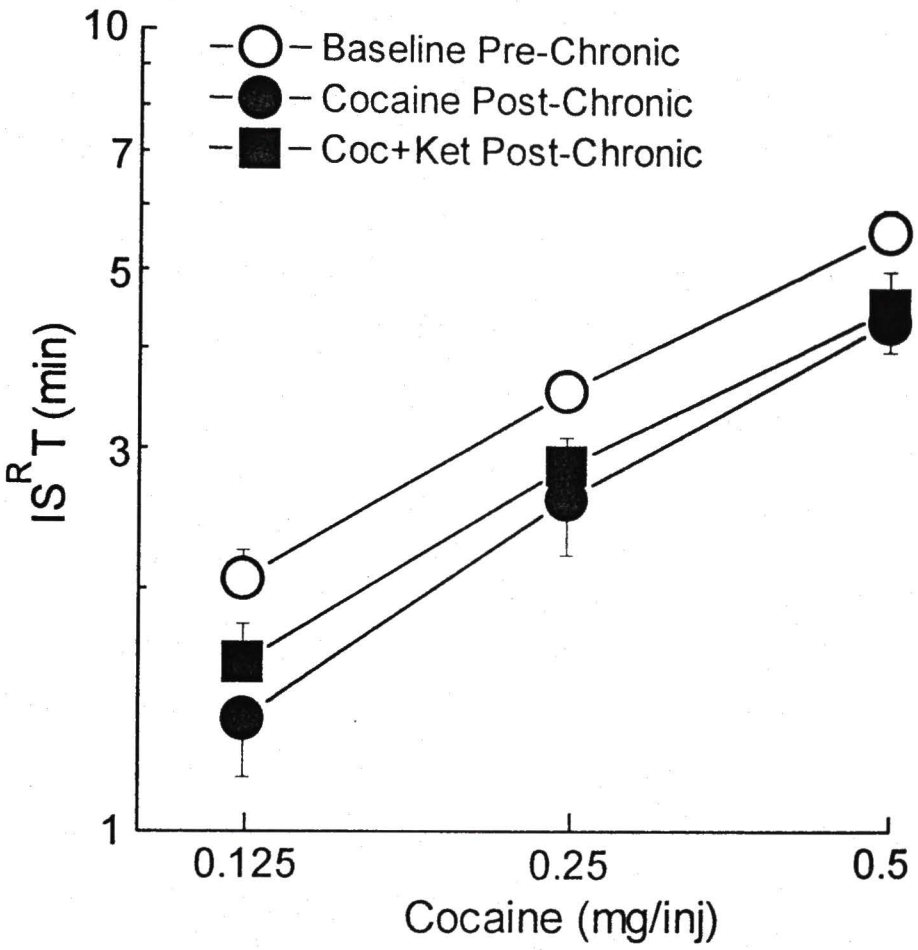


Fig. 6. Effect of chronic cocaine or cocaine plus ketamine on cocaine self-administration under a FR2 paradigm. Abscissa: Dose of cocaine made available for self-administration. Ordinate: inter-reinforcer time (min). Each symbol represents the inter-reinforcer time in a session, and vertical bars represent S.E.M. Open circles indicate self-administration of cocaine baseline prior to chronic treatment; closed circles indicate self-administration of cocaine 24 hr following the last chronic injection of cocaine; closed squares indicate self-administration of cocaine 24 hr following the last chronic injection of cocaine plus ketamine. The same subjects received all treatments in a randomized order. N=7.

alone versus cocaine combined ketamine ($F_{1,7}$ =1.99, P>0.1; Fig 6).

PR paradigm Three way analysis of variance with dose of cocaine as a repeated measure indicated that there was a significant shift of the cocaine dose response curves by chronic treatment with either cocaine alone or in combination with ketamine ($F_{1,32}$=4.836, p=0.035). The two drug treatment groups did not differ ($F_{1,32}$=1.999, p=0.167) at either the pre- or post-treatment tests and there was no significant interaction between treatment group or pre and post testing data ($F_{1,32}$=0.222, p=0.641). Within subjects, the dose effect of cocaine was highly significant ($F_{2,64}$=215.637, p<0.0001; Fig 7).

DISCUSSION

Only the high dose of ketamine (1.0 mg/inj) reduced the IS^RT of cocaine self-administration. However, the breaking point for cocaine self-administration under the PR schedule was reduced at all doses of ketamine. The combination of cocaine and ketamine produced an intoxication syndrome: tremor, jumping around inside their cages, and drowsiness. Under the FR2 schedule of reinforcement, the dependent measure, IS^RT, is a rate dependent measurement. It is hypothesized in our experiments that ketamine may produce motor incoordination which will increase the IS^RT. However, at the same time, ketamine decreases the reinforcing properties of cocaine which will decrease IS^RT under a FR schedule. Therefore, the overall effect of ketamine on cocaine self-administration will be unchanged. Because breaking point is not a rate dependent measurement, reduction of ketamine's reinforcing properties is observed at all doses. These data support the hypothesis that ketamine reduces the reinforcing properties of cocaine.

Chronic treatment with ketamine at 0.32 mg/inj for seven days failed to induce a change in either the IS^RTs or the breaking points of cocaine self-administration under both schedule. Ketamine also failed to inhibit the development of tolerance caused by chronic administration of cocaine. These results do not support the hypothesis that the NMDA receptor system is involved

