NEUROPHARMACOLOGICAL CHARACTERISTICS OF TOLERANCE FOR COCAINE USED AS A DISCRIMINATIVE STIMULUS

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The main purpose of this research was to investigate the phenomenon of tolerance to cocaine. Tolerance is operationally defined as a decreased drug effect due to prior history of drug administration. The animal model that was chosen to investigate tolerance to cocaine was the drug discrimination model, which is an animal analogue of human subjective drug effects. In the drug discrimination procedure, animals are trained to emit one behavior when injected with drug, and a different behavior when injected with saline. In the present experiments, rats were trained to press one lever when injected with cocaine, 10 mg/kg, and a different lever when injected with saline for food reinforcement. Once rats are trained, they can accurately detect the cocaine stimulus greater than 95% of the time.

Using this model, tolerance to the cocaine stimulus was investigated, and the following research objectives were established: 1) factors governing the occurrence of tolerance and cross-tolerance to cocaine, 2) the role of dopamine-receptor stimulation in the development of tolerance to cocaine, 3) the role of specific brain sites in

mediating the cocaine cue, and 4) whether behavioral tolerance occurs simultaneously with tolerance to the discriminative stimulus.

To meet these objectives, 15 experiments were conducted to investigate these questions. Results indicated that 1) tolerance develops to cocaine, 2) rats recover spontaneously from tolerance following cessation of cocaine administration, 3) amphetamine anologs produce tolerance and cross-tolerance profiles similar to cocaine, 4) tolerance to the cocaine stimulus is mediated by dopamine receptors, possibly specifically related by D2 receptors, 5) the cocaine stimulus is centrally mediated, with site specificity in the nucleus accumbens, and 6) chronic cocaine administration produces simultaneous tolerance to the behaviorally disruptive effects of cocaine. These findings suggest that investigations of tolerance in the drug discrimination procedure may have potential for establishing a comprehensive evaluation of the abuse liability of cocaine, and neurological mechanisms mediating tolerance to the cocaine stimulus.

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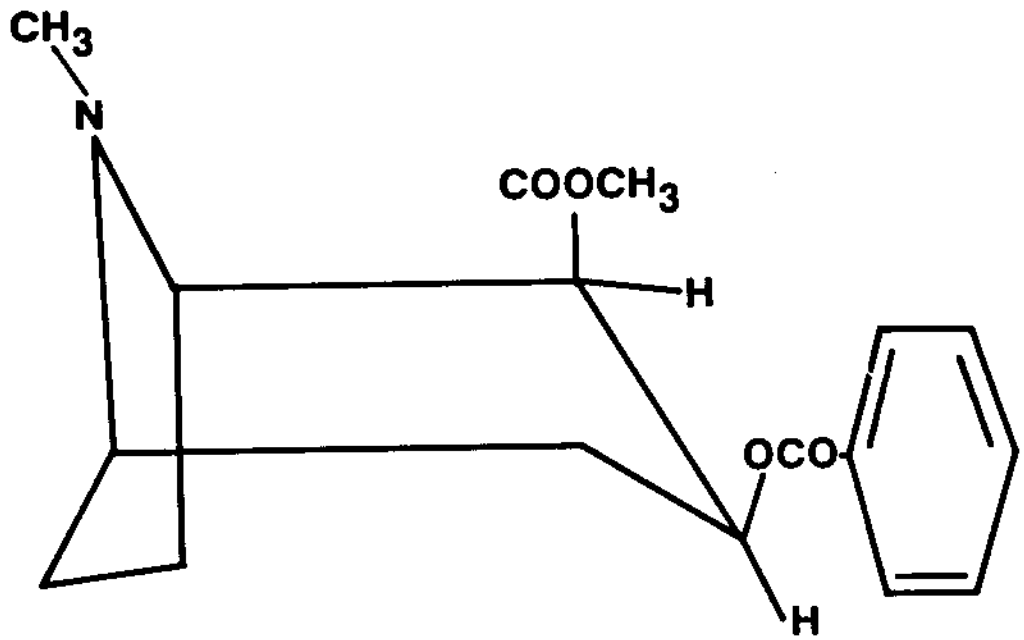
CHAPTER I

INTRODUCTION

Cocaine is a naturally occurring alkoloid obtained from the coca leaves of the shrub Erythroxylon coca. Its chemical nomenclature (in Chemical Abstracts) is 8-azo-bicyclo <3.2.1> octane-2-carboxylic acid, 3-benzoyloxy-8-methyl methyl ester <1-R-(exo-exo)> (Figure 1). The coca shrub normally grows in western South America and is also cultivated in Java and Mexico. The use of cocaine in man extends over several centuries. According to Inca records, in South America some 3,000 years ago coca leaves were given as a reward for special services (Cohen, 1975). Coca leaves were also used to increase stamina, and when food was scarce, the leaves were used as a dietary supplement.

Cocaine was first chemically isolated from coca leaves in 1860, and shortly thereafter, one of the first scientific studies on the subjective effects of cocaine was conducted by Sigmund Freud. Since that time cocaine has retained its popularity as the drug of choice over all other types of recreational drugs. Standard subjective questionaires, including the Profile of Mood States (POMS) have consistently reported the stimulatory and euphoric effects of cocaine (Fischman, Schuster, and Hatano, 1983a; Fischman,



COCAINE

Figure 1. Chemical structure of cocaine.

Schuster, Javaid, and Hatano, 1983b). These subjective effects are similar to those produced by amphetamine, and in comparison studies, cocaine and amphetamine cannot be differentiated (Fischman and Schuster, 1982). Other subjective effects described by humans include increased scores on scales of "pleasantness" (Resnick, Kestenbaum, and Schwartz, 1977), "high" (Fischman et al., 1983a; Fischman et al., 1983b), and "stimulation" (Fischman et al., 1983a; Fischman et al., 1983b). Decreased scores have been reported for "hunger" (Resnick et al., 1977) and "sedation" (Fischman, 1977).

The euphoria obtained from cocaine injected intravenously peaks approximately 3-5 minutes after injection, and is dissipated within 30-40 minutes (Javaid, Fischman, and Schuster, 1978). Similarly, subjective effects reported after intranasal administration of cocaine are quite similar to those after intravenous administration, but are less intense (Resnick et al., 1977), have a slower onset of action (15-20 min.)(Van Dyke, Jatlow, Ungerer, Barash, and Byck, 1979), and are of longer duration (60-90 min.)(Javaid et al, 1978). Cocaine can be also administered orally, or smoked as the base, and the effects of these routes of administration are similar to those of cocaine administered intranasally (Perez-Reyes, DiGuiseppi, Ondrusek, Jeffcoat, and Cook, 1982).

While there have been numerous studies investigating

the subjective effects of cocaine in humans, there have been few studies of these effects using animal models. A major factor limiting pre-clinical measurement of subjective aspects of cocaine dependence has been a lack of a suitable methodology for testing such questions. By definition, subjective effects are not directly verifiable by experimenter observation, and because of the dangers of anthropomorphism, animal investigations of subjective events are particularly difficult. However, Lal and Emmett-Oglesby (1983) have recently argued that subjective events can be tested experimentally in animals if behavioral responses can be made specifically contingent upon detection of the subjective occurrence by the test subject. For example, subjects can be trained to use the internal discriminative stimuli (IDS) arising from drug injections as the basis for choosing which of several potential responses is correct. When only two responses are available, the response emitted, be it human-verbal or animal-choice behavior, resolves to "yes, the stimulus is present," or "no, it is not." The qualitative nature of such a binary decision can then be quantified through the method of population analysis; that is, the percent of subjects reporting the presence of the subjective event is a function of the stimulus intensity (Swanson, and Kinsbourne, 1978). In the past decade, many investigations have shown that subjective events arising from drug administration can be detected using

discriminative stimulus methodology, and where direct comparisons have been made, drug effects thus measured are classified in parallel (e.g., LSD-like, narcotic-like) by humans and animals (Chait, Uhlenhuth and Johanson, 1984; Glennon and Rosecrans, 1981; Griffiths, Roache, Ator, Lamb and Lukas, 1985; Schuster and Balster, 1977).

Cocaine as a Discriminative Stimulus

The relationship between a particular stimulus and the resulting behavioral response has been extensively analyzed. The term "stimulus control of behavior" has emerged to relate these concepts. One such stimulus is the discriminative stimulus, where one set of behaviors is reinforced under one set of conditions (e.g., presence of a drug) and a second set of behaviors is reinforced under another set of conditions (e.g., absence of a drug). For example, rats can be trained to discriminate an injection of cocaine from saline using an operant procedure where responses on one lever are reinforced with food reward only in the presence of cocaine, and responses on the second lever are reinforced only in the presence of saline. Various scheduling contingencies of reinforcement can be employed which provide sensitive behavioral baselines against which the drug effects can be measured. For example, a frequently used reinforcement contigency is the fixed-ratio schedule (FR). The FR schedule requires that a

behavior is reinforced after a specified number of responses regardless of time. For example, a FR10 schedule of bar pressing would require a rat to press the bar ten times before a food reward would be given. Other scheduling contigencies include variable-ratio (VR), where a behavior is reinforced after a random number of responses; fixed-interval (FI), where a behavior is reinforced after a designated time period; and variable-interval (VI), where a behavior is reinforced after a random time interval. Cocaine's effect on operant responding depends on the rate of ongoing responding. For example, cocaine tends to increase low rates of responding using a fixed-interval schedule. However, cocaine tends to decrease or leave unchanged high rates of responding such as the fixed-ratio schedule (Seiden and Dykstra, 1981). For this reason the fixed-ratio schedule is a popular contigency schedule with drug discrimination procedures.

Another important feature of the discriminative stimulus produced by cocaine is the relationship of lower doses of cocaine compared to the cocaine training drug, which is commonly called stimulus generalization. Stimulus generalization is considered to reflect perceptual similarity of the test stimulus to the discriminative stimulus (Colpaert, 1978). For example, in rats trained to detect 5 mg/kg cocaine, when lower doses of cocaine are tested for cocaine lever selection, there is a dose-

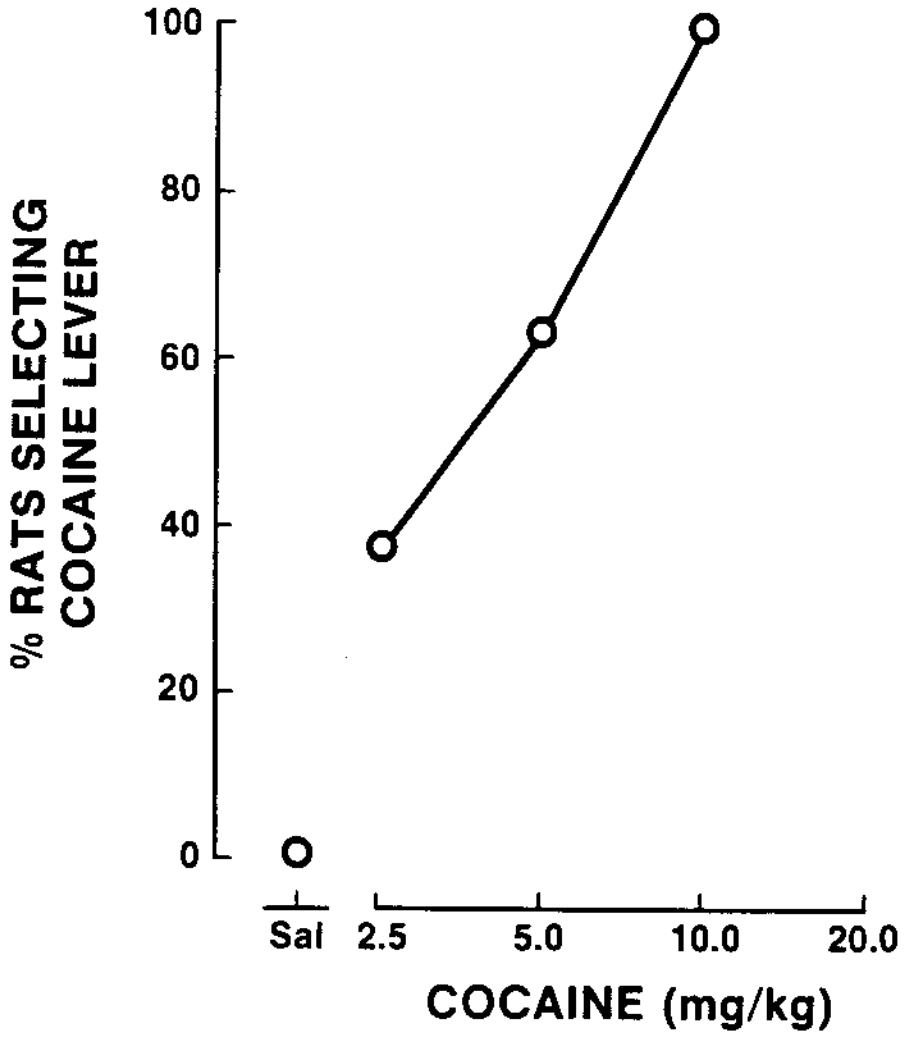


Figure 2. Generalization of cocaine to the cocaine stimulus. Abscissa: dose of cocaine. Ordinate: percentage of rats completing the first ten responses on the cocaine-appropriate lever. Rats were trained to detect cocaine (10 mg/kg) for approximately 80 prior to testing with lower doses of cocaine. Eight rats were tested at all points.

dependent relationship such that decreasing doses of cocaine produce a decrease in cocaine-lever selection (Figure 2). These data support the hypothesis that the stimulus produced by cocaine is orderly and related to its physical intensity.

Specificity of Cocaine as a Discriminative Stimulus

One of the major strengths of the drug discrimination procedure is that the stimulus produced by cocaine is associated with specific pharmacological actions. The major pharmacological actions produced by cocaine are its anesthetic, vasoconstrictive, and euphoric properties. Barry (1974) has proposed that the discriminative stimulus properties of drugs may be used to classify them since closely related substances typically produce similar discriminative cues. Following this rationale, once the discriminative stimulus of cocaine has been established, other drugs can be substituted, and the test subjects will substitute for drugs with CNS stimulant properties similar to cocaine. For example, in one study (Colpaert, Niemegeers and Janssen, 1978a), rats were trained to discriminate 10 mg/kg cocaine from saline and a variety of drugs was tested for substitution for the cocaine discriminative stimulus. Results indicated that amphetamine-type drugs such as methylphenidate, nomifensine, d-amphetamine, and methamphetamine would substitute for the cocaine stimulus. Other studies which have confirmed these results include

substitution of amphetamine (Colpaert, Niemegeers and Janssen, 1978b; D'Mello and Stolerman, 1977), and methylphenidate (Ho and Silverman, 1978; Emmett-Oglesby et al., 1983) for the cocaine stimulus (Table I). Conversely, drugs such as LSD, morphine, and pentobarbital (Jarbe, 1984); THC (Jarbe 1981); oxazepam (de la Garza and Johanson, 1981; imipramine, fenfluramine, strychnine or catepresan would not substitute for the cocaine stimulus (Table II). Thus, cocaine and amphetamine possess similar discriminative stimulus properties, and the specificity of the cocaine stimulus supports Barry's (1974) hypothesis that the discrimination paradigm could quantitatively assess a drug's pharmacological effects and classify them according to similar discriminative stimulus properties.

Tolerance to the Subjective Effects of Cocaine

There has been much controversy as to whether the pharmacological actions of cocaine are subject to tolerance. Tolerance often develops upon repeated administration of drugs, and is usually described as a decreased drug-effect related to a prior history of drug administration (Goldstein, Aronow and Kalman, 1974, Goodman and Gillman, 1985). Tolerance can also be described as the phenomenon whereby a greater amount of drug is required to obtain a response whose intensity is similar to that of the original response (Colpaert, 1978). Typically, when a particular

TABLE I

REPRESENTATIVE LIST OF DRUGS THAT SUBSTITUTE FOR COCAINE

Amantadine
Apomorphine
Bromocryptine
Piribedil
Methylphenidate
Nomifensine
d-amphetamine
Methamphetamine
Diethylpropion
Phentermine
Phendimetrazine
Phenylpropanolamine
Cathinone
Benzoylnorecognine
Norcocaine
Tranylcypromine
Pheniprazine
Deprenyl
Pargyline
Nialamide

TABLE II

REPRESENTATIVE LIST OF DRUGS THAT DO NOT SUBSTITUTE FOR COCAINE

Isoproternol
Epinephrine
Propanolol
Practolol
Salbutamol
Atropine
Morphine
Fentanyl
LSD
Mescaline
Imipramine
Desipramine
Chlorodiazepoxide
Lidocaine
Procaine
Fenfluramine
Hydroxyamphetamine

dose is administered repeatedly, tolerance is graphically assessed as a decreased effect of that dose and/or a shift to the right of the entire dose-effect curve. However, with respect to cocaine, it is apparent that repeated administration per se does not guarantee tolerance.