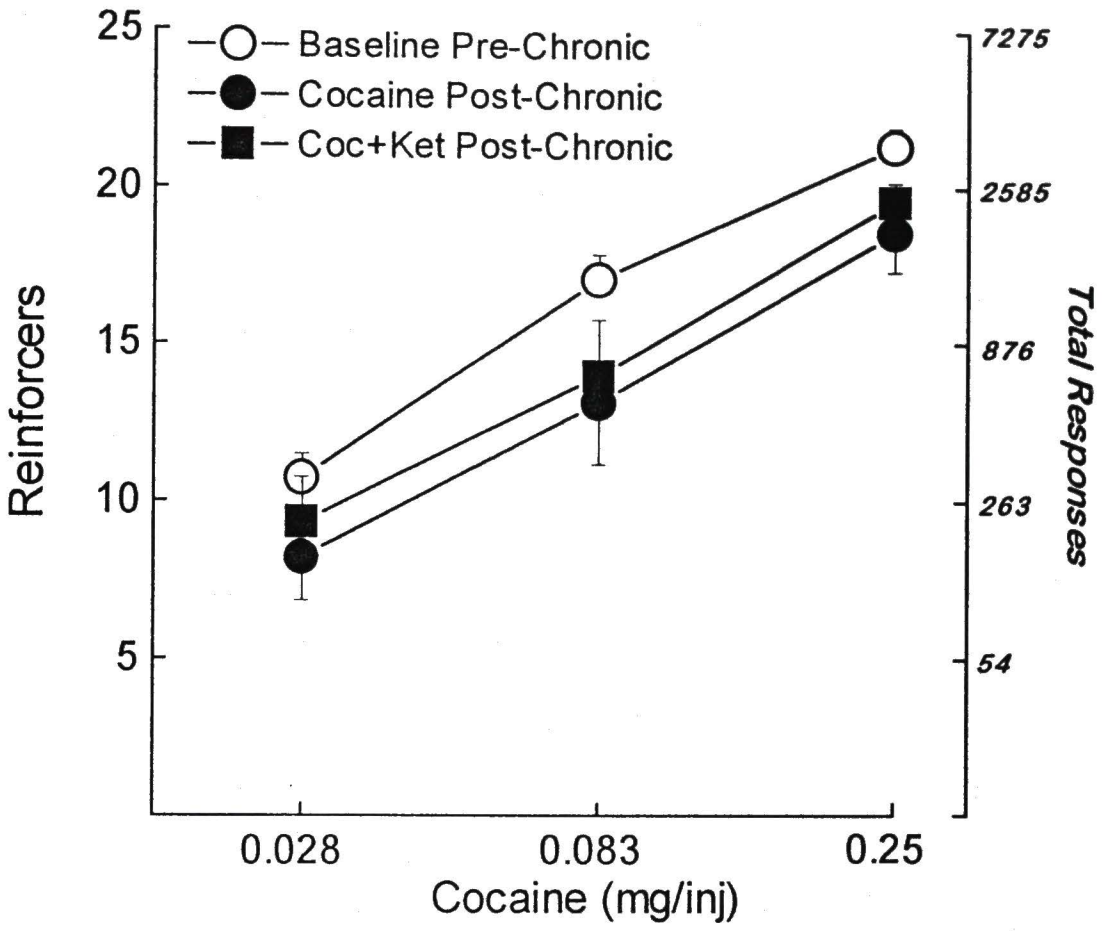


Fig. 7. Effect of chronic treatment with cocaine or cocaine plus ketamine on the breaking point obtained for various doses of cocaine. Abscissa: dose of cocaine available for self-administration. Ordinate: the number of reinforcers obtained. Each symbol represents the average number of reinforcers obtained in a session, and vertical bars represent S.E.M. Dose-response data were obtained over a three day period: baseline prior to chronic treatment (open circle), immediately after chronic cocaine (closed circle) and immediately after chronic cocaine plus ketamine (closed squares). N = 9.

in producing the reinforcing properties of cocaine. However, these data also indicate the ketamine does not block the action of cocaine required to produce tolerance to cocaine. Ketamine actually prevented saline induced sensitization, which is sometimes seen after suspension of cocaine administration for one week. The effect of NMDA antagonists on cocaine sensitization has been previously studied. Karler and co-workers indicate that pretreatment with antagonists of the NMDA receptor subtype (e.g., MK-801) prevents the initiation of behavioral sensitization to cocaine or amphetamine (Karler et al, 1989; Karler et al, 1990; Wolf and Khansa 1991). Others (Wolf and Khansa, 1991; Kalivas and Alesdatter, 1993; Stewart and Druhan, 1993) also confirm this finding and demonstrate that antagonists of non-NMDA receptors also prevent the initiation and expression of behavioral sensitization (Karler et al 1991). Thus, our data are consistent with published reports that blockade of NMDA receptors prevents the development of sensitization to cocaine.

Chronic treatment with cocaine, 20 mg/kg/8-hr for a week, produced tolerance to cocaine as indicated by a decrease of both the $IS^{R}Ts$ and the breaking point for cocaine self-administration. Concurrent treatment with ketamine did not block the development of tolerance to cocaine. Tolerance was reversible upon the termination of chronic cocaine or the combined treatment with ketamine, and the time course of recovery from tolerance parallels data of previous studies using this chronic regimen (Emmett-Oglesby *et al*, 1993; Li *et al*, 1994).

The neurochemical basis for the tolerance phenomenon is still unknown. Many hypotheses have been focused in the changes in the mesolimbic dopamine system to explain tolerance to cocaine. Cocaine acts at sites in the NAc and the striatum that are terminal fields of dopamine neurons (Izenwasser and Cox, 1992). These systems have been implicated in cocaine self-administration because neurotoxic lesions to the terminals regions (Roberts et al, 1977) or cell bodies (Zito et al, 1985) disrupt cocaine self-administration. However, the direct actions of cocaine at dopamine neurons (i.e., decreased sensitivity of autoreceptors, decreased uptake mechanisms or increased release) cannot account for all of the aspects of cocaine action in the development of both sensitization and tolerance (Zahniser and Peris, 1992). There are likely to

be non-dopaminergic systems involved in the development of tolerance to cocaine (Schenk *et al*, 1993).

Though our experiment failed to support the hypothesis that NMDA receptors are involved with the development of tolerance to the reinforcing effect of cocaine, we did demonstrate a blockade of cocaine's reinforcing properties. Since interactions between glutamatergic and dopamine systems have been documented (Stock *et al*, 1983; Overton and Clark 1992), and the fact that dopaminergic systems are widely accepted as being important in the mediation of cocaine's positive reinforcing effects (Roberts *et al*, 1977; Zito *et al*, 1985), it is reasonable to speculate that the tolerance produced by stimulant preexposure may involve interactions between these two systems (Schenk *et al*, 1993). Since some evidence demonstrates that intra-NAc injection of various glutamate receptor antagonists appears to reduce the activating properties of psychostimulant drugs (Pulvirenti *et al*, 1992), and that EAA participate in the mechanism of the release of dopamine in the striatum (Freed and Cannon-Spoor, 1990; Rao *et al*, 1991), we may expect that the proposed interaction between DA cells and glutamate NMDA neurotransmitter system may be involved in these areas. Additional studies should be conducted using microdialysis and microinjection techniques to determine which areas of the brain are important in these responses. We also need to apply some other NMDA antagonists which possess unique property in blocking particular glutamatergic receptors to study the interaction, since ketamine may have complicated mechanisms to induce behavioral effects on animals. From our data we can not determine if the blockade of cocaine's reinforcing properties is due to the a reduction in glutamate transmission in the terminal fields of dopamine neurons (Nac) or in the reward pathway before or after the release of dopamine. However, based on published data cited earlier, it is likely that glutamate reduced dopamine release via a calcium mediated mechanism that does not disrupt the development of tolerance to cocaine.

REFERENCES

Cox BM (1990) Drug tolerance and physical dependence. In: Pratt WB, Taylor P (eds) Principles of drug action, the basis of pharmacology, 3rd edition. Churchill Livingstone, New York, pp 639-690

De Montis MG, Devoto P, Meloni D, Gambarana C, Giorgi G & Tagliamonte A (1992) NMDA receptor inhibition prevents tolerance to cocaine. *Pharmacol Biochem Behav.* 42(1):179-82

Depoortere RY, Li D-H, Lane JD, Emmett-Oglesby MW (1993) Parameters of self-administration of cocaine in rats under a progressive-ratio schedule. Pharmacol Biochem Behav 45: 539-548

Emmett-Oglesby MW, Peltier RL, Depoortere RY, Pickering CL, Hooper ML, Gong YH, Lane JD (1993) Tolerance to self-administration of cocaine in rats: time course and dose-response determination using a multi-dose method. Drug Alcohol Depend 32: 247-256

Emmett-Oglesby MW, Lane JD (1992) Tolerance to the reinforcing effects of cocaine. Behav Pharmacol 3: 193-200

Fischmen MW, Schuster CR, Javaid J, Hatano Y, Davis J (1985) Acute tolerance development to the cardiovascular and subjective effects of cocaine. J Pharmacol Exp Ther 235: 677-682

Freed WJ and Cannon-Spoor HE (1990) A possible role of AA2 excitatory amino acid receptors in the expression of stimulant drug effects, *Psychopharmacology* 101:456-464

Griffiths RR, Bradford LD, Brady JV (1979) Progressive ratio and fixed ratio schedules of cocaine-maintained responding in baboons. Psychopharmacology 65: 125-136

Hodos W (1961) Progressive ratio as a measure of reward strength. Science 134: 943-944

Horger BA, Shelton K, Schenk S (1990) Preexposure sensitizes rats to the rewarding effects of cocaine. Pharmacol Biochem Behav 37: 707-711

Hubner CB, Moreton JE (1991) Effects of selective D1 and D2 dopamine antagonists on cocaine self-administration in the rat. Psychopharmacology 105: 151-156

Hurd YL and Ungerstedt U (1989) Cocaine: an in vivo microdialysis evaluation of its acute action on dopamine transmission in rat striatum. *Synapse* 3:48-54

Meisch RA, Lemaire GA (1993) Drug self-administration. In: van Haaren F (ed). Methods in behavioral pharmacology. Elsevier Science Publishers, NY, pp 257-300

Misra AL (1976) Cocaine: Chemical, Biological, Clinical, Social and Treatment Aspects. In: Mule, SJ (ed), CRC Press, Cleveland, pp 73-90

Nielsen JA, Chapin DS and Moore KE (1983) Differential effects of d-amphetamine, beta-phenylethylamine, cocaine and methylphenidate on the rate of dopamine synthesis in terminals of nigrostriatal and mesolimbic neurons and on the efflux of dopamine metabolites into cerebroventricular perfusates of rats. *Life Sci* 33:1899-1907

Overton P and Clark D (1992) Electrophysiological evidence that intrastriatally administered N-methyl-d-aspartate augments striatal dopamine tone in the rat. J Neural Transm 4:1-14

Peltier RL, Emmett-Oglesby MW, Lane JD (1993) Cross-tolerance between CNS stimulants in a self-administration paradigm in rats. Soc Neurosci Abstr 19 (3): 761.5

Peltier RL, Li D-H, Lytle D, Taylor CM, and Emmett-Oglesby MW (1996) Chronic *d*-amphetamine or methamphetamine produces cross-tolerance to the discriminative and reinforcing stimulus effects of cocaine. in press in J Pharmacol Exp Ther

Piazza PV, Deminiere JM, LeMoal M , Simon H (1990) Stress- and pharmacologically-induced behavioral sensitization increases vulnerability to acquisition of amphetamine self-administration. Brain Research, 514: 22-26

Pulvirenti L, Maldonado-Lopez R and Koob GF (1992) NMDA receptors in the nucleus accumbens modulate intravenous cocaine but not heroin self-administration in the rat. *Brain Res.* 594:327-330

Rao TS, Cler JA, Mick SJ, Emmett MR, Farah Jr. Jm, Contreras PC, Iyengar S and Wood PL (1991) Neurochemical interactions of competitive N-methyl-D-aspartate antagonists with dopaminergic neurotransmission and the cerebellar cyclic GMP system: functional evidence for a phasic glutamatergic control of the nigrostriatal dpaminergic pathway. *J Neurochem* 56:907-913

Risner ME, Silcox DL (1981) Psychostimulant self-administration by beagle dogs in a progressive-ratio paradigm. Psychopharmacology 75: 25-30

Robinson TE, Berridge KC (1993) The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res Rev 18: 247-291

Roberts DCS, Corcoran ME and Fibiger HC (1977) On the role of ascending catecholamine systems in intravenous self-administration of cocaine. Pharmacol Biochem Behav 6:615-620

Roberts DCS, Richardson NR (1992) Self-administration of psychomotor stimulants using progressive ratio schedules of reinforcement. In: Boulton AA, Baker G B, Wu PH (eds) (Neuromethods series: vol 24) Animal models of drug addiction, Humana Press, Totowa NJ, pp 233-269

Roberts DCS, Loh EA, Vickers G (1989) Self-administration of cocaine on a progressive ratio schedule in rats: dose-response relationship and effect of haloperidol pretreatment. Psychopharmacology 97: 535-538

Roberts DCS, Corcoran ME, Fibiger HC (1977) On the role of ascending catecholamine systems in intravenous self-administration of cocaine. Pharmacol Biochem Behav 6: 615-620

SAS System for personal computers, Release 6.03 (1987) SAS Institute Inc., SAS Circle, Cary, NC 27512

Schenk S, Valadez A. McNamara C, House DT, Higley D, Bankson MG, Gibbs S and Horger BA (1993) Development and expression of sensitization to cocaine's reinforcing properties: role of NMDA receptors. Psychopharmacol 111: 332-338

Schenk S, Snow S, Horger B (1991) Pre-exposure to amphetamine but not nicotine sensitizes rats to the motor activating effect of cocaine. Psychopharmacology 103: 62-66

Siegel S (1989) Pharmacological conditioning and drug effects. In: Goudie AJ, Emmett-Oglesby MW (eds) Psychoactive drugs: tolerance and sensitization. Humana Press, Totowa NJ, pp 115-180

Stewart J and Badiani A (1993) Tolerance and sensitization to the behavioral effects of drugs. Behav Pharmacol 4: 289-312

Stewart J and Druhan JP (1993) The development of both conditioning and sensitization of the behavioral activating effects of amphetamine is blocked by the non-competitive NMDA receptor antagonist, MK-801. *Psychopharmacology* 110:125-132

Stock G, Kummer P, Stumpf H, Zenner K and Sturm V (1983) Involvement of dopamine in amygdaloid kidling. Exp Neurol 80:439-450

Wilson MC, Schuster CR (1973) Cholinergic influence on intravenous cocaine self-administration by rhesus monkeys. Pharmacol Biochem Behav 1: 643-649

Wise RA, Leeb K (1993) Psychomotor-stimulant sensitization: a unitary phenomenon? Behav Pharmacol 4(4): 339-349

Wise RA, Yokel RA, Hansson P and Gerber GJ (1977) Concurrent intracranial self-stimulation and amphetamine self-administration in rats. Pharmacol Biochem Behav 7: 459-461

Wolf ME and Khansa MR (1991) repeated administration of MK-801 produces sensitization to its own locomotor stimulant effects but blocks sensitization to amphetamine. *Brain Res* 562:164-168

Wolgin DL, Kinney GG (1989) The role of instrumental learning in behavioral tolerance to drugs. In: Goudie AJ, Emmett-Oglesby MW (eds) Psychoactive drugs: tolerance and sensitization. Humana Press, Totowa NJ, pp 17-114

Wood DM, Lal H, Emmett-Oglesby MW (1984) Acquisition and recovery of tolerance to the discriminative stimulus properties of cocaine. Neuropharmacology 23: 1419-1423

Wood DM, Emmett-Oglesby MW (1986) Characteristics of tolerance, recovery from tolerance, and cross-tolerance to cocaine used as a discriminative stimulus. J Pharmacol Exp Ther 237: 120-125

Wood DM, Emmett-Oglesby (1988) Substitution and cross-tolerance profiles of anorectic drugs in rats trained to detect the discriminative stimulus properties of cocaine. Psychopharmacology 95: 364-368

Yanagita T (1973) An experimental framework for evaluation of dependence liability of various types of drugs in Monkeys. Bulletin on Narcotics 25: 57- 64

Zito KA, Vickers G and Roberts DCS (1985) Disruption of cocaine and heroin self-administration following kianic acid lesions of the nucleus accumbens. Pharmacol Biochem Behav 23:1029-1036

GENERAL DISCUSSION

In my dissertation, a modified progressive-ratio schedule has been introduced and developed to test the changes in the reinforcing effect of cocaine. A systematic study was then applied of the fundamental parameters of this schedule, and the acquisition and stability were observed for a period of forty sessions. Under this schedule, a direct relationship between the number of reinforcers obtained and inter-reinforcer time within each ratio was observed. This finding supplied a tool for simultaneous monitoring of the breaking point and the IS^RT, unlike the FR2 schedule which depends solely on the rate dependent measure of IS^RT. The two variables could be used to determine if a increase in the IS^RT is a result of a motor-incapacitating side effect of a pretreatment. If the increase in the IS^RT is induced by a motor-incapacitating effect, then the breaking point will be decreased or unchanged instead of increased. Based on the data from the PR schedule, an interaction between the breaking point and IS^RT occurred in testing the effect of *d*-amphetamine, methamphetamine, opiates, or ketamine on cocaine self-administration. The pretreatment of *d*-amphetamine significantly increased the breaking point of cocaine, while the IS^RT of cocaine self-administration remained unchanged. This result supplied evidence that the reinforcing effect of *d*-amphetamine is additive with the effect of cocaine. However, the development of a potentiation effect of *d*-amphetamine on saline self-administration indicated that the increase in the breaking point of cocaine self-administration was due to the direct action of *d*-amphetamine alone. The breaking point of cocaine self-administration remained unchanged after the pretreatment of morphine and buprenorphine. However, the IS^RT under the PR schedule increased significantly after the pretreatment which indicated a behavioral disruptive effect of morphine and buprenorphine on cocaine self-administration.