Tolerance to cocaine in man has been reported only rarely. There have been several anecdotal reports suggesting that tolerance may develop, since substantial amounts of cocaine, up to 10 gm per day, have been used by chronic cocaine users. Because the minimum lethal dose of cocaine is approximately 1.2 gm, these reported increases in drug administration were suggestive of a tolerance phenomenon, although probably through a metabolic mechanism (Caldwell and Seever, 1974; Jaffe, 1975). Fischman and Schuster (1982) reported that acute tolerance to cocaine developed if patients self-administered 32 mg cocaine within a one-hour period. In this study, the discriminability of cocaine was tested every 15 minutes by using the Profile of Mood States and a subjective effects questionnaire. The data indicated that as chronic administration of cocaine continued, patients detected a decrease in euphoria. This study was extended in 1985 (Fischman, Schuster, Javaid, Hatano and Davis), in which 8 subjects received an intranasal pretreatment of placebo or 96 mg of cocaine. Sixty minutes later 16, 32 or 48 mg of cocaine were injected i.v. Cocaine plasma levels were determined periodically

over a 2-hr period and both cardiovascular effects and verbal report of drug effects, using Profile of Mood States and subjective questionnaire, were monitored. The plasma concentrations of cocaine were always related to the dose administered. However, when i.v. cocaine was given after 96 mg intranasal pretreatment, both heart rate and the subjective effects were diminished compared to the control group. These results suggested that there is a decrease in physiological and subjective effects of cocaine when administered repeatedly in humans.

Tolerance to the Discriminative Stimulus Properties of Drugs

The problem of whether tolerance develops to the discriminative stimulus properties of drugs has been an area of much controversy. For example, York and Winter (1975) trained rats to detect 80 mg/kg sodium barbital from saline. During training, additional daily doses of barbital (240 mg) were administered for 8 days. Tolerance developed to the hypnotic effect, but not to the discriminative stimulus properties of barbital. However, Hirschorn and Rosecrans (1974) trained rats to discriminate 10 mg/kg morphine and 4 mg/kg delta-9 tetrahydrocannibol from saline. During training, additional morphine or delta-9 tetrahydrocannibol injections were given as doses up to 16 times the training dose following a training session for a period of 2 months.

Results indicated that additional injections of 40 or 80 mg/kg, but not of 8 or 160 mg/kg of morphine decreased morphine lever selection. Similarly, additional injections of 32 mg/kg, but not of 8 or 16 mg/kg delta-9 tetrahydrocannibol decreased delta-9 tetrahydrocannibol lever selection. Following testing, naloxone was injected in the morphine group, and withdrawal symptoms occurred. These data were the first data demonstrating tolerance to the stimulus effects of morphine and delta-9 tetrahydrocannibol, respectively.

In another experiment, Colpaert, Kuyps, Niemegeers, and Janssen (1976) trained rats to discriminate 0.04 mg/kg fentanyl from saline. During the course of training dose-effect data were determined for various doses of fentanyl (0.0025-0.02 mg/kg), and substitution tests were conducted with morphine (2.5-20 mg/kg). It was found that although 0.04 mg/kg fentanyl injections were regularly continued as part of the training sequence, the dose-effect curves for both fentanyl and morphine did not significantly decrease after four months of training. Nevertheless, significant tolerance to the rate decreasing as well as to the analgesic effect of fentanyl had developed, but none of the subjects showed any sign of physical dependence after naloxone administration. These data were interpreted as evidence that tolerance does not develop to the discriminative effects of narcotic drugs. Colpaert et al. (1976) suggested

four alternatives accounting for the contradictory results. One argument is that the training drug, fentanyl, may produce less tolerance than morphine, or that the amount of narcotic drugs injected during the course of the experiments was too low to produce any tolerance at all. Second, the tolerance to morphine's stimulus properties is related to the induction of dependence demonstrated by withdrawal symptoms following naloxone administration. Colpaert argued that morphine dependence may disrupt discriminative responding. Third, the occurrence of tolerance to the stimulus properties of fentanyl and morphine may be related to an adaptive learning process. That is, by increasing additional doses of morphine higher than the training dose, the subjects may be learning to attenuate to the higher morphine dose as part of an adaptive learning process. Fourth, the tolerance observed in Hirschorn and Rosecrans (1974) study may relate to tolerance to state-dependent learning effects because in the above study, the measurement of lever selection consists of the percentage of responding on the morphine-appropriate lever, assessed during an extinction trial (Colpaert, Niemegeers and Janssen, 1976).

Shannon and Holtzman (1976) and Miksic and Lal (1977), in a similar experimental design, trained rats to discriminate morphine from saline. In both studies, high doses of morphine were administered in a period during which the animals were withdrawn from training. In the Shannon

and Holtzman (1976) study, 10 mg/kg morphine, saline, or 17.5 mg/kg pentobarbital were injected every 12 hours for three days. Redetermination of the dose-effect curve for morphine was significantly shifted to the right, whereas the dose-effect curve following chronic saline or pentobarbital administration was not shifted. Similarly, in the Miksic and Lal (1977) study, increasing amounts of morphine were administered (10 mg/kg were increased by 10 mg/kg/injection daily until a terminal dose of 110 mg/kg was reached on the 11th day). At the end of chronic administration, all animals were tested for morphine lever selection and analgesia (tail-withdrawal latency--See Janssen, Niemegeers, and Dory, 1963). Results indicated both tolerance to the discriminative stimulus of morphine and to the analgesic action of morphine. These results were considered as evidence that tolerance develops to the narcotic cue since tolerance developed following chronic administration of morphine, but not pentobarbital. These data further support the hypothesis that the discriminative stimulus properties of narcotic drugs are relatively specific.

Colpaert, Niemegeers, and Janssen (1978) in two further experiments attempted to determined whether tolerance develops to the physiological actions underlying the discriminative stimulus properties of narcotic analgesic drugs. In the first study, rats were given daily exposure to 0.06 mg/kg fentanyl for 30 days before they were trained

to discriminate 0.04 mg/kg fentanyl from saline. Results indicated that, as compared to control group, there was no increase in either the number of training sessions to criterion, or in the ED50 for generalization of fentanyl doses lower than the training dose. In addition, the experimental group failed to show an increase of omission errors (reporting saline in the presence of the fentanyl training dose). In the second study, in rats with a 10-month history of 0.04 mg/kg fentanyl discrimination, additional injections of increasing (0.06-0.16 mg/kg) fentanyl doses followed regular training sessions. Results indicated that there was no increase of errors reporting saline in the presence of the training dose, and no effect on the generalization curve for fentanyl. Based on these results, Colpaert concluded that the tolerance observed in previous experiments is an artifact, for by terminating training and injecting high doses of the training drug, the subjects may actually be learning to attend to the higher magnitude of these doses.

In another experiment, Witkin, Dykstra, and Carter (1981) trained pigeons to discriminate intramuscular injections of 1.0 mg/kg morphine from water. Following dose-effect generalization studies with various doses of morphine (0.1-5.6 mg/kg), a single injection of 10 mg/kg morphine shifted the morphine dose-effect curve to the right; thus these data demonstrate acute tolerance to the

discriminative stimulus properties of morphine. Tolerance to the morphine stimulus was reversible within five days of the single injection of morphine. Tolerance did not develop to the effects of morphine on response rate. The authors concluded that tolerance may occur for different behavioral entities, and the magnitude of tolerance that develops may depend on the amount of drug administered and the behavior being measured. Furthermore, they argued that tolerance to the discriminative stimulus properties of opioids has parallels with the subjective effects of opioids and with opioid self-administration and abuse. For example, Martin and Fraser (1961), in their study of ex-narcotic addicts, demonstrated pronounced tolerance to the subjective intensity of heroin or morphine when subjects were given progressively larger doses of these drugs. Tolerance also appears to develop during self-administration of opioids. Deneau, Yanagita and Seevers (1969) reported increases in daily intake of morphine over a period of weeks in rhesus monkeys. Likewise, opioid abuse in man is often characterized by an escalation in dose with repeated administration (Jaffe, 1980).

Tolerance to the Discriminative Stimulus Properties of Cocaine

With respect to central nervous system stimulants, a similar controversy has arisen concerning tolerance to their

discriminative stimulus, although there have been fewer studies investigating tolerance to CNS stimulants compared to opioids. McKenna and Ho (1977) trained rats to discriminate 10 mg/kg cocaine from saline. Subsequently, training was halted and a subgroup of rats was injected with 20 mg/kg/8hr cocaine for 7 days, while the second subgroup of rats was injected with saline. Following chronic administration, dose-effect data were redetermined for selection of the cocaine lever following 2.5 and 5.0 mg/kg cocaine. Results indicated that there was no significant difference in the cocaine lever selection before and after repeated administration of saline; however, there was a significant decrease in cocaine lever selection before and after repeated administration of cocaine.

Colpaert (1978) argued that the methodology demonstrating tolerance observed with cocaine is similar to the methodology demonstrating tolerance to morphine (Shannon and Holtzman, 1976; Miksic and Lal, 1977) where training is halted, and high doses of drug are administered without training; thus Colpaert (1978) concluded that the animals are being retrained to a higher magnitude of the drug stimulus. Colpaert, Niemegeers and Janssen (1978) reported that sensitivity to the cocaine discriminative stimulus remained constant for 8 months. These results are similar to the Colpaert et al. (1976) study, where fentanyl sensitivity did not diminish after 4 months of training.

Thus, Colpaert argues that tolerance does not occur to the discriminative stimulus properties of cocaine.

A similar report concerning tolerance to the discriminative stimulus properties of d-amphetamine was described by Barrett and Leith (1981). In this experiment three groups of rats were trained to discriminate three doses of d-amphetamine (0.50, 1.0, or 1.5 mg/kg) from saline. Following completion of the dose-effect curve for generalization of various doses of d-amphetamine, testing was halted and subjects were injected chronically with d-amphetamine in escalating doses for 4 days (1 mg/kg initially, followed by increments of 1 mg/kg every 8 hours, so that the final injection was 12 mg/kg, totalling 78 mg/kg for the entire regimen). Dose-effect data were redetermined, and the dose-effect curve for generalization to d-amphetamine was significantly shifted to the right. Barrett and Leith (1981) concluded that the data demonstrate tolerance to the discriminative stimulus properties of d-amphetamine, and they further categorized tolerance in this paradigm as a "pharmacodynamic" tolerance compared to "pharmacokinetic" or "learned" tolerance.

In the laboratory (Wood, Lal and Emmett-Oglesby, 1984), rats were trained to discriminate the stimulus properties of 10 mg/kg cocaine (Figure 3). Using a procedure similar to McKenna and Ho (1977), training was suspended, and cocaine, 20 mg/kg, was injected every 8 hours. Tolerance developed

progressively to the discriminative stimulus properties of cocaine (Figure 4). After 6 days of chronic administration, redetermination of dose-effect data demonstrated tolerance to the discriminative stimulus of cocaine (Figure 5). After termination of chronic administration of cocaine, without further training, the tolerance was lost at the same rate at which it was acquired. Thus, these data strongly suggest that tolerance does occur to the discriminative stimulus properties of cocaine.

In a previous investigation, the effect of cocaine on the behavioral variables of time to obtain first reinforcement and response rate were also recorded. Cocaine dose-dependently produced a decrease in response rate and time to the first reinforcement. Chronic administration of cocaine, 20 mg/kg/8-hr for 6 days, produced no tolerance or sensitization to the disruptive effect of cocaine on response rate or time to the first reinforcement (Figures 6 and 7). These results agree with the Woolverton, Kandel, and Schuster study (1978a), which reported no tolerance to the disruption of response rate on a FR 20 schedule following chronic cocaine administration; however, these results disagree with the above study since no tolerance was found for time to first reinforcement, whereas Woolverton et al. reported tolerance for the duration of pause of first lever responding. Possible explanations for the the lack of tolerance that was observed for time to the first

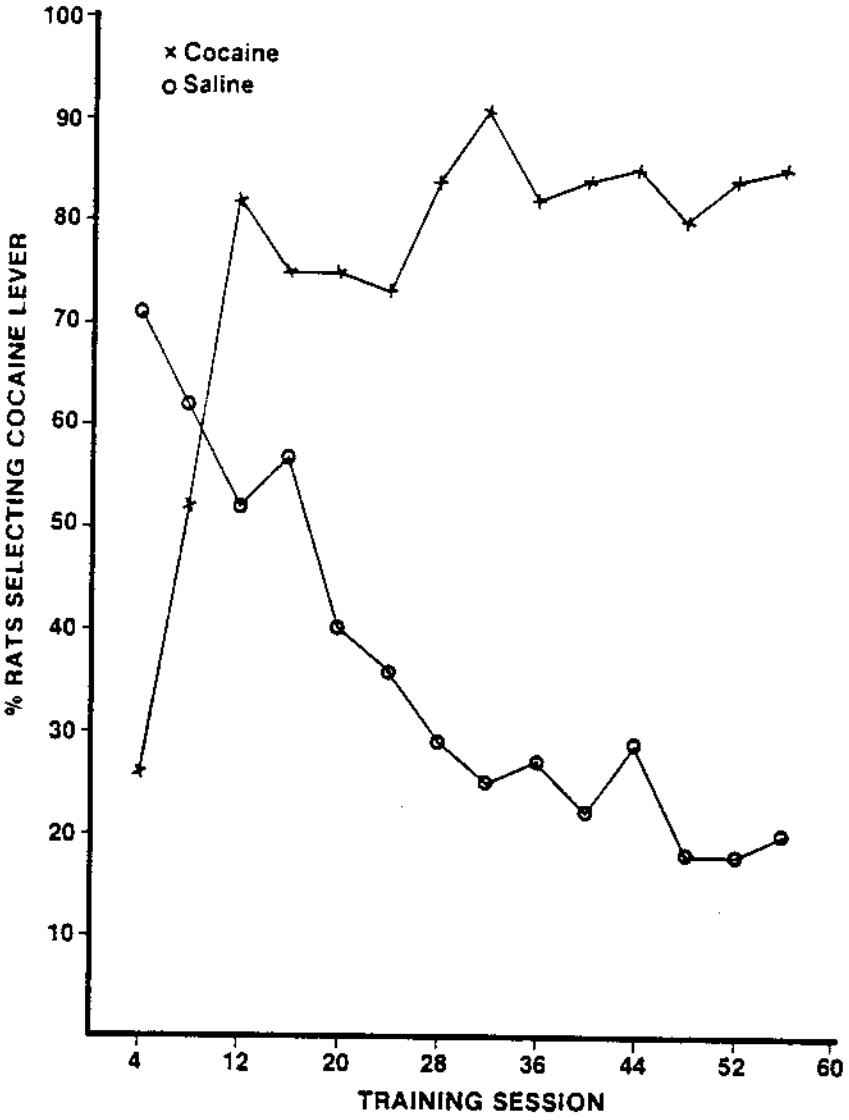


Figure 3. Acquisition of discriminated responding of 5 mg/kg cocaine. Abscissa: 110 total training sessions as shown as 55 sessions with saline and 55 sessions with cocaine. Ordinate: percentage of rats selecting the cocaine-lever. The (×) indicates the % of rats selecting the cocaine lever following a 5.0 mg/kg injection of cocaine; (○) indicates the % of rats selecting the cocaine lever following an injection of saline. Lever selection was defined as the lever in which the first 10 responses occurred in the session. N = 16.