In our first study we demonstrated that the reinforcing properties of cocaine were blocked by prior administration of either D1 (SCH 23390) or D2 (eticlopride) dopamine antagonist as indicated by a reduction in the breaking point under the PR schedule. Under low

value FR schedules, it has been repeatedly demonstrated that prior administration of a dopamine antagonist produces an increase in stimulant self-administration rate (Johason et al, 1976; Wit and Wise 1977), which is the reciprocal of the time spent between each reinforcer. Thus, PR results confirm the requirement for dopamine action for the reinforcing effects of cocaine. These data indicate that animals will compensate for the dopamine receptor blockade by increasing their drug intake when the drug injections can be "earned" with little effort, but at higher ratios the response extinguishes.

Historically there are few discrepancies between the results obtained under FR and PR schedules. However, some reports indicate that these two schedules represent different aspects of reward. When bilateral intracerebral injections of SCH 23390 were administered into NAc and amygdala (AMY), injection into both sites produced a dose-dependent increase in the rate of cocaine self-administration under a FR schedule. However, injection into the AMY produced a significantly greater increase in rate of cocaine self-administration than into the NAc (McGregor and Roberts, 1993). In contrast, under a PR schedule, SCH 23390 had very little effect within the AMY but greatly reduced the breaking point following injection into the Nac (McGregor and Roberts, 1993). This result suggests that FR and PR schedules may measure different aspects of cocaine's CNS action which support self-administration behavior. Our results (Chapter 2) also indicated a discrepancy of the two schedules when measuring the effect of MOR or BUP on cocaine self-administration.

Other parts of my dissertation describe the studies in tolerance and cross-tolerance to the reinforcing effect of cocaine. These experiments supplied the evidence that chronic treatment with a high dose of cocaine or amphetamines caused a reduction in the breaking point of cocaine self-administration, which supports the hypothesis that tolerance and cross-tolerance occurs to the reinforcing effects of cocaine. Following 7 days of recovery from chronic treatment, cocaine dose-response curves returned to their baseline levels which indicated the reversibility of tolerance.

Acute treatment with ketamine produced a dose-related decrease in the breaking point for cocaine self-administration, but ketamine was less effective in reducing the IS^RT of cocaine self-administration under the FR2 schedule. The chronic treatment of ketamine failed to increase either the breaking point or the IS^RT for cocaine self-administration. When animals were chronically treated with a combination of cocaine and ketamine , ketamine failed to block the development of tolerance to cocaine. When the same chronic paradigm was applied under a FR 2 schedule, a similar result was obtained. These results indicate that the PR schedule is useful in the testing the change of the reinforcing effect of a cocaine. After acute pretreatment of ketamine, there is no change in IS^RT under the FR2 schedule, but the breaking point under the PR schedule was reduced in a dose related manner.

It has long been known that cocaine and amphetamine are indirect monoaminergic agonists. Amphetamine causes impulse-independent (Carboni et al, 1989) release of norepinephrine, DA, and serotonin from brain stem cells that project widely to the diencephalon and telencephalon. Amphetamine also blocks the uptake mechanism that normally clears these transmitters from the extracellular space (Heikkila et al, 1975). Although cocaine does not cause monoamine release, cocaine blocks monoamine uptake mechanisms (Heikkila et al, 1975) and thus, like amphetamine, causes accumulation of monoamines near monoamine-containing nerve terminals (Carboni et al, 1989; Kalivas and Duffy 1990) and dendrites (Kalivas and Duffy 1988). Studies have established that the reinforcing properties of cocaine derive primarily from its ability to block neurotransmitter reuptake, and thus enhance neurotransmitter action in the mesocorticolimbic dopamine system (Koob and Bloom 1988; Wise and Bozarth 1987; Wise and Rompre 1989). Wise and colleagues (1984, 1987; 1989) proposed the hypothesis that a single neural circuit is critical in mediating the "reward" effects of many, if not all, drugs of abuse. This circuitry is thought to depend critically upon the functioning of the medial forebrain bundle, and particularly upon the interaction of dopamine with its receptors in the mesolimbic areas of the brain. In contrast, Koob and others (Koob 1992; Koob and Bloom, 1988; Petit et al, 1984; Di Chiara and North 1992) have provided a

model in which mesolimbic dopamine is important for mediating the reinforcing effects of CNS stimulants, but for other classes of drugs of abuse, they suggest that other areas of the brain are perhaps more important.

Based on the above evidence, it has been suggested that brain regions of mesolimbic reward system receiving non-dopaminergic projections from the Nac may function as part of a dopamine dependent common reward pathway during both opiate and cocaine reinforcement (Koob et al, 1987). However, it has been also suggested that the reinforcing actions of opiates may involve an additional dopamine-independent pathway which may employ mechanisms not used in the action of cocaine (Koob 1992). Two aspects of natural reward can be distinguished (see Figure 3 next page):

1) incentive or preparatory aspect -- provided by the distinctive, identifying sensory properties of the reward (smell, color, shape, taste, temperature, etc.); and

2) consummatory aspect -- mainly involves the physiological and metabolic consequences of the contact, interaction and consumption of the rewarding stimulus itself.

Each of these aspects is pleasurable (i.e. elicits a positive affective state) but both are necessary for natural reward to be fully reinforcing. The incentive aspect of reward stimuli involves ergotropic changes such as arousal and activation of motor behavior, the sympathetic nervous system and catabolism. While the consummatory aspect involves trophotropic changes such as rest, sedation, anabolism and activation of the parasympathetic nervous system. Incentive properties are essential in learning a behavioral response directed to approach of the reward stimulus itself, and dopamine might play an important role in this process (Koob 1992). Therefore, psychostimulants and most of abused drugs stimulate the central dopamine reward system mainly through this process. However, the stimulation of dopaminergic transmission in mesolimbic brain areas may not be the critical component of the consummatory aspect, which might involve the activation of non-dopaminergic mechanisms such as the proposed central opioid reward system. Activation of this system has been related