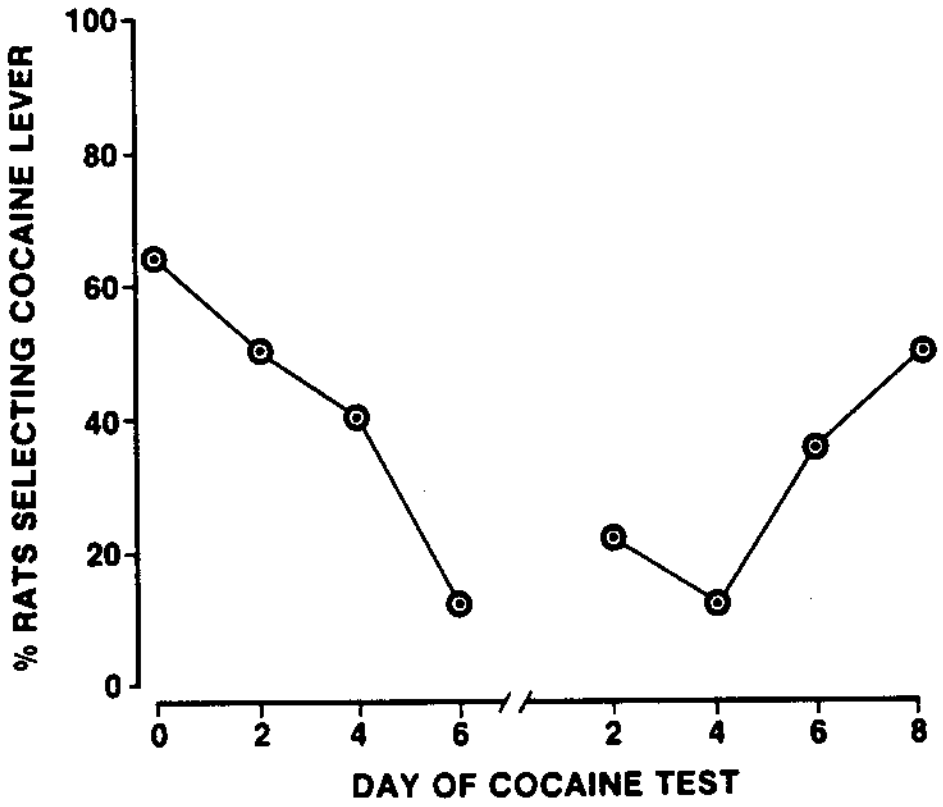


Figure 4. Generalization of 5 mg/kg of cocaine to the stimulus for cocaine before and during chronic administration of cocaine. Abscissa: left half, number of days of chronic administration of 20.0 mg/kg/8-hr; right half, number of days after chronic administration of cocaine was terminated. Ordinate: percentage of rats completing the first 10 responses on the cocaine-lever after a 5 mg/kg dose of cocaine. Eight rats were tested at all points.

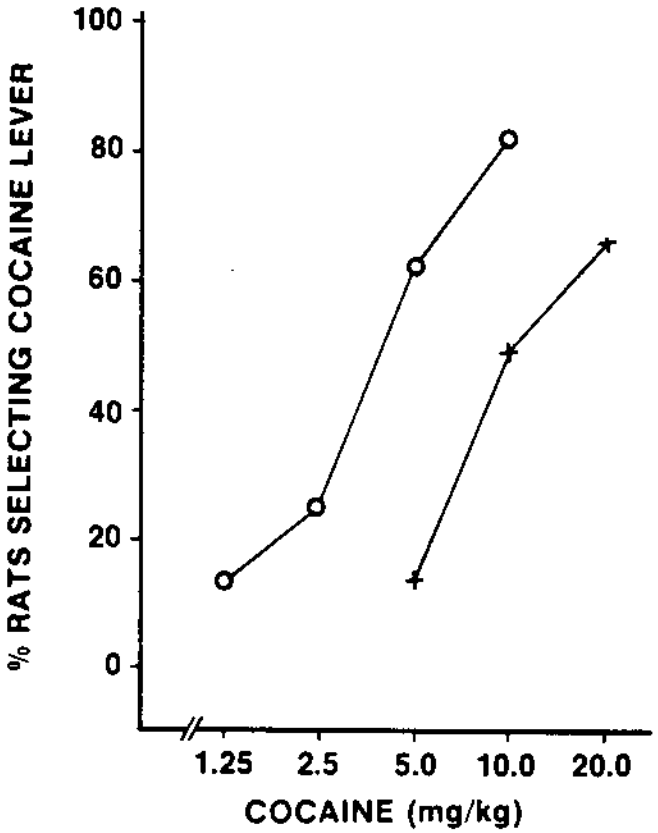


Figure 5. Dose-effect data for the detection of cocaine before (O) and after (×) 20 mg/kg/8-hr of cocaine. During chronic administration, dose-effect data were redetermined on days 7,8, and 9. N = 8 at all points.

reinforcement include the difference in the duration of drug exposure. This would require that a longer duration of chronic administration of cocaine would be needed to produce tolerance to the behavioral effects of cocaine. Also, although the duration of pause of first lever responding and time to first reinforcement are related behaviors, they may be differentially affected. Thus, there may be a difference between the development of tolerance to a subjective effect (discriminative stimulus) and to a behavioral effect.

In conclusion, there seems to be some controversy regarding the development of tolerance to the discriminative stimulus properties of drugs. There are many factors which appear to determine whether tolerance does or does not develop. For example, parameters such as whether chronic administration occurs during training, or after training is halted; the amount of drug administered; the frequency of administration; and the training drug itself, all seem to affect the development of tolerance. With respect to cocaine, there are little data as to whether tolerance develops; therefore, one of the aims of this research is to investigate, parametrically, some of the parameters which affect the development of tolerance to the discriminative stimulus properties of cocaine. Another aim of this research is to explore some of the behavioral variables affecting chronic cocaine administration.

Role of Neurotransmitters in the Discriminative Stimulus Properties of Cocaine

Cocaine is known to affect various central neurotransmission processes due to its interfence with reuptake, indirect release of neurotransmitters, or mimicking neurotransmitters at the receptor sites. Consequently, alterations of neurotransmitter function are thought to mediate specific pharmacological effects of cocaine. The neurotransmitter systems most often implicated as playing a significant role in cocaine's action include dopamine, noradreneline, 5-hydroxytryptamine, and phenylethylamine (Groppetti and Di Giulio, 1976). Because of these considerations, investigations of the possible neurochemical mechanisms involved in producing the discriminative stimulus properties of cocaine have been studied. The ultimate aim of such research is to uncover the neural processing through which a drug can act as a discriminative stimulus (Colpaert, 1978).

Ross and Renyi (1967) provided evidence that cocaine inhibited dopamine uptake in brain slices. Similarly, Farnebo and Hamberger (1971) showed that cocaine increases the electrical field stimulation which correspond to increased labeling of tritiated dopamine from neostriatal brain slices. These data suggest that an inhibition of the transmitter uptake and/or an increase of release from dopamine terminals may cause this effect. <u>In vitro</u> studies

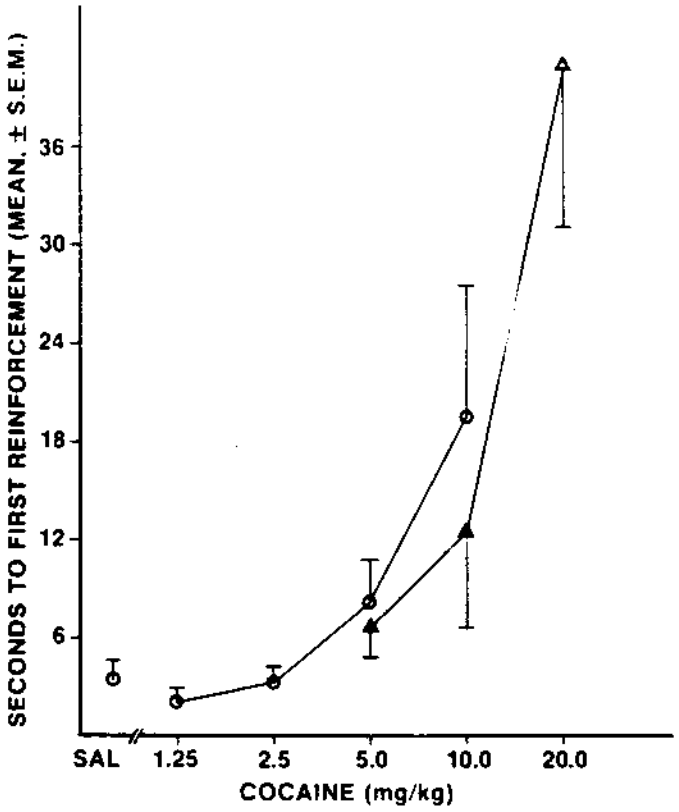


Figure 6. Effect of cocaine on the time to obtain reinforcement before and during chronic administration of cocaine. Abscissa: dose of cocaine or saline (SAL). Ordinate: time to complete 10 responses resulting in reinforcement. Response-rate data obtained for various doses of cocaine before (O) and during (△) chronic treatment with 20 mg/kg/8-hr for 6 days of cocaine. N = 8.

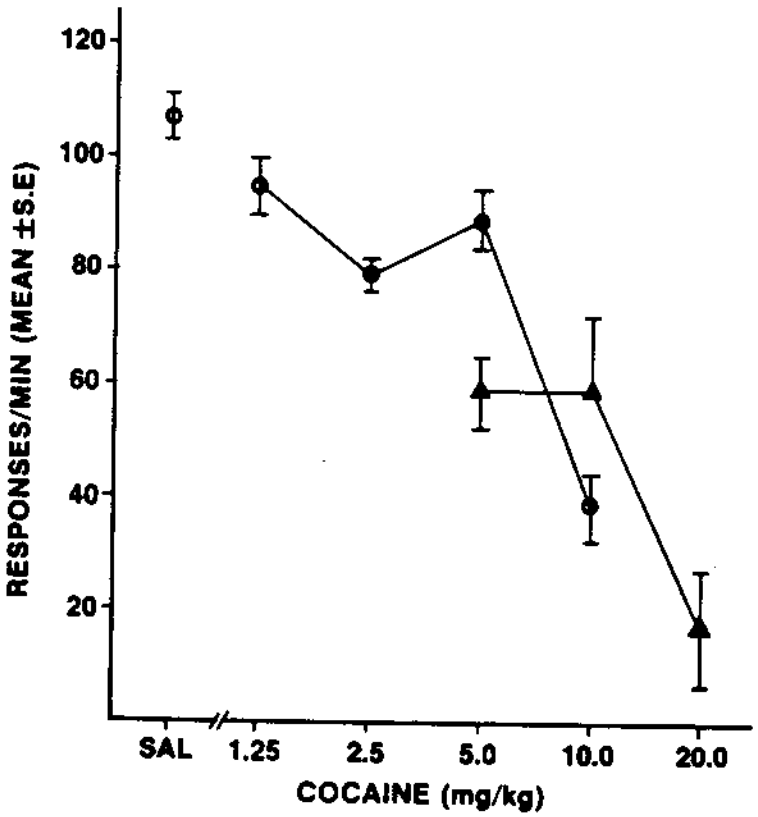


Figure 7. Effect of cocaine on response rate before and during chronic administration of cocaine. Abscissa: dose of cocaine or saline (SAL). Ordinate: number of responses per unit time resulting in reinforcement. Response-rate data obtained for various doses of cocaine before (O) and during (▲) chronic treatment with 20 mg/kg/8-hr for 6 days of cocaine. N = 8.

have also demonstrated increases in concentrations of brain dopamine following cocaine administration (Patrick and Barchas, 1976).

Several studies have used the discriminative stimulus procedure to examine the role of dopamine in producing stimulus generalization with cocaine as a cue (Colpaert, Niemegeers and Janssen, 1976; McKenna and Ho, 1980; Colpaert, Niemegeers and Janssen, 1978b). These experiments have used the rationale that if amphetamine and cocaine produce interchangeable stimulus properties, and if these two drugs indirectly increase brain dopamine, then direct dopaminergic receptor agonists should also mimic the cocaine stimulus.

Apomorphine is presumed to mimic the intrinsic action of endogenous dopamine at the dopamine receptor sites (Colpaert, Van Bever and Leysen, 1976; Creese and Seeman, 1982). In several experiments using cocaine as a discriminative stimulus, apomorphine substituted 100% for the cocaine stimulus, and the dopamine receptor antagonist, haloperidol, blocked the substitution of apomorphine for cocaine in rats (Colpaert et al., 1978b; McKenna and Ho, 1980; Stolerman and D'Mello, 1981), and pigeons (Jarbe, 1984). Similarly, other dopaminergic receptor agonists have substituted for the cocaine stimulus, including piribedil, bromocryptine and amantadine (Colpaert et al., 1978b). Blockade of the cocaine stimulus by the dopaminergic

receptor antagonist, pimozide, and the amine depletor, reserpine, have also been described (McKenna, Ho and Englert, 1978). Furthermore, central dopamine depletion by 6-hydroxydopamine on the d-amphetamine discriminative stimulus in rats has been reported to attenuate amphetamine lever responding. A similar decrease for the detection of cocaine has also been reported following administration of the amine depletor alpha-methyl-para-tyrosine (McKenna and Ho, 1980). These data strongly suggest that a central dopaminergic mechanism is involved in mediating the discriminative stimulus properties of cocaine.

There has been some controversy as to whether cocaine produces significant norepinephrine receptor involvement. For example, in vitro studies have demonstrated no change (Callingham and Cass, 1962), slight reduction (Higuchi, Matsuo and Shimmamoto, 1962) and slight increases (Pradhan, Roy and Pradhan, 1978) in norepinephine concentrations following cocaine administration. Based on data from drug discriminations studies, it has been suggested that noradrenergic and adrenergic mechanisms play a minor role in the discriminative stimulus properties of cocaine. In drug substitution studies, beta-adrenergic-receptor agonists, isoproternol and salbutamol do not substitute for the cocaine stimulus (Colpaert et al., 1978). Similarly, the alpha-2 receptor agonist clonidine also does not substitute for the cocaine stimulus (McKenna and Ho, 1980). In

experiments attempting to block the cocaine stimulus, neither the alpha-receptor antagonists, phentolamine and phenoxybenzamine, nor the beta-receptor antagonists, practolol and propanolol have attenuated generalization of the cocaine stimulus (Colpaert et al., 1978; McKenna and Ho, 1980).

It has been reported that cocaine produces decreased 5-hydroxytrptamine (5-HT) synthesis (Groppetti and Di Giulio, 1976), and inhibition of 5-HT uptake in the brain (Friedman, Gershon, Rotrose, 1975). The decrease in 5-HT has been associated with an increase in cocaine-induced stereotpyic behavior (Roy, Bhattacharyya, Pradhan and Pradhan, 1978). However, the role of 5-HT in mediating the discriminative stimulus properties of cocaine appears to be minimal. For example, p- chlorophenylalanine (5-HT depletor via inhibiting typtophan hydroxylase) administered to rats trained to discriminate cocaine did produce marked behavioral disturbances (decreased responding and hyperactivity), but did not alter correct lever responding following cocaine or saline administration. In another experiment, pretreatment with the 5-HT precursor, l-typtophan before cocaine injection was tested in cocaine-discriminated rats. Tryptophan significantly increases brain 5-HT levels (Fernstron and Wurtman, 1971), but it did not affect cocaine discrimination responding. Similarly, experiments assessing the effects of blocking the 5-HT

receptor sites also failed to alter cocaine discrimination. The 5-HT receptor antagonists, cinanserin, cyproheptadine, and methysergide decreased rate of responding, but did not attenuate cocaine discrimination (Colpaert, Niemegeers and Janssen 1976; McKenna and Ho, 1980). Therefore, it is suggested that 5-HT is not the major neurotransmitter involved in mediating the cocaine stimulus.

Phenethylamine is an endogenous trace amine whose behavioral effects in animals have been reported to mimic amphetamine (Jackson, 1972; Reisner and Jones, 1977; Braestrup, 1977). It has been proposed that phenyethylamine is an endogenous amphetamine which mediates the actions of amphetamine-related compounds (Borison, Mosnaim and Sabelli, 1975; Chuang, Karoum and Perlow, 1981). Colpaert, Niemegeers, and Janssen (1980) suggested that phenyethylamine may be responsible for mediating the discriminative stimulus properties of cocaine. In that study, rats were trained to discriminate 5.0 mg/kg cocaine from saline. Drugs which inhibited the enzyme monoamine oxidase type B generalized to the cocaine stimulus. Since phenyethylamine is a preferred substrate for this enzyme, Colpaert concluded that phenethylamine may be responsible for mediating discriminative stimulus properties of cocaine and possibly other central nervous stimulants.

Huang and Ho (1974) reported that in rats trained to discriminate amphetamine, neither phenethylamine (1.0 mg/kg)

nor iproniazid, a MAO inhibitor, substituted for amphetamine. In contrast, Goudie (1982) trained rats to discriminate 30 mg/kg phenethylamine from saline. Substitution tests with cocaine and amphetamine demonstrated that partial selection of the phenethylamine lever occurred for both drugs. Recently, Wood et al. (1984) trained rats to discrimate cocaine, 10 mg/kg (Table III). Phenethylamine substituted partially (60%) for the cocaine stimulus at high doses, with maximal substitution occurring at 80 mg/kg. Chronic administration of cocaine resulted in tolerance to the discriminative stimus of cocaine; however, there was a lack of cross-tolerance between cocaine and phenethylamine. While there is some support that phenethylamine may play a minor role in the cocaine stimulus, there is no evidence for involvement in the mediation of tolerance.