Figure 3. Two aspects of natural rewards

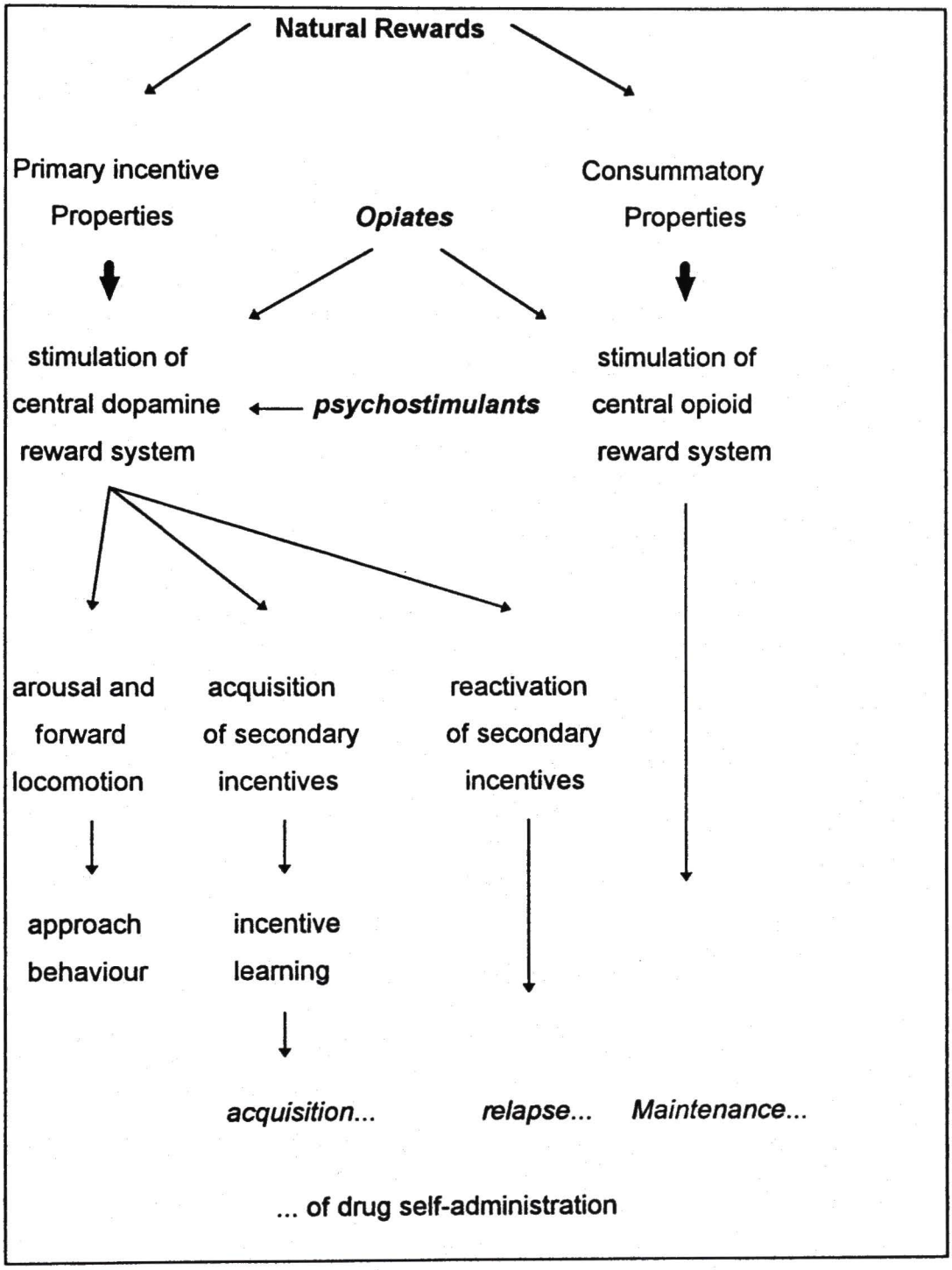


Figure from Chiara GD and North RA (1992) Neurobiology of opiate abuse. TIPS 13: 190

to the sedative, anabolic and reduced-drive state typical of the consummatory aspect of natural reward and accounts for the sedative, narcotic and analgesic properties of opiates (Belluzzi and Stein, 1977). Opiates might mimic the incentive as well as the consummatory aspects of natural reinforcers, given their ability to stimulate dopaminergic transmission in addition to the proposed endogenous μ-opioid reward system.

Our experiments showed that morphine or buprenorphine failed to modify the breaking points of cocaine self-administration. However, the combination of the two classes of drug caused a significant increase in inter-reinforcer time (IS^RT) of cocaine self-administration. These results indicated that the reinforcing effect of cocaine is not potentiated by the co-administration of opioid. The increase in the IS^RT also indicate a consummatory aspect of the opioid being added to the reward properties of cocaine. These results demonstrate that combination of the PR schedule and FR schedule provides more information than either measure alone.

Another explanation for the prolonged ISRT in the case of the morphine experiment could be a prolonged half-life of cocaine (pharmacokinetic mechanism) in the presence of morphine. In human studies, among cocaine-related fatalities, blood cocaine concentrations were found to be significantly lower in those cases where morphine was also detected (Finkle & McCloskey, 1977). These data suggest that morphine increased the lethality of cocaine, but this was not due to an increased concentration of cocaine in blood. Therefore, it is unlikely that the co-administration of morphine caused a longer half life of cocaine which would lead to the increase of IS^RT in cocaine self-administration by rats.

Tolerance to the subjective effects of drugs is widely regarded as a key component of the dependence process and, as such, it has been extensively studied over many years (Khanna et al., 1982; Jaffe, 1990). Investigators are interested in studying tolerance for at least three reasons (Kalant and Khanna, 1990): 1) to adjust dosage during treatment if tolerance has resulted in a gradual loss of effect of the dosage initially employed, or to facilitate the forensic interpretation of measured drug levels, with respect to the possible degree of impairment or

other effect in a particular individual; 2) to determine its temporal correlation with physical dependence, and the effect of both of these processes on drug self-administration, as factors in the production or maintenance of addiction; and 3) to elucidate the mechanisms and kinetics of tolerance, as they may serve to illustrate the fundamental processes of biological adaptive response. In this connection, tolerance is seen as simply one example of a broad biological adaptive capability, to which physiological adaptation, learning, and other forms of biological adjustment belong. Emmett-Oglesby (1992) first demonstrated that tolerance to the reinforcing effects of cocaine occurred in a rodent model of i.v. drug self-administration under a FR 2 schedule. Currently, the demonstration of a reduction in the breaking point under the PR schedule after repeated exposure of cocaine provides further evidence of tolerance to the reinforcing effect of cocaine.

The mechanisms that underlie the changes in the behavioral effect of cocaine that occur when the drug is administered repeatedly have not been clearly established. The literature concerning CNS effects of repeated administration of cocaine is inconsistent. However, previous experiments indicated that the disappearance of an acute effect with repeated administration is tolerance, while the appearance of a novel effect with repeated administration may be related to sensitization (Woolverton and Johnson, 1992). Although sensitization to the ability of cocaine to inhibit [^{3}H]dopamine uptake was observed only in the nucleus accumbens (NAc) with no change in the striatum, tolerance to the inhibition of [^{3}H]dopamine uptake occurred in both brain regions (Izenwasser and Cox, 1992). Another possible explanation is that the right shifting of cocaine dose-response curve is caused by the increasing of cocaine metabolism induced by the chronic exposure of cocaine. Previous experiments demonstrated that this mechanism is not responsible for changes in cocaine response after chronic administration because metabolism of cocaine in rats is not changed (Misra, 1976; Katz *et al.*, 1993). Peltier (1996) treated rats chronically with cocaine (20 mg/kg/8 hr, i.p.) for seven days and clearly demonstrated that the same treatment protocol that

produced significant tolerance to the reinforcing effects of cocaine did not produce a reduction in the concentration of cocaine in either plasma or brain.

The other possible explanation for these results is that the development of sensitization is mediated by inputs into the NAc which are either not present or not activated in the striatum. The mechanism by which tolerance is produced, however, appears to function in both brain regions. Although the neurochemical basis for tolerance to psychostimulants is still unclear, my experiments at least indicated that tolerance may be caused by changing DA activity in the mesolimbic DA system. The synergistic action of cocaine and amphetamines on the DA system leads to an expectation of cross-tolerance between the two drugs which was observed. Since glutamatergic corticostriatal projections synapse with the stiatal neurons which also receive DA input from the substantia nigra, the mechanism that ketamine inhibits the reinforcing effect of cocaine may due to either the nigrostratal DA system or the DA mesolimbic system or both.

There are several reasons to hypothesize that a glutamate-dopamine interaction within the NAc may represent the neurochemical basis of the acute and chronic action of cocaine (Pulvirenti et al, 1992). First, anatomical evidence suggests that afferent fibers to the NAc originate within the amygdala and the hippocampus and they appear to be glutamatergic (Fuller et al, 1987). Electron microscopy studies also showed that, within the NAc, these fibers of hippocampal origin lay in close apposition with tyrosine hydroxylase-positive nerve terminals, originating in the VTA, which represents the main source of dopamine innervation of the NAc (Sesack et al, 1990). Second, both in vitro and in vivo neurochemical studies suggest that infusion of glutamate or glutamate agonists within the NAc induces release of dopamine (Payson et al, 1989). Third, electro-physiological evidence indicates that NAc neurons show excitatory response to hippocampal stimulation (Yang and Mogenson, 1984). Finally, behavioral evidence suggests that microinfusion of glutamate within the NAc induces locomotor hyperactivity, which is blocked by administration of dopamine receptor antagonists (Donzanti and Uretsky, 1983). Furthermore, intra-NAc injection of various glutamate receptor antagonists appears to reduce the activating properties of psychostimulant drugs (Pulvirenti et

al, 1992) and the rewarding properties of ethanol (Rassnick et al, 1992). In addition to these results, some experiments also supplied evidence of interactions between dopamine and EAA in behavioral sensitization to psychostimulants (Kalivas 1995; Karler et al, 1989). MK-801 was found to be able to block sensitization to the locomotor-stimulant effect of cocaine and amphetamine (Karler et al, 1989). Khnna (Khanna et al, 1992) also demonstrated that ketamine retards chronic but not acute tolerance to ethanol in a tilted-plane test.

The data from my experiments indicated that co-administration of ketamine reduced the breaking point of cocaine in a dose-related manner. Ketamine reduced the IS^RT for cocaine only at the high dose, at which an effect of anesthesia or motor disruption started to inhibit the animal from responding. Therefore, the PR study demonstrated that ketamine reduced the reinforcing effect of cocaine. FR2 schedule was unable to show the change of reinforcing effect of cocaine due to ketamine, indicating that the FR schedule is less sensitive for measuring changes of the reinforcing effect of cocaine in the presence of a drug producing motor deficits. Chronic treatment with ketamine failed to modify the dose-response curve of cocaine self-administration. Chronic treatment with ketamine also failed to prevent tolerance to the reinforcing effect of cocaine after the chronic treatment of high dose cocaine. These results indicated that ketamine failed to block all of the actions of cocaine. However, in other experiments in this laboratory, it has been demonstrated that there is rapid development of tolerance to many of the actions of ketamine (Ward, 1995). Therefore, it is unclear if chronic ketamine treatment failed to block a critical action of cocaine for the development of tolerance or if ketamine lost effectiveness to block any action of cocaine to tolerance to ketamine itself.

The cocaine-induced increase in DA neurotransmission appears to be particularly prominent in its acute behavioral effects (Wise 1984; Woolverton and Kleven 1988). Early structure-activity relationship studies suggested that the locomotor effects of cocaine were related to indirect DA agonist effect (Sonsalla et al, 1988). Indirect DA agonist actions are also responsible for cocaine like effects on operant behavior (McKearney 1982), discriminative stimulus effects (Woolverton 1991), and reinforcing effects (Ritz et al, 1987). Since both D1

and D2 receptors appear to play a role in the behavioral effects of cocaine (Woolverton 1992), the study of the effects of D1 and D2 antagonists on the breaking point of cocaine self-administration and the results of cross-tolerance between amphetamines and cocaine strengthen the conclusion that DA plays a major role in the reinforcing effect of cocaine. Our experiments also demonstrate stimulation of opiate receptors modified the rate of cocaine self-administration, supporting the hypothesis of a common final pathway of reward among the drugs of abuse. Acute ketamine reduced the reinforcing effect of cocaine in our experiment, but the role of NMDA, GABA systems and calcium channels in DA rewarding system still needs to be investigated. The mesolimbic DA system appears to synapse on GABAergic cells and perhaps cholinergic cells having synapses in the pallidum, substantia nigra, and superior colliculus which project to the pedunculo-pontin nucleus in the mesencephalic locomotor region. These nuclei have reward-relevant functions and may be part of a final common pathway for behavioral output from the forebrain (Bechara and van der Kooy 1992). Study of the involvement of other biochemical mechanisms in the mesolimbic DA system will help us understand the mechanism of drug dependence and, in return, may help us find some way to control the problem of drug abuse.

BIBILIOGRAPHY

Bedford JA, Bailey LP, and Wilson MC (1978) Cocaine reinforced progressive-ratio performance in the rhesus monkey. Pharmacol. Biochem. Behav. 9: 631-638.

Belluzzi JD and Stein L (1977) Elkephaline may mediate euphoria and drive-reduction reward. *Nature* 266:556-558

Carboni E, Imperato A, Perzzani L, and Di Chiara G (1989) Amphetamine, cocaine, phencyclidine and nomifensine increase extracellular dopamine concentrations preferentially in the nucleus accumbens of freely moving rats. Neuroscience 28:635-661

Cunningham KA, Dworkin SI and Smith JE (1992) Neurobiology of cocaine: reinforcing and stimulus effects. In: Cocaine: pharmacology, physiology, and clinical strategies. Lakoski JM, Galloway MP and White FJ, Eds: 91-113. CRC Press Inc. Boca Raton Ann Arbor London.

Dantzer R (1976) Effect of diazepam on performance of pigs in a progressive-ratio schedule. Physiol. Behav. 17:161-163.

De Montis MG, Devoto P, Meloni D, Gambarana C, Giorgi G & Tagliamonte A (1992) NMDA receptor inhibition prevents tolerance to cocaine. *Pharmacol Biochem Behav.* 42(1):179-82.

Di Chiara G and North AR (1992) Neurobiology of opiate abuse. Trends Pharmacol Sci 13: 185-193.

Di Chiara G, and Imperato A (1988) Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc Natl Acad Sci* (USA) 85:5274-5278

Donzanti BA and Uretsky NJ (1983) Effects of excitatory amino acids on locomotor activity after bilateral microinjection into the rat nucleus accumbens: possible dependence on dopaminergic mechanisms. *Neuropharmacology* 22:971-81

Emmet-Oglesby,M.W., Peltier, R.L., Depoortere, R.Y., Pickering, C.L., Hooper, M.L., Gong, Y.H. and Lane, J.D. (1993) Tolerance to self-administration of cocaine in rats: time course and dose-response determination using a multi-dose method. *Drug. Alcohol Depend.*

Emmett-Oglesby, M.W. and Lane, J.D. Tolerance to the reinforcing effects of cocaine. *Behav. Pharmacol.* 3:193-200, 1992.

Ettenberg A, Pettit HO, Bloom FE and Koob GF (1982) Heroin and cocaine intravenous self-administration in rats: mediation by separate neural systems. Psychopharmacol 78:204-9

Finkle BS, McCloskey KL (1977) The forensic toxicology of cocaine. In: Cocaine: 1977. Washington, DC: National Institute on Drug Abuse Research Monograph 13, Department of Health, Deucation, and Welfare Publication No. (ADM) 79-741, US Government Printing Office; 153-192

Foltin RW, Woolverton WL and Schuster CR (1983) Effects of psychomotorstimulants, alone and in pairs, on milk drinking in the rat after intraperitoneal and intragastric administration. J Pharmacol Exp Ther 226:411-418

Foltin RW and Fischman MW (1992) The cardiovascular and subjective effects of intravenous cocaine and morphine combinations in humans. J Pharmacol Exp Ther 261:623-632.