The mechanism mediating tolerance to the discriminative stimulus properties of cocaine is unclear. Although, there is indirect evidence that dopamine is the neurotransmitter involved in the cocaine stimulus, there have been no experiments testing this hypothesis. In a recent report, Nielsen and Scheel-Kruger (1986) reported that d-amphetamine administered directly into the nucleus accumbens generalized to the stimulus properties of d-amphetamine trained by intraperitoneal (i.p.) injection. These results demonstrate that 1) the discriminative stimulus properties of amphetamine are centrally mediated, 2) the discriminative

TABLE III

SUBSTITUTION AND CROSS-TOLERANCE TO COCAINE

Drug	Dose (mg/kg)	% Rats selecting cocaine lever	Number of rats
Nontolerant			
methamphetamine	0.32	43	7
methamphetamine	0.625	71	7
phenethylamine	40.0	0	5
phenethylamine	80.0	60	5
Tolerant			
methamphetamine	0.32	20	10
methamphetamine	0.625	38	8
phenethylamine	40.0	30	10
phenethylamine	80.0	50	4

stimulus produced by amphetamine may be mediated in a specific brain region.

Because of the similarities between the stimulus properties of amphetamine and cocaine (D'Mello and Stolerman, 1977; Huang and Ho, 1974), it may be possible that the discriminative stimulus properties of cocaine are mediated in specific brain sites. Since Nielsen and Scheel-Kruger (1986) reported that amphetamine administered in the nucleus accumbens generalized to the discriminative stimulus properties of amphetamine, it is anticipated that the direct administration of cocaine may also be active in the nucleus accumbens.

Summary

Recently, drug discrimination methodology has established that animals and humans detect the stimulus properties of drugs in parallel. Furthermore, recent evidence indicates that the discriminative stimulus of a drug is related to the subjective effects experienced in humans. In this procedure, by differentially reinforcing separate behaviors, it is possible to train subjects to emit one behavior after injection with a drug, and a different behavior when injected with saline. With respect to cocaine's stimulus properties, the pattern of drugs that substitute for the cocaine stimulus suggests that the cue property of cocaine is related to its abuse potential as a CNS stimulant. The experiments reported here used drug

discrimination methodology to investigate tolerance to the discriminative stimulus properties of cocaine. Using food as a reinforcer, rats were trained to press one-lever when injected with cocaine, 10 mg/kg, and a different lever when injected with saline. After training the discrimination and establishing the generalization curve for the detection of cocaine, training was halted, and tolerance was produced by injecting cocaine, 20 mg/kg/8-hr for 7 days. This procedure has been shown to shift the dose-effect curve for the detection of the cocaine stimulus approximately two-fold to the right. In one series of experiments, the effect of different dosing regimens on the development of tolerance was tested. In another set of experiments, cross-tolerance profiles of amphetamine-type drugs was determined. These cross-tolerance experiments tested the hypothesis that amphetamine-type drugs with less abuse potential than d-amphetamine will show a greater degree of cross-tolerance. Another set of experiments investigated the role of dopamine receptors in the development of tolerance to the stimulus properties of cocaine. The hypothesis tested is that chronic administration of a dopamine-receptor agonist will produce tolerance to the stimulus properties of cocaine. Another set of experiments investigates the role of the nucleus accumbens, prefrontal cortex, and the striatum in the detection of the cocaine stimulus. These experiments employ a bilateral cannululation technique to

directly administer cocaine into areas of the brain that are rich in dopamine nerve terminals. A final set of experiments investigated the role of chronic administration of cocaine, 20 mg/kg/8-hr, on behavioral parameters (locomotor activity and stereotypy). It was hypothesized that the drug discrimination procedure can differentiate the subjective and behavioral aspects of cocaine.

The significance of these experiments is that since the discriminative stimulus produced by cocaine is similar to the subjective effects of cocaine in man, the drug discrimination procedure may be a valid methodology to 1) assess the abuse potential of various potential psychomotor stimulants, and 2) serve as a neurobiological assay to determine the mechanism of action of psychomotor stimulants.

CHAPTER II

METHODS

Subjects

Male hooded rats of the Long Evans strain (Charles River Breeding Laboratories, Inc., Wilmington, MA) were used in all experiments. They were housed individually in a large room of constant temperature (21 $\pm$ 1^{0}C). The rats, initially sixty days old and weighing between 250 and 275g, received ad libitum food (Purina Rat Chow) until body weights were stable at 320$\pm$10g. Thereafter, body weights were maintained at 320 $\pm$5g by limiting daily access to food; water was freely available.

Apparatus

Discrimination training was conducted in standard operant chambers (Coulbourn Instruments Inc., Columbus, OH). Each chamber was housed in a light- and sound-attenuating box that was fan-ventilated. On one wall of the chamber a house light was mounted centrally above a food cup, which was located between two response levers. Food reward (45 mg pellets, Bio-Serv Inc., Frenchtown, NJ) was delivered by a pellet dispenser. Recording of lever responses and scheduling of reinforcement contingencies were performed through TRS-80 Model III microcomputers and printers (Radio

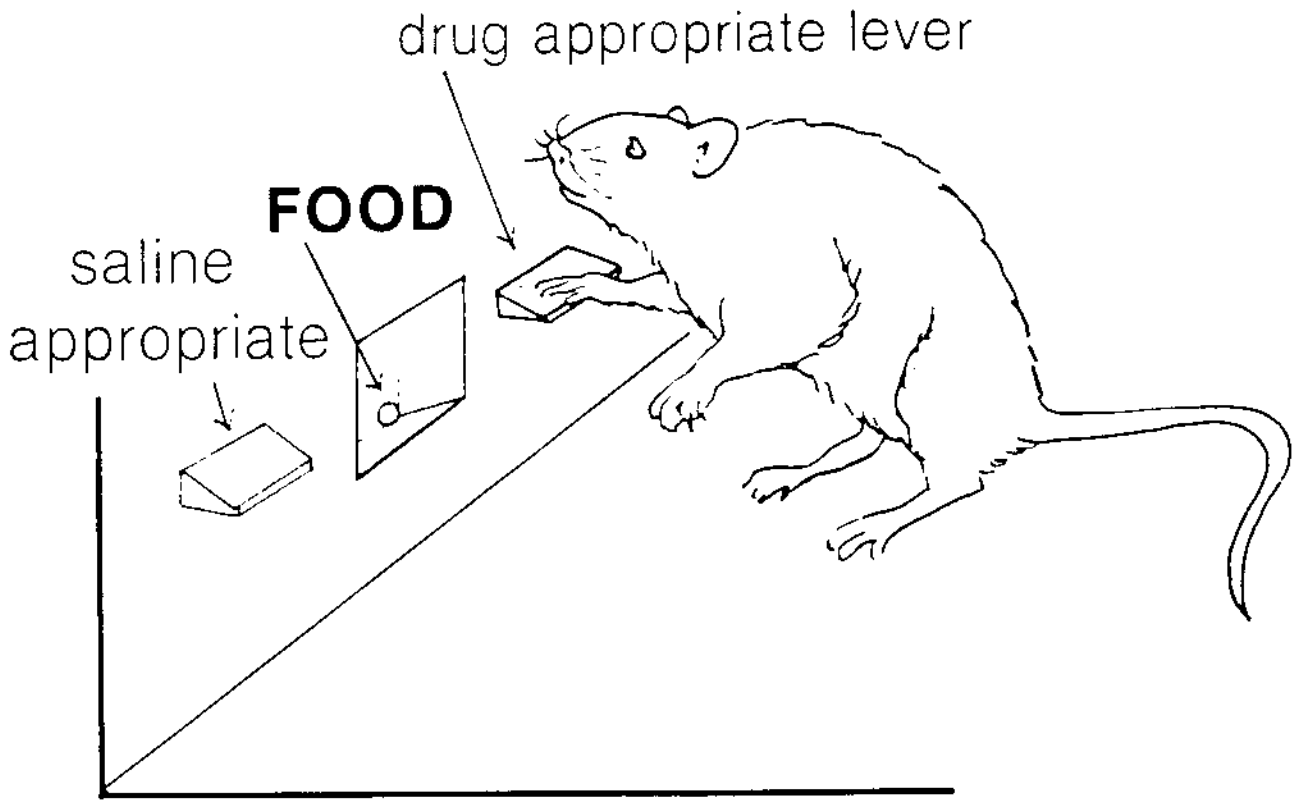


Figure 8. Illustration of a standard two-lever operant chamber. One lever is reinforced after administration of cocaine, 10 mg/kg, the other lever is reinforced after saline, 1 ml/kg. The rats need to press the appropriate lever ten times for food reinforcement.

Shack, Fort Worth, TX) connected to the chambers through LVB interfaces (Med Associates, East Fairfield, VT). The microprocessors were programmed to execute behavioral sessions and gather data using an OPN program developed by Emmett-Oglesby, Spencer and Arnoult, 1982; Spencer and Emmett-Oglesby,, 1985. This software program, OPN, executes in Z-80 assembly language, permitting real-time control of up to 8 stations per computer, and with its modular arrangement, readily permits flexible scheduling of operant contingencies.

Preliminary Training

Initially, the rats were placed overnight in the operant chambers, and they were trained to press a lever using food reward. Utilizing OPN, this training consisted of a series of components with a progressively increasing schedule of reinforcement. Initially, in the first component, each lever press was reinforced (CRF; continuous reinforcement schedule) for 50 reinforcements in 60 minutes. If this criterion was achieved, the rats were escalated to the second component. In the second component, every second press was reinforced (FR2; Fixed Ratio 2) for 50 reinforcements in 60 minutes. Similarly, if this criterion was met, the subjects were further escalated to the third and fourth components where every fifth and tenth press on either lever was reinforced, respectively. The rats

remained on the FR 10 schedule of reinforcement for the duration of the overnight session. If the subjects did not press the required fifty reinforcements in the 60 minute time period, they remained on the same component until they successfully reached this criterion (See Appendix A).

The second overnight session was similar to the first overnight session. However, there was no time contigency and the number of reinforcements on the lower FR schedules was shortened so that only 10 reinforcements were required to move to the final FR10 component. On the third overnight session, the procedure was shortened to two hours, and the lower FR schedules were again used, with the initial component consisting of a FR2 schedule for 5 reinforcements, followed by a FR 5 schedule for 5 reinforcements, to a final FR10 schedule for 50 reinforcements. However, in this session, saline was injected intraperitoneally (i.p.) 15 minutes before placing the subjects in the operant chambers. For the first two operant chambers, lever presses on the right lever resulted in the delivery of food reinforcement, and responses on the left lever were recorded, but not reinforced. Alternately, for the next two operant chambers, only responses on the left lever were reinforced. This pattern of alternation of correct saline lever selection was established for the thirty-two operant chambers. On the fourth shaping session, cocaine (10 mg/kg) was injected i.p., 15 minutes presession. The same schedule

as the third session was used, but the saline lever was extinguished and did not produce food reinforcement, and the other lever resulted in the delivery of food. The fifth training session was similar to the third training session, except that following saline injection, only an initial FR5 schedule for 5 reinforcements preceded the final FR10 schedule for reinforcements on the saline-correct lever. On the sixth shaping session, following cocaine injection, the same schedule as the fifth training session was used, except that only responses on the cocaine-correct lever were reinforced.

Discrimination Training

Following initial training, subjects were trained to press one of the levers after cocaine injection and the other lever after saline injection. For this training, saline or cocaine, 10 mg/kg, was injected i.p., 15 min before each 10-min session. After cocaine injection, only FR10 responses on one of the levers (the cocaine lever) were reinforced; responses on the saline lever were recorded but not reinforced. Similarly, after injection of saline, only FR10 responses on the saline lever were reinforced, and responses on the cocaine lever were recorded but not reinforced. There were equal numbers of cocaine and saline training sessions, which were presented in an irregular sequence so that no condition occurred in more than three successive training sessions. No cue other than the effect

of the drug was available to guide appropriate lever selection.

Only responses emitted before obtaining the first reinforcement were used to determine which lever was selected, and the first lever on which 10 responses occurred was considered the selected lever. Discriminative control was defined as 10 successive sessions of correct-lever responding in which when saline was injected, 10 responses were emitted on the saline lever with fewer than 10 responses on the cocaine lever, and when cocaine was injected, 10 responses occurred on the cocaine lever with fewer than 10 responses on the saline lever. Once this criterion had been achieved, tests were conducted whenever the correct lever was selected for four consecutive sessions.

Discrimination Testing

The testing procedure was identical to the training procedure, except that 10 responses on either lever produced food reinforcement, and sessions were conducted only until one reinforcement was obtained or 10 min had elapsed. For all test sessions, drugs were injected 15 min pretest, and the lever on which 10 responses were first emitted was recorded as the selected lever.

During chronic drug administration, dose-effect curves were determined by drug discrimination tests performed 8 hr

after a previous dose of drugs. Immediately after these tests, rats were injected with a supplemental dose of cocaine to maintain a constant level of chronic drug administration. For example, if the chronic dose was 20.0 mg/kg/injection and the test dose was 10.0 mg/kg, rats would receive a supplemental injection of 10.0 mg/kg of cocaine.

Chronic Injection

Except where noted, chronic administration of cocaine consisted of administering cocaine, 20 mg/kg/8-hr, for at least 7 days. In previous investigations, this regimen has been found to reliably shift the dose-effect curve to the right for the detection of the cocaine stimulus, thus demonstrating tolerance to the discriminative stimulus properties of cocaine (McKenna and Ho, 1977: Wood, Lal and Emmett-Oglesby, 1984). During this administration, animals are withheld from training.

Drugs

The following drugs were obtained from their respective sources; cocaine HCL (Mallinckrodt Inc., St.Louis, M.O.); apomorphine HCL, d-Amphetamine sulfate, diethylpropion HCL, fenfluramine HCL, morphine sulfate, naloxone HCL, phentermine HCL, sulpiride (Sigma Chemical Co., St. Louis, MO); phenmetrazine HCL (Boehringer Ingelheim, Ridgefield, CT)); methylphenidate (CIBA-Geigy, Summit, NJ); SKF-38393 (Smith, Kline & French, Philadelphia, PA); piribedil (Les

Laboratoires Servier, Gidy, France); and, haloperidol (Janssen Pharmaceuticals, Beerse, Belgium). All drugs were dissolved in 0.9% saline, except for sulpiride and SKF-38393. For these drugs a minimum volume of lactic acid was added to the solution to solubilize these compounds. All injections were administered i.p..

Experiment 1: The Role of Training Sessions and Number of Food Reinforcers on Acquiring Cocaine Discrimination

Hooded rats of the Long Evans strain were shaped according to the procedure discussed in the above preliminary training section. Four groups of rats were trained to detect cocaine, 10.0 mg/kg. In the first group (N=32), training sessions were conducted daily, five days per week. In the second group (N=32), an extra training session (either saline or cocaine) was conducted after a saline session. In the third group, training sessions were conducted daily (N=26), five days per week, and the number of reinforcers per session was reduced from 50 to 25. Similarly, in the fourth group (N=25), supplemental sessions of cocaine or saline were conducted after saline sessions and the number of reinforcers per session was reduced from 50 to 25.

Experiment 2: Effect of Cocaine Dose on the Development of Tolerance for the Discriminative Stimulus Produced by Cocaine

Initial dose-effect data for the generalization of cocaine (2.5, 5.0 and 10.0 mg/kg) were determined in eight rats. Subsequently, training was halted, and the rats were chronically injected with cocaine (20 mg/kg/8-hr). On days 7, 8 and 9 of cocaine administration, the dose-effect curve for the detection of the cocaine stimulus was redetermined. After this determination, chronic injections were terminated, and the rats were neither trained nor tested for 7 days, after which they were retrained for at least 25 sessions.

When initial sensitivity to the cocaine training dose was reestablished, the entire procedure was repeated with a chronic dose of cocaine (10 mg/kg). After testing and retraining, the procedure was repeated for 5 mg/kg/8-hr. However, when the training dose was tested on day 7 of chronic administration of the latter dose, the percentage of subjects selecting the cocaine lever was unchanged. Therefore, the injection procedure was continued for one more week, and the cocaine dose-effect curve was redetermined on days Fourteen through Sixteen.