Freed WJ and Cannon-Spoor HE (1990) A possible role of AA2 excitatory amino acid receptors in the expression of stimulant drug effects, *Psychopharmacology* 101:456-464

FullerTA, Ruschen FT and Price JL (1987) Source of presumptive glutamatergic/aspartergic afferents to the rats ventral striatopallidal region. *J Comp Neurol* 258: 317-338

Gastfriend DR, Mendelson JH, Mello NK, Teoh SK and Reif S (1993) Buprenorphine pharmacotherapy for concurrent heroin and cocaine dependence. Am J Addict 2:269-278.

Griffiths RR, Bigelow GE, and Henningfield JE (1980) Similarities in animal and human drug-taking behavior. In: Mello, N.K., ed. Advances in Substance Abuse: Behavioral and Biological Research, vol. 1. Greenwich: JAI Press; 1-90.

Griffiths RR, Brady JV, and Snell JD (1978) Progressive-ratio performance maintained by drug infusions: comparison of cocaine, diethylpropion, chlorphentermine, and fenfluramine. Psychopharmacology. 56: 5-13.

Sonsalla PK. Manzino L. Heikkila RE (1988) Interactions of D1 and D2 dopamine receptors on the ipsilateral vs. contralateral side in rats with unilateral lesions of the dopaminergic nigrostriatal pathway. J Pharmacol Exp Ther 247:180-5

Heikkila RE, Manzino L and Cabbat FS (1981) Stereospecific effects of cocaine derivatives on 3H-dopamine uptake: correlations with behavioral effects. Sub Alcohol Actions Misuse 2:115-121.

Heikkila RE, Orlansky H, and Cohen G (1975) Studies on the distinction between uptake inhibition and release of (^{3}H)dopamine in rat brain tissue slices. Biochem Pharmacol 24:847-852.

Hodos W (1961) Progressive-ratio as a measure of reward strength. Science 134: 943-944.

Hubner CB and Moreton JE (1991) Effects of selective D1 and D2 dopamine antagonists on cocaine self-administration in the rat. Psychopharmacology. 105: 151-156.

Hubner CB and Koob GF (1990) The ventral pallidum plays a role in mediating cocaine and heroin self-administration in the rat. *Brain Res* 508:20-29

Iwamoto ME and Martin W (1988) A critique of drug self-administration as a method for predicting abuse potential of drugs. In: Harris, L.S., ed. Problems of Drug Dependence. Proceedings of the Committee on Problems of Drug Dependence. Washington: U S. Government Printing Office; pp. 457-465.

Izenwasser S and Cox BM (1992) Inhibition of dopamine uptakeby cocaine and nicotine: tolerance to chronic treatment. *Brain Res* 573:119-125

Jaffe J (1990) Drug addiction and drug abuse. In: *Goodman and Gilman's the Pharmacological Basis of Therapeutics,* Gilman AG, Rall TW, Nies AS & Taylor P, eds. pp 522-573, Pergamon Press, New York, 1990.

Johanson CE, Vaccarino F, Amalric M and Bloom FE (1987) Positive reinforcement properties of drus: search for neural substrates. In: Engel J, Oreland L (eds) Brain reward systems and abuse. Raven Press, New York: 35-50

Johanson CE, Schuster CR (1981) A comparison of the behavioral effects of *l*- and *dl*-cathinone and *d*-amphetamine. J Pharmacol Exp Ther 219:355-362

Kalant H and Khanna JM (1990) Methods for the study of tolerance, . In: Adler C, Cowan A (eds) Testing and evaluation of drugs of abuse, Vol 6, *Modern Methods in Pharmacology*, Wiley-Liss, New York, pp. 43-66

Kalivas PW and Alesdatter JE (1993) Involvement of NMDA receptor stimulation in the VTA and amygdala in behavioral sensitization to cocaine. *J Pharmacol Exp Ther* 267:486-495

Kalivas PW and Duffy P (1990) Effect of acute and daily cocaine treatment on extracellular dopamine in the nucleus accumbens. Synapse 5:48-58

Kalivas PW and Duffy P (1988) Effects of daily cocaine and morphine treatment on somatodendritic and terminal field dopamine release. J Neurochem 50:1498-1504

Karler R and Calder LD (1992) Excitatory amino acids and the actions of cocaine. *Brain Res* 582:143-146

Karler R, Calder LD, and Turkanis SA (1991) DNQX blockade of amphetamine behavioral sensitization. *Brain Res* 552:295-300

Karler R, Calder LD, Chaudhry IA, and Turkanis SA (1989) Blockade of reverse tolerance to cocaine and amphetamine by MK-801. *Life Sci* 45:599-606

Katz JL, Griffiths JW, Sharpe LG, De Souza EB Witkin JM (1993) Cocaine tolerance and cross-tolerance. J Pharmacol Exp Ther 264: 183-192

Katz JL (1990) Models of relative reinforcing efficacy of drugs and their predictive utility. Behav. Pharmacol. 1: 283-301.

Khanna JM, LeBlanc AE & Le AD (1982) Overview: historical overview of tolerance and physical dependece. In: *Ethanol Tolerance and Dependence*: Endocrinological Aspects, Cicero, TJ, ed, pp 415. US Govt Printing Office, Washington, DC, 1982

Koob GF (1992a) Neural mechanisms of drug reinforcement. *Ann NY Acad Sci* 654: 171-191

Koob GF (1992b) Drugs of abuse: anatomy, pharmacology and function of reward pathways. Trends Pharmacol Sci 13: 177-184.

Koob GF and Bloom FE (1988) Cellular and molecular mechanisms of drug dependence. Science 242:715-723

Koob GF, Vaccarino FJ, Amalric M, and Swerdlow, NR (1987) Neural substrates for cocaine and opiate reinforcement. In: Fisher S, Raskin A, and uhlenhuth EH, ed. *Cocaine:Clinical and Behavioral Aspects*. New York: Oxford, 1987, pp. 80-107

Kosten TR, Rounsaville BJ, Gawin FH and Kleber HD (1986) Cocaine abuse among opioid addicts: Demographic and diagnostic factors in treatment. Am J Drug Alcohol Abuse 12:1-16.

Kozel NJ and Adams EH (1986) Epidemiology of drug abuse: An overview. Science 34:970-974.

Kuhar MJ, Ritz MC, Grigoriadis D and Lew R (1992) A cocaine receptor associated with dopamine transport and drug self-administration. In: Cocaine: pharmacology, physiology, and clinical strategies. Lakoski JM, Galloway MP and White FJ, Eds: 191-202. CRC Press Inc. Boca Raton Ann Arbor London.

Loh EA and Roberts DCS (1990) Breakpoints on a progressive-ratio schedule reinforced by intravenous cocaine increase following depletion of forebrain serotonin. Psychopharmacology. 101: 262-266.

LeMoal M and Simon H (1991) Mesocorticolimbic dopaminergic network: functional and regulatory roles. Physiol Rev 77:155-234

McGregor A & Roberts DCS (1993) Dopaminergic antagonism within the nucleus accumbens or the amygdala produces differential effects on intravenous cocaine self-administration under fixed and progressive ratio schedules of reinforcement. Brain Research, 624:245-252

McKearney JW (1982) Effects of dopamine uptake inhibitors on schedule-controlled behavior in the squirrel monkey . Psychopharmacol 78:377-379

Mello NK, Negus SS, Lukas SE, Mendelson JH, Sholar JW and Drieze J (1995) A primate model of polydrug abuse: cocaine and heroin combination. J Pharmacol Exp Ther 274:1325-1337

Misra AL (1976) Cocaine: Chemical, Biological, Clinical, Social and Treatment Aspects. In: Mule, SJ (ed), CRC Press, Cleveland, pp 73-90

Mogenson GJ, Wu M, and Tsai CT (1989) Subpallidal-pendunculopontine nucleus projections, but not subpallidal-mediodorsal thalamus projections contribute to spontaneous exploratory locomotor activity. *Brain Res* 485:396-8

Payson MM and Donzanti BA (1989) Effect of excitatory amino acids on in vivo dopamine release and metabolism in the nucleus accumbens. *Soc Neurosci Abstr.* 584.