Experiment 3: Effect of Duration of Chronic Administration of 20.0 mg/kg of Cocaine on the Development of Tolerance for the Cocaine Cue

Initial dose-effect data for the generalization of cocaine (2.5, 5.0 and 10 mg/kg) were determined in 16 rats

naive to the tolerance procedure. Subsequently, training and testing were halted, and they were assigned to two groups. Both groups were injected with cocaine (20 mg/kg/8-hr). The cocaine dose-effect curve was redetermined in one group on days 7 through 9 of chronic administration and in the other group on days 14 through 16 of chronic administration.

Experiment 4: Time Course for the Recovery from Tolerance After Termination of Chronic Cocaine

Initial dose-effect data for the generalization of cocaine 2.5, 5.0 and 10 mg/kg) were determined in 24 rats naive to the tolerance procedure. Subsequently, training was halted, and the animals were injected with cocaine (20 mg/kg) for 12 days. The cocaine dose-effect curve was redetermined on days 7 through 9. After termination of chronic injection, recovery from tolerance was assessed by testing cocaine lever selection after injection of the training dose (10.0 mg/kg). These tests were conducted once every 3 days for 18 days, and no injections, training or testing occurred on intervening days. Stimulus control was confirmed 2 days after this portion of the experiment was terminated, when saline injection produced 14 of 21 saline lever selections.

Experiment 5: Tolerance and Cross-Tolerance Characteristics of Anorectic Drugs

Rats were assigned to six subgroups of 8 subjects each. Using one subgroup for each drug, initial dose-effect data were obtained for cocaine generalization (2.5-10 mg/kg) and substitution of diethylpropion (0.32-2.5 mg/kg), fenfluramine (1.25-5 mg/kg), methylphenidate (1.25-10 mg/kg), phenmetrazine (0.64-10 mg/kg), and phentermine (0.64-10 mg/kg) for the cocaine training stimulus. Subsequently, training was halted, and all rats were injected with cocaine, 20 mg/kg/8-hr for 7 days. On days 7-9, generalization and substitution data were redetermined for all drugs tested. During generalization and substitution testing, a dose of one of the drugs was given at the time of a regularly scheduled injection of cocaine, 20 mg/kg (i.e. 8 hr after a previous dose of cocaine, 20 mg/kg), and subjects were tested 15 minutes later. Otherwise, the rats continued to receive the 20 mg/kg dose of cocaine every 8 hours during these tests. After dose-effect curves were redetermined, chronic injections of cocaine were halted, and subjects were not trained or tested for at least 14 days. Stimulus control was demonstrated by testing the discrimination of the training dose of cocaine (10 mg/kg) and saline.

Experiment 6: Effect of Chronic Administration of d-amphetamine on the Detection of Cocaine and d-amphetamine

When initial sensitivity to the cocaine cue was reestablished in rats used in experiment 1, 14 rats were

assigned to two groups of seven each, one to be used in d-amphetamine tests and one to be used in cocaine tests. Initial dose-effect curves were determined for the substitution of d-amphetamine (0.32, 0.64 and 1.25 mg/kg), and the generalization of cocaine (2.5, 5.0 and 10.0 mg/kg) to the cocaine training stimulus. Subsequently, training and testing were halted, and both groups were injected with d-amphetamine (2.5 mg/kg/8-hr). On days 7 through 9, d-amphetamine and cocaine dose-effect curves were redetermined.

Experiment 7: Effect of Morphine Dependence on the Discrimination of Cocaine

Initial dose-effect data were determined in eight rats for the generalization of cocaine (2.5, 5.0 and 10.0 mg/kg) to the cocaine stimulus. The effect of morphine (5 mg/kg 30 min presession) on the detection of the cocaine training dose (10.0 lmg/kg) was also tested. Subsequently, training was halted, and morphine dependence was produced by injecting increasing doses of morphine for 9 days. On days 1 and 2, rats received 10.0 mg/kg/8 hr; on days 3 and 4, rats received 20.0 mg/kg/8-hr; and on days 5 through 9, rats received 30.0 mg/kg/8-hr. On days 7 through 9, the cocaine dose-effect curve was determined as in experiment 1, except that rats received a postsession injection of morphine (30 mg/kg) rather than cocaine. After the last cocaine test,

all rats received naloxone (0.64 mg/kg) and were observed for signs of narcotic withdrawal.

Experiment 8: Tolerance and Cross-Tolerance Characteristics Of Dopamine Receptor Agonists To The Discriminative Stimulus Properties of Cocaine

Thirty-two naive rats were assigned to 4 subgroups of 8 rats. Using one subgroup for each drug tested, initial dose-effect data were obtained for the generalization of cocaine (2.5, 5.0 and 10.0 mg/kg), and the substitution of apomorphine (0.64, 1.25 and 2.5 mg/kg), piribedil (2.5, 5.0, 10.0 and 20.0 mg/kg), SKF 38393 (5.0, 10.0, 20.0 and 40.0), and amantadine (10.0, 20.0, 40.0 and 80.0 mg/kg) for the cocaine training stimulus. Subsequently, training was halted, and all rats were injected with cocaine, 20 mg/kg every 8 hours. On days 7-9 of chronic cocaine administration, generalization and substitution data were redetermined for cocaine (5.0, 10.0 and 20.0 mg/kg), apomorphine (1.25, 2.5 and 5.0 mg/kg), piribedil (5.0, 10.0, 20.0 and 40.0 mg/kg), and SKF 38393 (10.0, 20.0, 40.0, and 80.0 mg/kg) using the same rats in which initial dose-effect data were determined. During generalization and substitution testing, a dose of one of the drugs was substituted for a regularly scheduled injection of 20 mg/kg of cocaine, and subjects were tested 15 minutes later. Otherwise, the rats continued to receive the 20 mg/kg dose of cocaine every 8 hours during these tests. After dose-

effect data were redetermined, chronic injection of cocaine was halted, and subjects were not trained or tested for at least 14 days. Stimulus control was demonstrated by testing for discrimination of the training dose (10.0 mg/kg of cocaine) at this time.

Experiment 9: The Effects of Chronic Administration of Haloperidol and Sulpiride and Cocaine on the Discriminative Stimulus Properties of Cocaine

Sixteen rats were assigned to two subgroups of 8 rats. Using one subgroup for each drug, initial dose-effect data were obtained for the generalization of cocaine (1.25, 2.5, 5.0 and 10.0 mg/kg), and the substitution of haloperidol (0.08, 0.16, 0.32 and 0.64) and sulpiride (10.0, 20.0, 40.0 and 80.0 mg/kg) for the cocaine training stimulus. Haloperidol and sulpiride was administered one hour pre-test session. Subsequently, generalization of cocaine (2.5-10.0 mg/kg) was determined one hour following i.p. administration of a dose of haloperidol (0.08, 0.16, 0.32 and 0.64), or sulpiride (10.0, 20.0, 40.0 and 80.0 mg/kg). Subsequently, training was halted, and all rats were injected with cocaine, 20 mg/kg every 8 hours, but each injection followed administration of either sulpiride, 20 mg/kg, or haloperidol, 0.64 mg/kg. On days 7-9 of chronic administration, generalization of cocaine (5.0, 10.0 and 20.0 mg/kg), was redetermined.

Experiment 10: The Effects of Chronic Administration of Haloperidol and Sulpiride on the Discriminative Stimulus Properties of Cocaine

Sixteen rats were assigned to two subgroups of 8 rats. Using one subgroup for each drug, initial dose-effect data were obtained for the generalization of cocaine (1.25, 2.5, 5.0 and 10.0 mg/kg). Training was halted, and one group of rats was injected with haloperidol (0.64 mg/kg), and the second group was injected with sulpiride (20 mg/kg) daily for 6 days. On days 7-9 of chronic administration, generalization of cocaine (1.25-10.0) were redetermined. Rats continued to receive supplemental administration of haloperidol or sulpiride until testing was completed. After dose-effect data were redetermined, chronic injections of cocaine were halted, and subjects were not trained or tested for at least 2 months.

Experiment 11: Effect of Chronic Administration of Apomorphine, Piribedil, or SKF-38393 on the Detection of Cocaine

When initial sensitivity to the cocaine cue was reestablished in rats used in Experiment 10, 24 rats were assigned to three groups of eight each. Initial dose-effect curves were determined for the generalization of cocaine (1.25-10.0 mg/kg) in all rats and each group was tested for substitution of apomorphine (0.64-2.5 mg/kg), piribedil (2.5 -10 mg/kg), or SKF 38393 (5.0-40.0 mg/kg), respectively.

Subsequently, training and testing were halted, and each group was injected with apomorphine (2.5 mg/kg/8hr), piribedil (20 mg/kg/8hr), or SKF 38393 (40.0 mg/kg/8hr) for 6 days using the same rats that initial dose-effect data were determined. On days 7-12 of chronic administration, apomorphine, piribedil, SKF-38393, and cocaine dose-effect curves were redetermined.

Experiment 12: Tolerance to the Discriminative Stimulus Properties of Cocaine Administered Intraventricularly

Cannulation Procedure. Rats were cannulated bilaterally in the lateral ventricles, using sterile stereotaxic technique. Bilateral guide cannulae assemblies were made from #26 gauge stainless steel hypodermic tubing and 10 mm teflon molds according to the procedure of Czech and Stein (1984). Cannulae were implanted using coordinates from Paxino and Watson (1982). Non-performing animals were implanted with the bilateral cannulae in the lateral ventricles, and the results verified histologically for accuracy of the coordinates before any implants were made in trained rats. From bregma coordinates used for the lateral ventricles were A=-0.8, L=1.5, V=3.0.

Intraventricular Microinjection Procedure. Micro-injections were made using #33 gauge cannulae which were inserted inside the guide cannulae. The injection cannulae were attached to #22 polyethylene tubing using acrylic glue.

TABLE IV

Composition of Artificial CSF

NaCl	2.025 g
KCl	62.5 mg
$CaCl_2$	35.0 mg
$MgCl_2$	27.5 mg
$NaHCO_3$	440.0 mg
NaH_2Po	17.5 mg
UREA	32.5 mg
D-GLUCOSE	152.5 mg

Adjust pH to 7.2
Bring total volume to 250 ml.
Run through 0.45 micron millipore filter.

The injection cannulae were cut 1 mm longer than the guide cannulae, so that when injection occurred, drug did not diffuse back up the guide cannulae. Once cannulae were inserted, injections were made over a 30 second period using a volume of 5ul per side. For these injections, the #22 gauge tubing was connected to a 5 ul Hamilton syringe. Cocaine (20.0, 40.0, 80.0, 160.0, and 320.0 ug) was dissolved in artificial CSF prepared by this laboratory (Table IV). Rats were placed in the operant chambers 5 min. following injection and the lever on which 10 responses were first emitted was recorded as the selected lever. In preliminary data, 5 minutes produced maximum generalization to the cocaine training stimulus. Following generalization testing, tolerance was induced using the same procedure described above, and the dose-effect curve for intraventricular administration of cocaine was redetermined. Eight rats were cannulated in this experiment, and following testing, rats were sacrificed, and cannula placement was verified histologically. One animal died during the experiment and one cannula was implanted superior to the ventricles. Data were tabulated for only those animals which had accurate cannulae placements.

Experiment 13: Brain Sites Mediating Cocaine Discriminative Stimulus

Sixteen hooded rats of the Long Evans strain were cannulated bilaterally in the prefrontal cortex, nucleus

accumbens, and caudate-putamen using sterile stereotaxic technique. Cannulae were implanted using coordinates from Paxino and Watson (1982). Non-performing animals were initially implanted with the bilateral cannulae in the brain sites, and the results were verified histologically for accuracy of the coordinates before any implants were made in trained rats. From bregma, midline and the dura as reference, coordinates for the cannulation prefrontal cortex were Anterior = 2.0 mm; Lateral = 2 mm; and Ventral 3.0 mm; nucleus accumbens was A = 0.2 mm; L = 3.0 mm; and V = 5.5 mm; and caudate-putamen was A = 0.2; L = 3.0 mm; and V = 4.0 mm. After recovery from surgery and restabilization on the peripheral detection of cocaine, dose-effect data for the detection of centrally administered cocaine were determined as described in general microinjection testing technique above. Rats were placed in the operant chambers 5 min. following injection and the lever on which 10 responses were first emitted was recorded as the selected lever. Subsequently, tolerance was produced as decribed in general Methods, and tolerance via specific brain sites was tested.

Experiment 14: Analysis of Locomotor Activity Following 6 Days of Chronic Cocaine Administration

Locomotor activity was monitored using a Digiscan Animal Monitoring System (Omnitech Electronics, Inc.

Columbus, OH) which consisted of individual acrylic activity cages (40 X 40 X 30.5 cm) surrounded by red-filtered horizontal and vertical activity sensors, and a digiscan analyzer for collection of up to 23 locomotor variables. The apparatus used 16 X 16 X 16 photocell arrays to generate measures related to ambulation (Sanberg, Hagenmeyer, and Henault, 1985), and rearing (Sandberg, Moran, Kubos, and Coyle, 1984). The apparatus also allowed for quantification of the amount of time spent in any of 9 floor zones, or within the center zone and peripheral zones. Data collection, storage, and analysis were done using an IBMPC-XT microcomputer.

Twenty-eight naive male Long-Evans hooded rats were used to assess locomotor activity. Rats were placed in the locomotor chambers 15 min. following drug administration. Locomotor activity was assessed at 5 min. intervals for 20 minutes. Initially, the rats were habituated to the locomotor activity chambers for 7 sessions. Subsequently, cocaine (2.5-20 mg/kg), or saline was administered and subjects were tested for locomotor activity. Testing was then halted, and cocaine, 20 mg/kg/8-hr, was administered to all rats. Following chronic administration, locomotor activity was reassessed using the same drug doses described above.

Experiment 15: Analysis of Stereotypy Following 6 Days of Chronic Cocaine Administration

The same rats used in Experiment 14 were used to assess stereotypy. Stereotypy was measured according to the procedure of Ernst (1967) and Gianutsos, Drawbaugh, Hynes and Lal (1974). Animals were placed singly in the locomotor activity chamber which was made of plexiglass sides and wire-mesh top. They were observed and rated according to the following scale.

Score

1. Normal Stationary Behaviors--Normal grooming and licking with minimal movement.
2. Normal Activity--Moving about cage, sniffing and rearing.
3. Hyperactive Activity--Rapid, jerky movements around the cage with marked rearing and sniffing
4. Slow-Pattern Stereotypy--Slow repetitive exagerated movements of sniffing, licking, and chewing
5. Fast-Pattern Stereotypy--Same as above with increased intensity and hyperactivity
6. Restriction of Movement--Subject remaining in the same place in cage with fast repetitive head and/or foreleg movements (includes licking, chewing, or gnawing stereotypy).

The rats were observed for a 5 min. periods with a 5 min. intermissions between observations. They were scored during 5 consecutive observation periods during the 45 min.

session.

Data Analysis

Data are presented in terms of percentage of subjects selecting the cocaine lever, which is the percentage of subjects emitting 10 responses on the cocaine lever before emitting 10 or more responses on the saline lever. As shown by Colpaert (1978), FR10 discrimination responding produces bimodal data which should be presented and analyzed as quantal data. Drugs were considered to have properties similar to the training stimulus if the percentage of subjects selecting the cocaine lever was greater than 80%. Reduced sensitivity to the discriminative stimulus properties of cocaine was defined as at least a 2-fold shift to the right of the dose necessary to produce cocaine lever selection.

The data based upon the the acquisition of cocaine discrimination were analyzed by the Kolgomerov-Smirnov Two Sample test which is comparable to the parametric Chi-square test. Data obtained from locomotor activity and stereotypy experiments were compiled in 5 min. bins. The averages and standard errors were tabulated for each group. These data are continuous and were analyzed by repeated measures analysis of variance (Hayes, 1981) using a computerized statistical package developed by M.L. Brecht and J.A. Woodward (General Univariate and Multivariate Analysis of

Variance, 1983). This program is an interactive microcomputer program that performs general univariate and multivariate analysis of variance.

CHAPTER III

RESULTS

Experiment 1: Acquisition of stimulus control.

Rats required approximately 60 sessions of training to discriminate cocaine (10 mg/kg) from saline and meet the criterion of selecting the correct lever on 10 consecutive sessions for both groups being trained daily (25 and 50 food reinforcers per session, respectively), and the group receiving an extra training session following a saline training session using 25 reinforcers. The multiple training session using 25 reinforcers per session shortened the time for cocaine discrimination, but this effect was due to the increased number of training sessions per unit time, rather than to an increased rate of learning per session. When acquisition was assessed as a function of number of training sessions, no difference was found between the groups trained by the two methods. However, the group receiving an extra training session following a saline training session using fifty reinforcers per session required approximately 30 sessions of training (Figure 9). Thus, there was a significant two-fold increased rate of learning with multiple training sessions per day with 50 reinforcers (Kolgomerov-Smirnov Two Sample test, K(d) =12.8, n=32, p

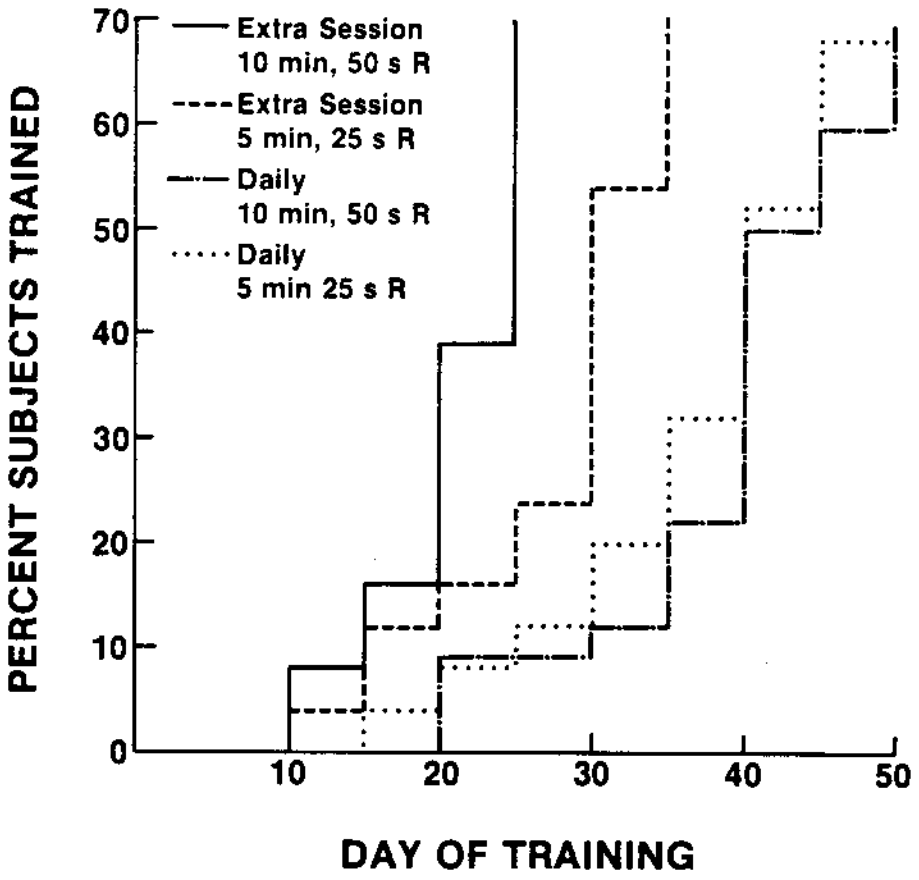


Figure 9. Acquisition of the cocaine discrimination, plotted by days of training. Abscissa: midpoints of 5-day bins. Ordinate: percent subjects meeting the criterion of 10 correct lever selections. The (—) represents an extra training session (N = 32) following saline session using 50 S^R; (---) extra training session following saline session (N = 25) using 25 S^R; (-·-) daily training session using 25 S^R; (····) daily training session (N = 32) using 50 S^R; and daily training session (N = 26) using 25 S^R.

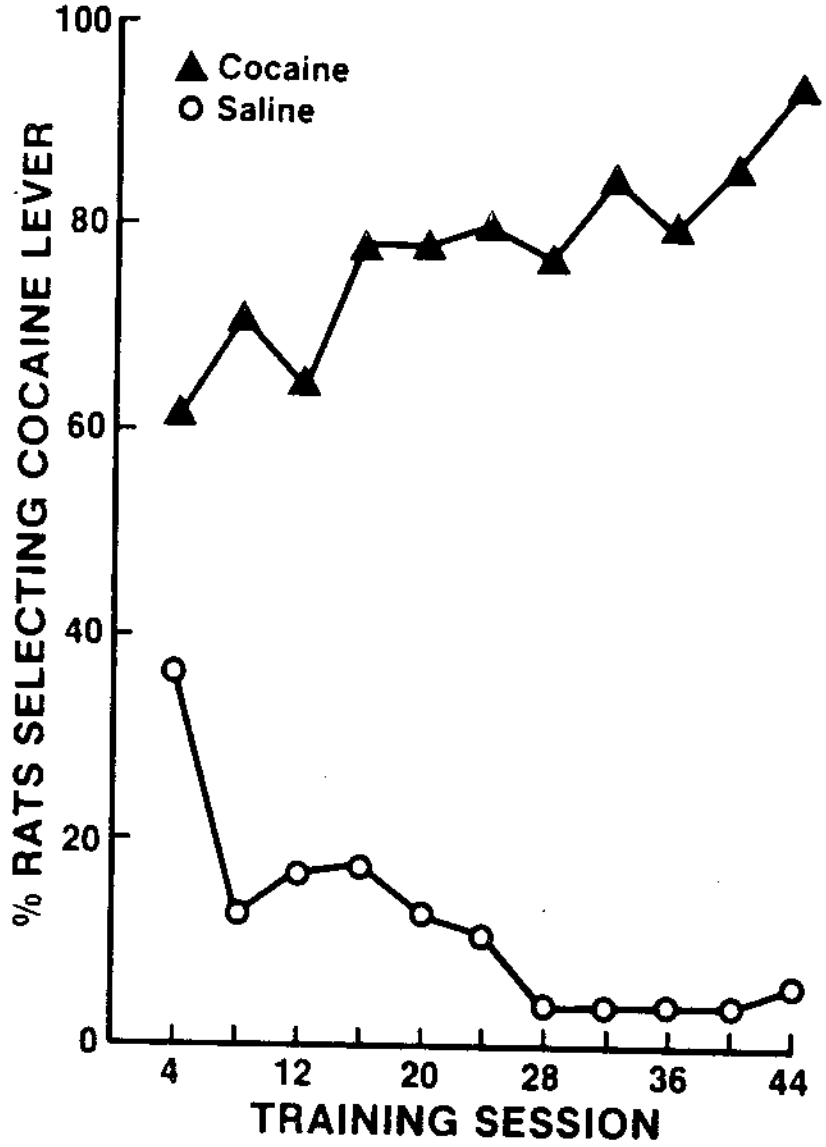


Figure 10. Training data for the acquisition of cocaine as a discriminative stimulus. Abscissa: sessions of training of cocaine (10.0 mg/kg and on 0.9% saline (1 ml/kg) Ordinate: percentage of subjects completing 10 responses on the cocaine lever before receiving reinforcement. Each point represents the mean of four administrations of cocaine (▲) or saline (O) in 48 rats.

$\leq$.01). For all groups of rats, they were trained for another 30 sessions, and by the onset of experiments, the discriminability of cocaine, 10 mg/kg fluctuated between 90 - 100% selection of the cocaine-appropriate lever (for a typical cocaine acquisition curve, See Figure 10).

Experiment 2: Tolerance for Cocaine: Dose.

Before chronic administration, cocaine (2.5-10 mg/kg) was generalized to the cocaine training stimulus in a dose-dependent manner. Chronic administration of cocaine, either 10 or 20 mg/kg/8-hr for 7 days, resulted in a 2-fold shift to the right of the dose-effect curve for the detection of the cocaine stimulus (Figure 11). In contrast, chronic administration of cocaine, 5 mg/kg/8-hr for 14 days, produced no shift in the dose-effect curve. All subjects made lever selections at all doses of cocaine tested.

Experiment 3: Tolerance for Cocaine: Duration.

Whether cocaine (20 mg/kg) was administered for 1 or 2 weeks, the dose-effect curve for the detection of the cocaine stimulus was shifted comparably 2-fold to the right. The dose that produced cocaine-appropriate lever responding was increased from 10 to 20 mg/kg in both groups. Thus, increased duration of cocaine administration beyond 1 week did not produce a greater degree of tolerance (Figure 12). All subjects made lever selections at all doses of cocaine tested.

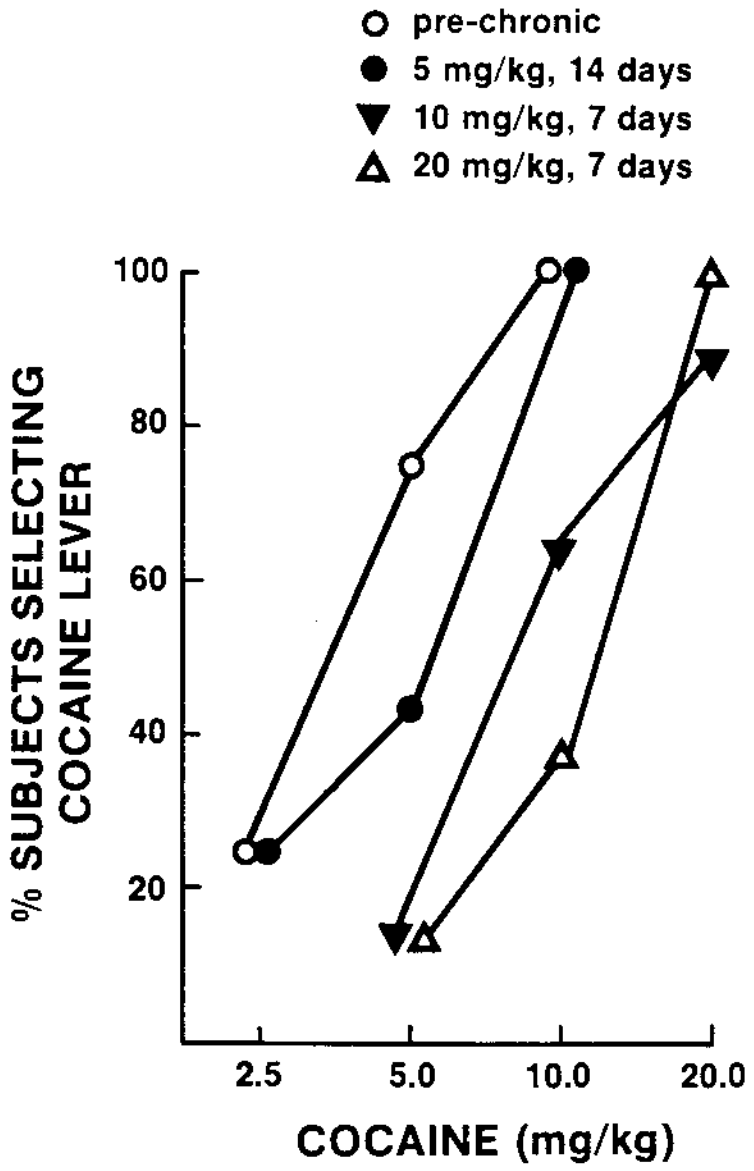


Figure 11. Dose-effect data for the detection of cocaine before (O) and after various doses of cocaine given 3 times daily. During chronic administration, dose-effect data were redetermined on days 7,8, and 9 (△▼) or on days 14,15 and 16 (●). N = 8 at all points.

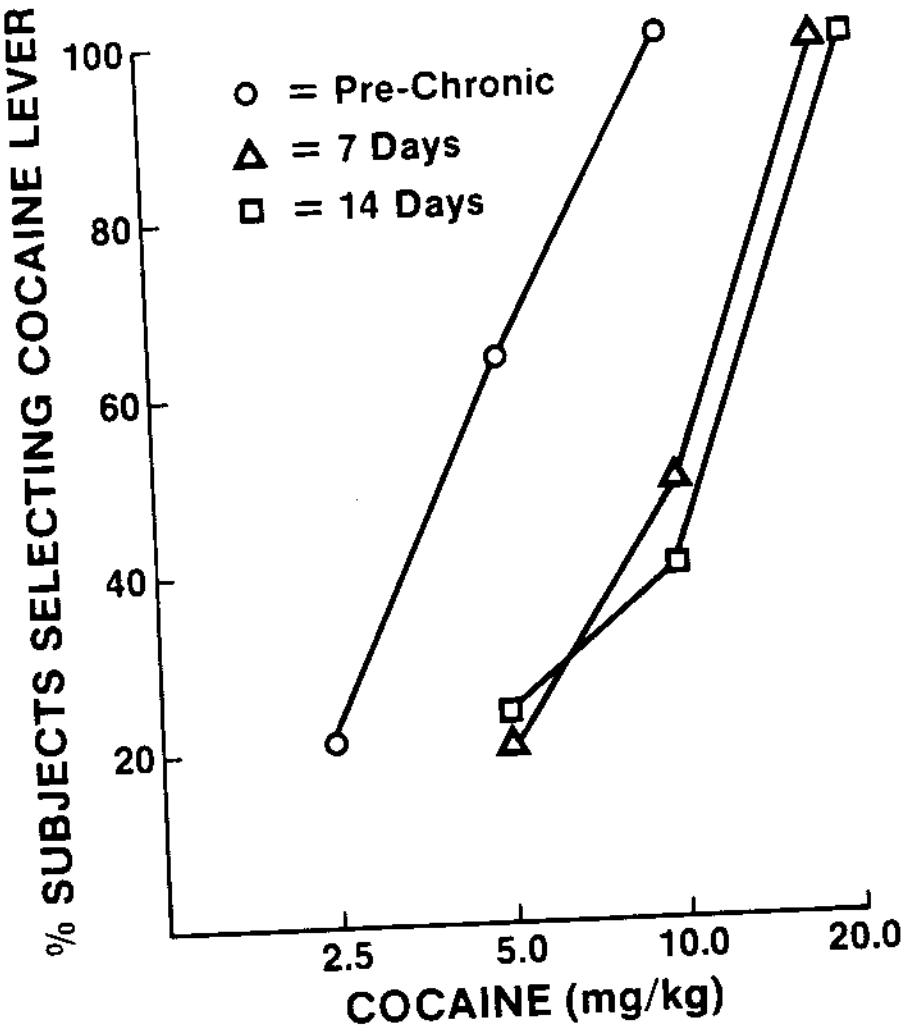


Figure 12. Dose-effect data for the detection of cocaine after 20 mg/kg/8-hr of cocaine. Dose-effect data were redetermined on days 7,8 and 9 (△) or on days 14,15 and 16 (□). N = 8 at all points.

Experiment 4: Recovery from cocaine tolerance.

Before chronic drug administration, 91% of the subjects selected the cocaine lever when tested with the training dose of cocaine (10 mg/kg). After 1 week of chronic administration, 37% of subjects selected the cocaine lever when tested with the training dose. After 12 days following termination of chronic cocaine administration, 20 mg/kg/8-hr, the percentage of subjects selecting the cocaine lever increased progressively until 86% of subjects made the correct discrimination on day 18 following the last cocaine dose (Figure 13). Thus the subjects recovered their initial sensitivity to the cocaine training stimulus without retraining.

Experiment 5: Tolerance and cross-tolerance for anorectic drugs.

Prior to chronic administration of cocaine, rats tested with cocaine (2.5-10 mg/kg) selected the cocaine-appropriate lever in a dose-dependent manner. Similarly, when diethylpropion (2.5 mg/kg), methylphenidate (5 mg/kg), phenmetrazine (10 mg/kg), and phentermine (10 mg/kg) were tested for substitution for the cocaine stimulus (Figure 14), rats selected the cocaine-appropriate lever in a dose-dependent manner. The potency of these drugs compared to the cocaine training stimulus was diethylpropion > methylphenidate > phenmetrazine = phentermine. After 7 days of cocaine administration (20 mg/kg/8-hr), the dose-effect

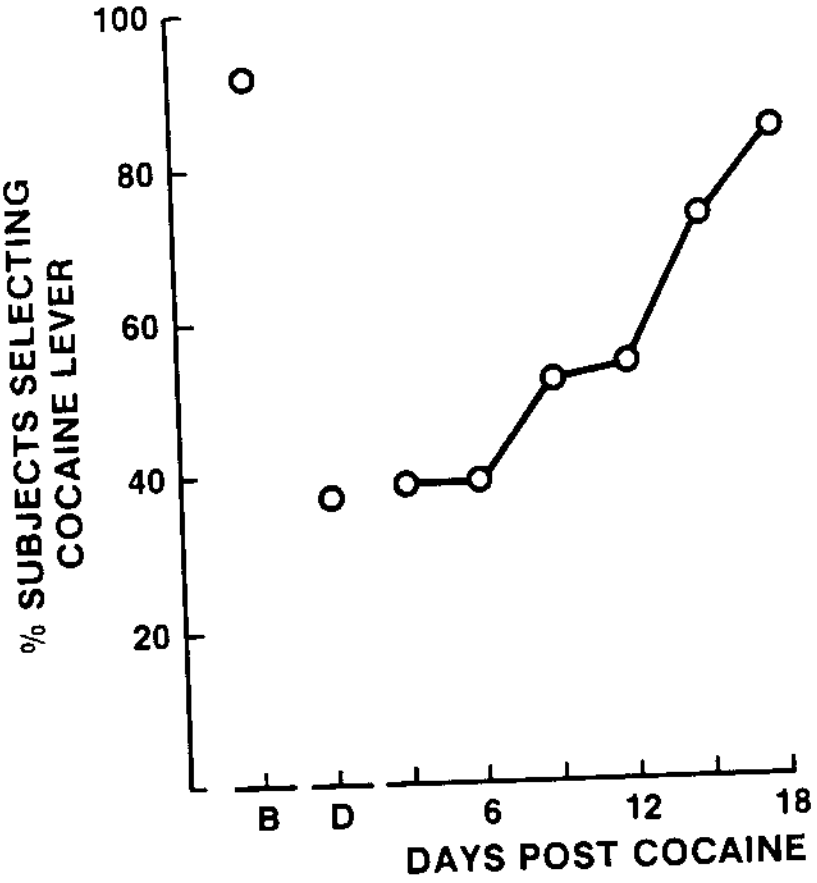


Figure 13. Spontaneous recovery from tolerance after termination of chronic cocaine administration. Each point represents the percentage of subjects selecting the cocaine lever after 10.0 mg/kg of cocaine. B, before chronic administration; D, day 7 of chronic administration. Cocaine (20.0 mg/kg/8-hr) was administered for 12 days. Recovery was tested at 3-day intervals. N = 24.

curves for the detection of cocaine, methylphenidate, phenmetrazine, and phentermine shifted approximately 2-fold to the right. The dose-effect curve for the detection of diethylpropion had also shifted to the right, but the magnitude of the shift was greater than 4-fold.