Peltier RL, Springfield A, Emmett-Oglesby MW, Lal H and Wallis CJ (1996) Chronic cocaine treatment does not produce pharmacokinetic tolerance to cocaine in a self-administration paradigm. Graduate dissertation and in preparation in Pharmacol Biochem Behav.

Peltier RL, Li D-H, Lytle D, Taylor CM, and Emmett-Oglesby MW (1996) Chronic *d*-amphetamine or methamphetamine produces cross-tolerance to the discriminative and reinforcing stimulus effects of cocaine. in press in J Pharmacol Exp Ther in press

Peltier RL and Emmett-Oglesby MW (1994) CNS stimulants produce cross-tolerance to cocaine in an FR 2 schedule of cocaine self-administration. *National Institute on Drug Abuse Research Monograph Series* 153:459

Peltier RL and Emmett-Oglesby (1993) Cross-tolerance between CNS stimulants in a self-administration paradigm in rats. *Abstract of Society for Neuroscience* 423

Petit HO, Ettenberg A, Bloom FE and Koob GF (1984) Destruction of dopamine in the nucleus accumbens selectively attenuates cocaine but not heroin self-administration in rats. Psychopharmacology 84:167-173.

Pulvirenti L, Maldonado-Lopez R and Koob GF (1992) NMDA receptors in the nucleus accumbens modulate intravenous cocaine but not heroin self-administration in the rat. *Brain Res.* 594:327-330

Rao TS, Cler JA, Mick SJ, Emmett MR, Farah Jr. Jm, Contreras PC, Iyengar S and Wood PL (1991) Neurochemical interactions of competitive N-methyl-D-aspartate antagonists with dopaminergic neurotransmission and the cerebellar cyclic GMP system: functional evidence for a phasic glutamatergic control of the nigrostriatal dpaminergic pathway. *J Neurochem* 56:907-913

Risner ME and Cone EJ (1986) Intravenous self-administration of fencamfamine and cocaine by beagle dogs under fixed-ratio and progressive-ratio schedules of reinforcement. Drug Alc. Dep. 17: 93-101.

Risner ME and Silcox DL (1981) Psychostimulant self-administration by beagle dogs in a progressive-ratio paradigm. Psychopharmacology. 75: 25-30.

Ritz MC, Cone EJ and Kuhar MJ (1990) Cocaine inhibition of ligand binding at dopamine, norepinephrine and serotonin transporters: A structure-activity study. *Life Sci* 46: 635-645

Ritz MC, Lamb RJ, Goldberg SR and Kuhar MJ (1987) Cocaine receptors on dopamine transporters are related to self-administration of cocaine. Science 237:1219-1223

Roberts DCS and Richardson NR (1992) Self-administration of psychomotor stimulants using progressive ratio schedules of reinforcement. In:Boulton AA, and Wu PH ed Animal Models of Drug addiction. *Neuromethods* 24: 233-269

Roberts DCS (1989) Breaking points on a progressive-ratio schedule reinforced by intravenous apomorphine increase daily following 6-hydroxydopamine lesions of the nucleus accumbens. Pharmacol. Biochem. Behav. 32: 43-47.

Roberts DCS, Bennett SAL, and Vickers GJ (1989) The estrous cycle affects cocaine self-administration on a progressive-ratio schedule in rats. Psychopharmacology. 98: 408-411.

Sample JC Concept of polydrug use. In: Richards, LG; Blevens, LB, eds. The epidemiology of drug abuse. Research Monograph No. 10. Rockville, MD: National Institute on Drug Abuse; 1977:19-31

Sesack SR and Pickel VM (1990) In the rat model nucleus accumbens, hippocampal and catecholaminergic terminals converge on spiny neurons and are in apposition to each other. *Brain Res* 527:266-279

Scheel-Kruger J, Braestrup C, Nielson M, Golembiowska K and Modilnicka E (1976) Cocaine: Discussion on the role of dopamine in the biochemical mechanism of action. In Cocaine and Other Stimulants. EH Ellinwood and Kilbey MM, Eds: 373-407. Plenum Press. New York.

Stewart J and Druhan JP (1993) The development of both conditioning and sensitization of the behavioral activating effects of amphetamine is blocked by the non-competitive NMDA receptor antagonist, MK-801. *Psychopharmacology* 110:125-132

Teoh SK, Mello NK, Mendelson JH, Kuehnle J, Castfriend DR, Rhoades E and Sholar W (1994) Buprenorphine effects on morphine and cocaine induced subjective responses by drug-dependent men. J Clin Psychopharmacol 14:15-27

Tutton CS and Crayton JW (1993) Current pharmacotherapies for cocaine abuse: A review. J Addict Dis 12: 109-127

Ward SA (1995) Characterization of tolerance and cross-tolerance between noncompetitive N-methyl-D-aspartate (NMDA) antagonist in rats trained to self-administer ketamine. Graduate Dissertation of fall

Wilson MC, Schuster CR (1973) Cholinergic influence on intravenous cocaine self-administration by rhesus monkeys. Pharmacol Biochem Behav 1: 643-649

Winger G and Woods JH (1985) Comparison of fixed-ratio and progressive-ratio schedules of maintenance of stimulant drug-reinforced responding. Drug Alc. Dep. 15: 123-130.

Wise RA (1992) Cocaine reward and cocaine craving: the role of dopamine in perspective. 191-206.

Wise RA and Rompre PP (1989) Brain dopamine and reward. *Ann Rev Psychol* 40:191-225.

Wise RA (1987) The role of reward pathways in the development of drug dependence. Pharmacol Ther 35: 227-263.

Wise RA and Bozarth MA (1987) A psychomotor stimulant theory of addiction. Psychol Rev 94:469-492.

Wise RA (1984) Neural mechanisms of the reinforcing actions of cocaine. NIDA Mgr. 50:15-33

Wise RA, Yokel RA, Hansson P and Gerber GJ (1977) Concurrent intracranial self-stimulation and amphetamine self-administration in rats. Pharmacol Biochem Behav 7: 459-461

de Wit H, Wise RA (1977) Blockade of cocaine reinforcement in rats with the dopamine receptor blocker pimozide, but not the noradrenergic blockers phentolamine and phenoxybenzamine. Can J Psychol 31:195-203

Withers NW, Pulvirenti L, Koon GF and Gillin JC (1995) Cocaine abuse and dependence. *J Clin Psychopharmacol* 15:63-78.

Wolf ME and Khansa MR (1991) repeated administration of MK-801 produces sensitization to its own locomotor stimulant effects but blocks sensitization to amphetamine. *Brain Res* 562:164-168

Wood DM & Emmett-Oglesby M.W. (1986) Characteristics of tolerance, recovery from tolerance, and cross-tolerance to cocaine used as a discriminative stimulus. *J. Pharmacol. Exp. Ther.* 237: 120-125.

Woolverton WL and Johnson KM (1992) Neurobiology of cocaine abuse. *TIPS* 13: 201-209

Woolverton WL (1991) Discriminative stimulus effects of cocaine. NIDA Res Mongr 116:61-74

Woolverton WL and Kleven MS (1988) Multiple dopamine receptors and the behavioral effects of cocaine. NIDA Res Monogr 88:160-184

Yanagita T (1973) An experimental framework for evaluation of dependence liability of various types of drugs in Monkeys. *Bulletin on Narcotics* 25: 57- 64

Young AM and Herling S (1986) Drug as reinforcers: Studies in laboratory animals. In Goldberg SR, Stolerman IP (eds): "Behavioral Analysis of Drug Dependence". New York : Academic Press:9-68.