Only one dose of fenfluramine (2.5 mg/kg) partially substituted for the cocaine stimulus (Figure 15). A larger dose of fenfluramine (10 mg/kg) was behaviorally disruptive and no rats made a lever selection during the test session. Chronic administration of cocaine failed to alter cocaine lever selection at the 2.5 mg/kg dose of fenfluramine.

One week after this experiment, without further training or testing, rats were tested with saline and the training dose of cocaine (10 mg/kg) to assess the retention of stimulus control. Neither test result differed from previous baseline levels.

Experiment 6: Tolerance for d-amphetamine and cross-tolerance for cocaine.

d-Amphetamine substituted for cocaine in a dose-dependent manner with an approximate ED50 of 0.58 mg/kg before chronic administration of d-amphetamine. d-Amphetamine was approximately 8 times as potent as cocaine, since a dose of 1.25 mg/kg of d-amphetamine was equivalent to the training dose (10 mg/kg) of cocaine. After 7 days of chronic administration of d-amphetamine (2.5 mg/kg/8-hr),

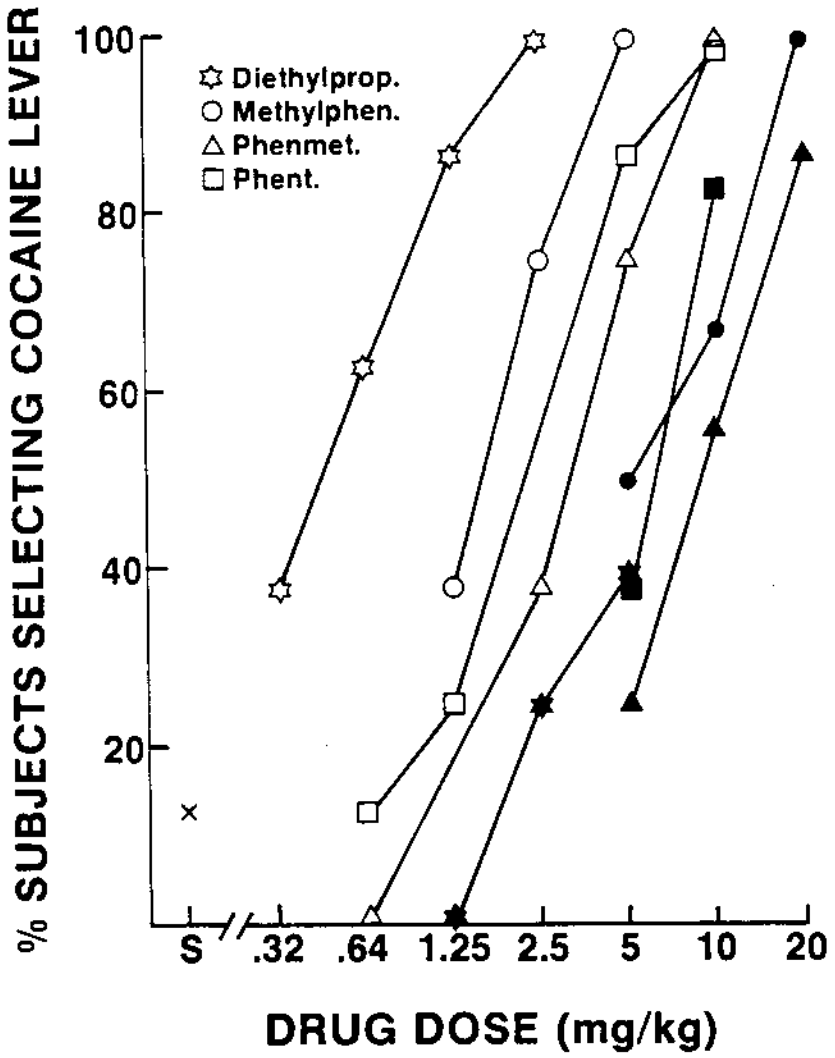


Figure 14. Full substitution profiles of selected anorectics before and during chronic administration of cocaine. Abscissa: dose of test drug. Ordinate: percentage of rats completing the first 10 responses on the cocaine lever. Data demonstrate cocaine-lever selection before (open symbols) and during (closed symbols) chronic administration of 20 mg/kg of cocaine for 7-9 days. The data points represent (✡) diethylpropion; (△) phenmetrazine; (□) phentermine; (○) methylphenidate. S = saline (1 ml/kg). Eight rats were tested at all points.

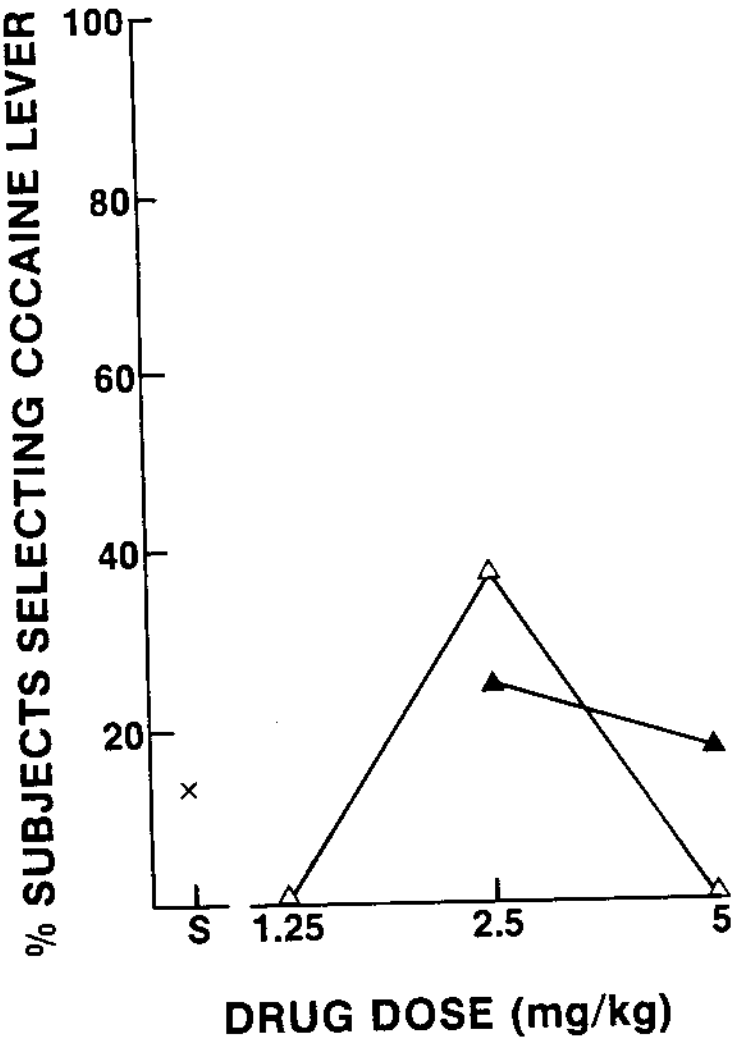


Figure 15. Substitution profile of fenfluramine before and during chronic administration of cocaine. Abscissa: dose of fenfluramine. Ordinate: percentage of rats completing the first 10 responses on the cocaine lever. Data demonstrate cocaine-lever selection before (open symbols) and during (closed symbols) chronic administration of 20 mg/kg of cocaine for 7-9 days. S = saline (1 ml/kg). Eight rats were tested at all points.

the dose-effect curve for the substitution of d-amphetamine for cocaine had shifted to the right 4-fold with an approximate ED50 of 2.5 mg/kg. In addition, tolerance for d-amphetamine conferred cross-tolerance for cocaine; the dose of cocaine that produced 100% cocaine-appropriate lever responding was increased from 10 to 40 mg/kg (Figure 16).

Experiment 7: Effect of morphine dependence on the detection of cocaine.

Given acutely, morphine (5 mg/kg) did not antagonize the cocaine training stimulus; seven of eight subjects selected the cocaine lever. After a 1-week course of morphine administration in escalating doses, the detection of the cocaine stimulus was unaltered (Figure 17). The administration of naloxone (0.64 mg/kg) at this time produced signs of narcotic withdrawal ranging from diarrhea to wet-dog shakes and tooth chattering in all rats, demonstrating that the morphine dosing regimen produced narcotic dependence.

Experiment 8: Tolerance and cross-tolerance characteristics of dopamine receptor agonists.

Comparable to the above studies, cocaine (1.25-10 mg/kg) produced dose-dependent generalization to the cocaine stimulus. Similarly, apomorphine (0.64-2.5 mg/kg), piribedil (2.5-20 mg/kg), and SKF-38393 (5-40 mg/kg) produced dose-dependent substitution for the cocaine

stimulus. The relationship between potency for these dopamine receptor agonists compared to the training dose of cocaine (10 mg/kg) was apomorphine > piribedil > SKF-38393; however, maximum substitution for the cocaine stimulus was piribedil > apomorphine > SKF-38393. Whereas piribedil produced full substitution for the cocaine stimulus (94%), apomorphine (72%) and SKF-38393 (67%) produced only partial substitution (Figure 18).

Chronic administration of cocaine (20 mg/kg/8-hr) for 6 days produced a 2-fold shift to the right of the dose-effect curve for the detection of cocaine. Similarly, the entire dose-effect curve for piribedil was also shifted 2-fold to the right. However, the substitution curve for apomorphine was shifted greater than 4-fold to the right such that no dose of apomorphine produced cocaine-lever responding (doses higher than 5 mg/kg produced no lever selection during the 15 min. test session). In contrast, the dose-effect curve for substitution of SKF-38393 for the cocaine stimulus was not shifted following chronic cocaine administration.

Experiment 9: Effects of chronic administration of sulpiride and haloperidol, and cocaine on the cocaine stimulus

Like the previous studies, cocaine produced dose-dependent generalization to the cocaine stimulus. Pre-treatment with the dopamine receptor antagonist,

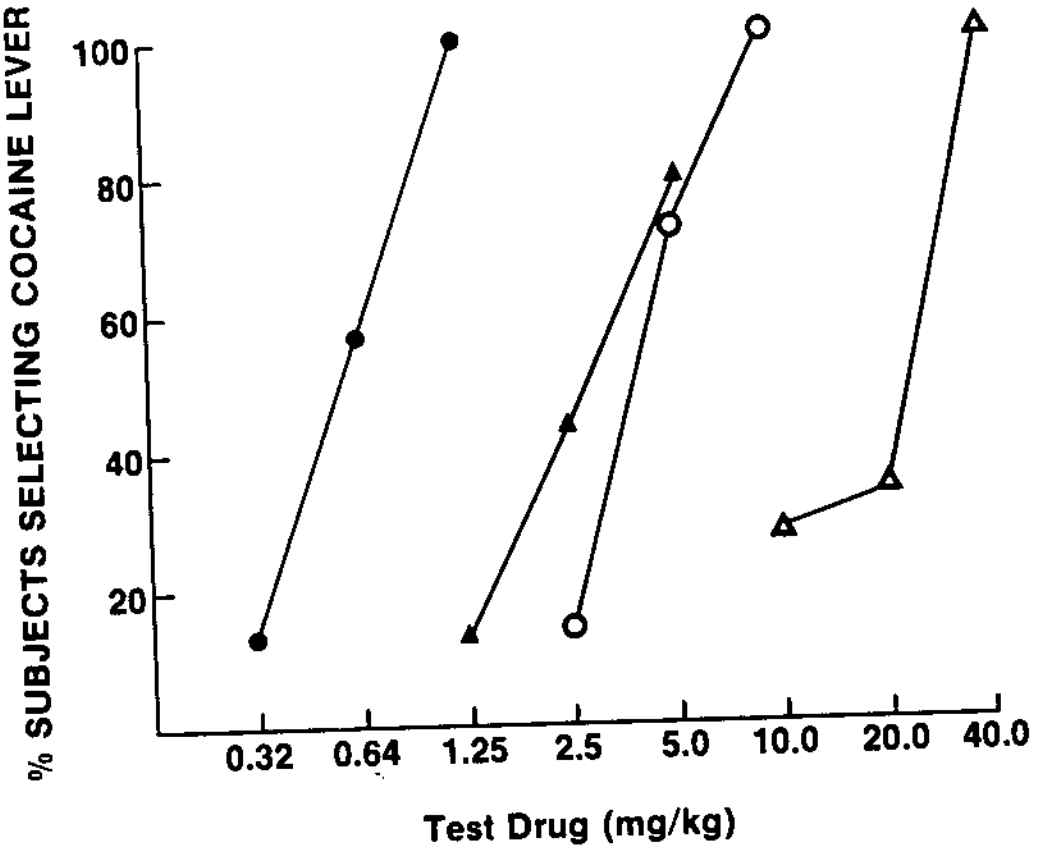


Figure 16. Chronic d-amphetamine produces tolerance for the substitution of d-amphetamine for cocaine and cross-tolerance for the stimulus properties of cocaine. d-Amphetamine substituted for cocaine before (●) and during (▲) days 7,8 and 9 of d-amphetamine (2.5 mg/kg/8-hr). Cocaine was generalized to cocaine before (○) and during (△) days 7,8, and 9 of d-amphetamine (2.5 mg/kg/8-hr). N = 7.

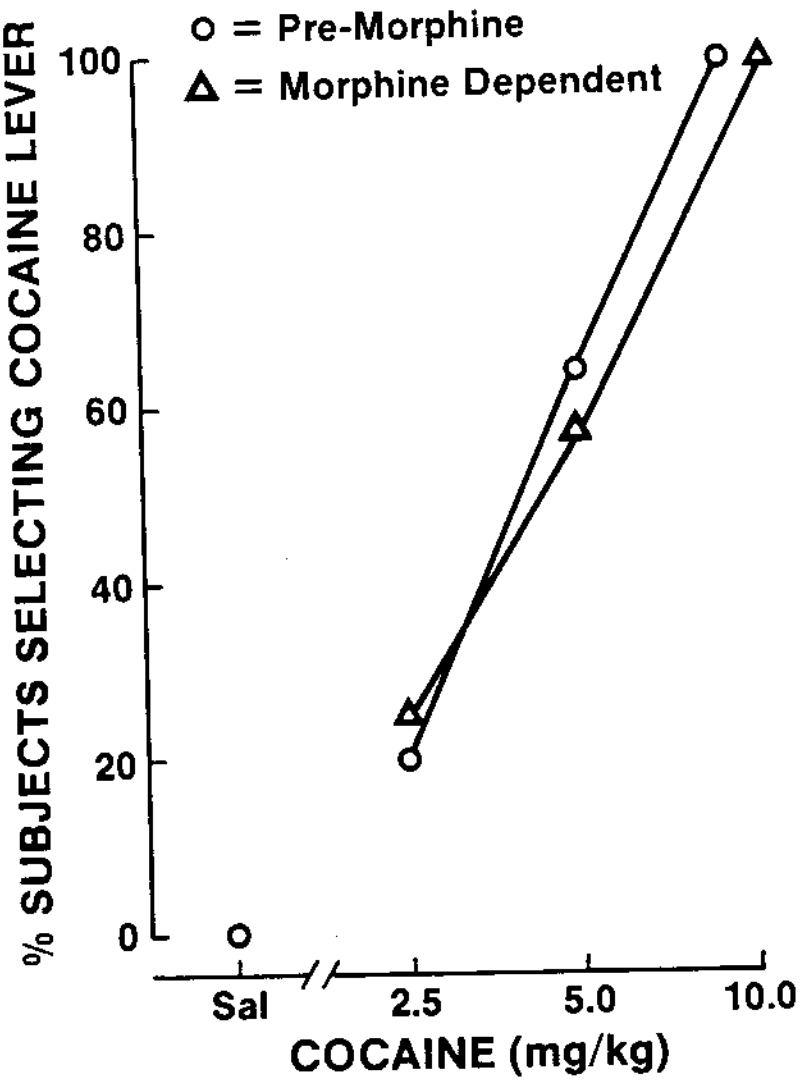


Figure 17. Morphine dependence does not alter the detectability of the cocaine stimulus. Cocaine was generalized to the cocaine training stimulus before (O) and during (△) days 7,8 and 9 of morphine administration. Sal, saline. N = 8 at all points.

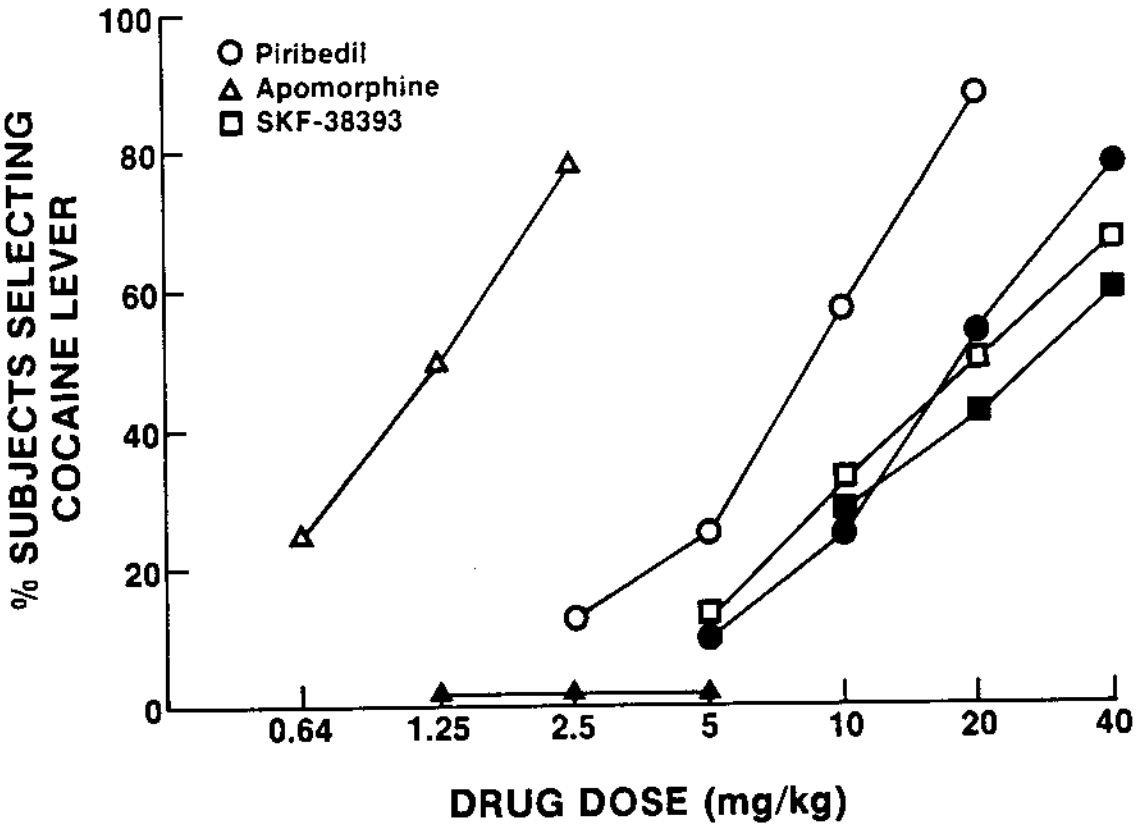


Figure 18. Full substitution profiles of selected dopamine receptor agonists before and during chronic administration of cocaine. Abscissa: dose of test drug. Ordinate: percentage of rats completing the first 10 responses on the cocaine lever. Data demonstrate cocaine-lever selection before (open symbols) and during (closed symbols) chronic administration of 20 mg/kg of cocaine for 7-9 days. The data points represent (O) piribedil; (△) apomorphine; and, (□) SKF-38393. S = saline (1 ml/kg). Eight rats were tested at all points.

haloperidol, produced a shift to the right of the cocaine generalization curve (Figure 19). Pre-treatment with the specific D2 receptor antagonist, sulpiride, produced a greater shift to the right of the generalization curve for the cocaine stimulus (Figure 20). Chronic administration of cocaine (20 mg/kg/8-hr) and haloperidol (0.64 mg/kg/8-hr) or sulpiride (20.0 mg/kg/8-hr) did not shift the original generalization curve for detection of the stimulus. As a control, the substitution of haloperidol (0.08-0.64) and sulpiride (10-80 mg/kg) did not generalize to the cocaine stimulus.

Experiment 10: The effects of chronic administration of haloperidol and sulpiride on the discriminative stimulus properties of cocaine

Cocaine produced dose-dependent generalization to the cocaine stimulus. Following 6 days of daily administration of haloperidol (0.64 mg/kg), the dose-effect curve for the generalization of cocaine for the cocaine stimulus shifted to the left. Similarly, 6 days of daily administration of sulpiride also shifted the cocaine dose-effect curve to the left to a lesser degree than the shift of the cocaine dose-effect curve following chronic haloperidol (Figure 21).

Experiment 11: Effects of chronic administration of dopamine receptor agonists to the cocaine stimulus

Cocaine (1.25-10 mg/kg) produced dose-dependent

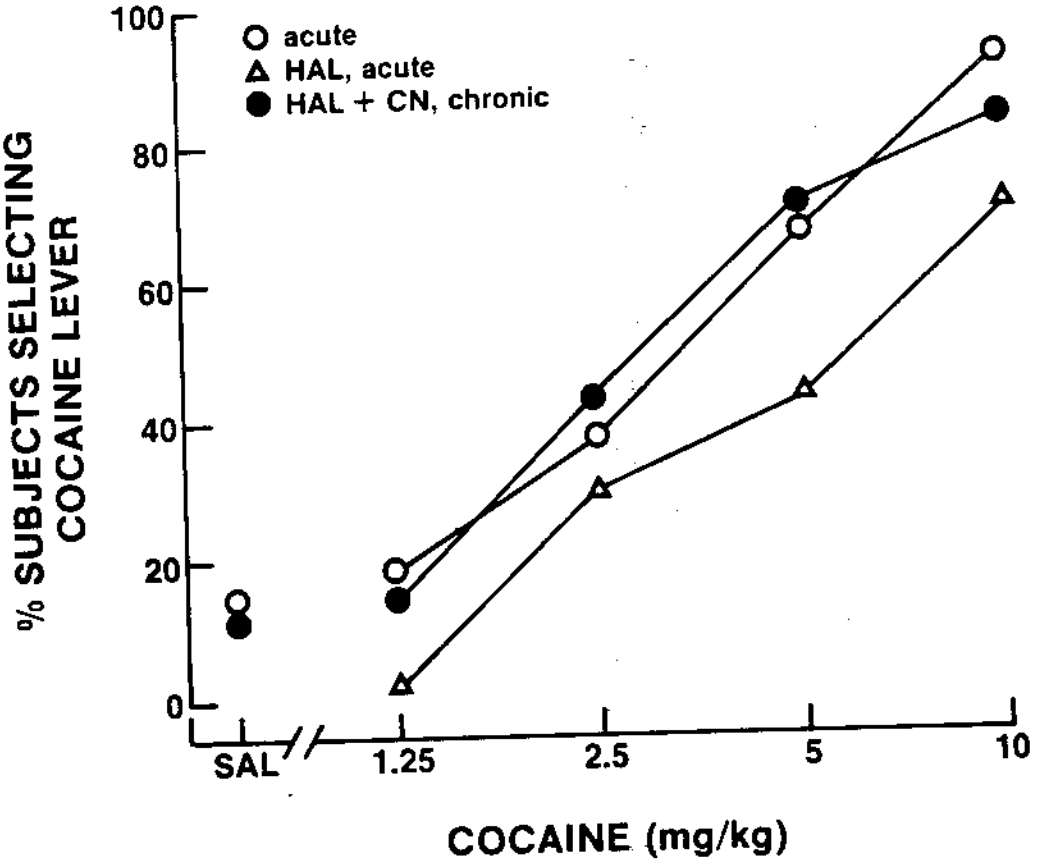


Figure 19. Chronic administration of haloperidol and cocaine blocks tolerance to the cocaine stimulus. Abscissa: dose of cocaine. Ordinate: percentage of rats completing the first 10 responses on the cocaine-appropriate lever. Data show selection of cocaine lever obtained before (O) and during (●) chronic pretreatment with haloperidol, 0.64 mg/kg, followed by cocaine, 20 mg/kg/8-hr, for 6 days. (△) demonstrate blockade of the cocaine stimulus with haloperidol, 0.64 mg/kg.

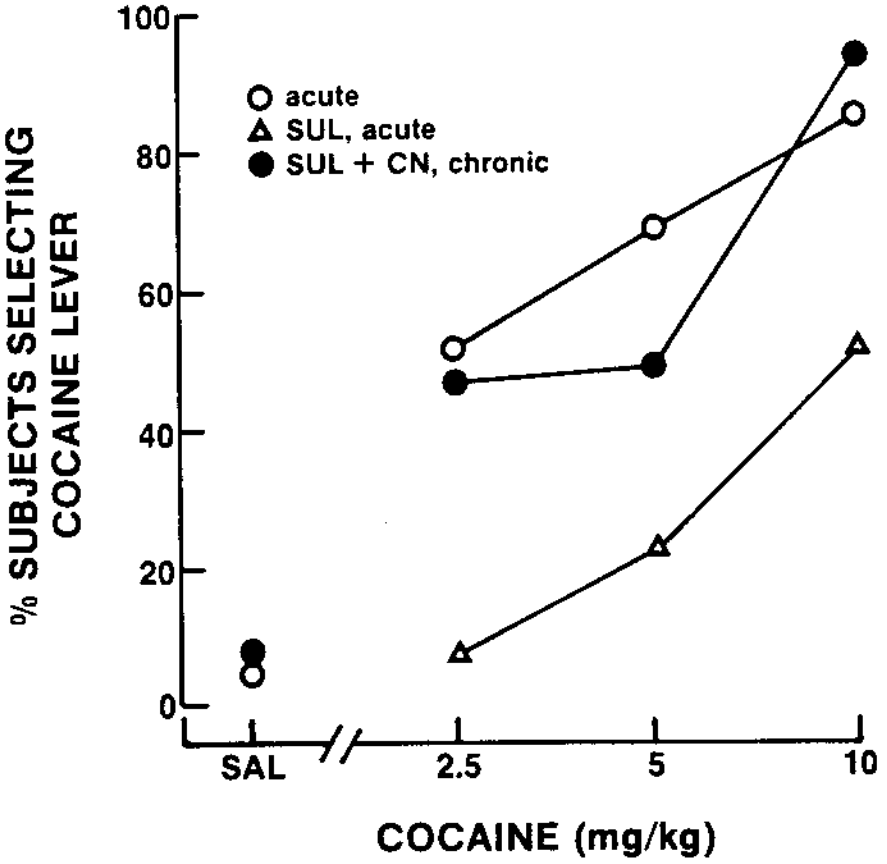


Figure 20. Chronic administration of sulpiride and cocaine blocks tolerance to the cocaine stimulus. Abscissa: dose of cocaine. Ordinate: percentage of rats completing the first 10 responses on the cocaine-appropriate lever. Data show selection of cocaine lever obtained before (O) and during (●) chronic daily pretreatment with sulpiride, 20 mg/kg, followed by cocaine, 20 mg/kg/8-hr, for 6 days. (△) demonstrate blockade of the cocaine stimulus with sulpiride, 20 mg/kg.

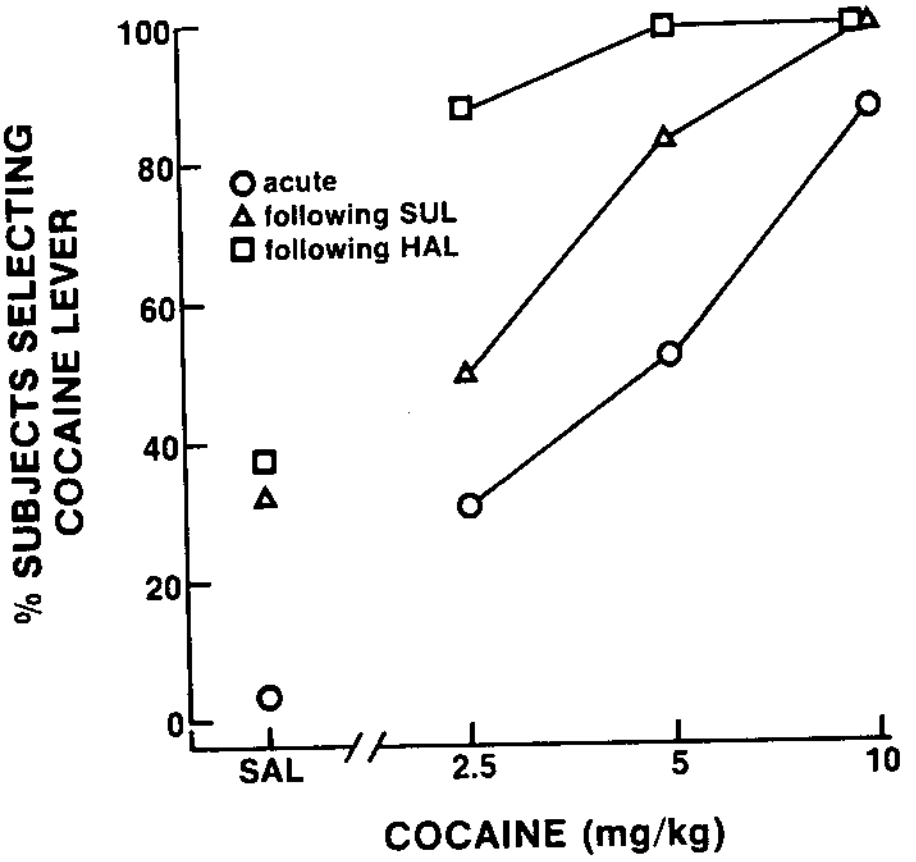


Figure 21. Chronic administration of haloperidol or sulpiride produce sensitivity to the cocaine stimulus. Abscissa: dose of cocaine. Ordinate: percentage of rats completing the first 10 responses on the cocaine-appropriate lever. Data show selection of cocaine lever obtained before (O) and during daily treatment with haloperidol (△), 0.64 mg/kg, or sulpiride (□), 20 mg/kg, for 6 days.

generalization to the cocaine stimulus. Similarly, apomorphine (0.64-2.5 mg/kg), piribedil (2.5-20 mg/kg), and SKF-38393 (5-40 mg/kg) produced dose-dependent substitution for the cocaine stimulus. Similar to the previous studies, the relationship between potency for these dopamine receptor agonists compared to the training dose of cocaine (10 mg/kg) was apomorphine > piribedil > SKF-38393; and, maximum substitution for the cocaine stimulus was piribedil > apomorphine > SKF-38393.

Chronic administration of apomorphine (0.64 mg/kg/8-hr) and piribedil (20 mg/kg) for 6 days produced a 2-fold shift to the right of the dose-effect curve for generalization to cocaine. However, the dose-effect curve was not shifted following chronic administration of SKF-38393 (Figure 22).

Experiment 12: Intraventricular administration of cocaine.

Prior to chronic administration, microinjections of intraventricular cocaine generalized to the cocaine stimulus in a dose-dependent manner, with maximum generalization occurring with 40 ug cocaine injected on each side (80%). Preliminary results indicated that maximum generalization to the cocaine stimulus occurred 5 min. after i.v.t. administration, compared to 15 min. after i.p. administration (Figure 23). Doses higher than 40 ug cocaine per side resulted in no lever selection during the 10 min.

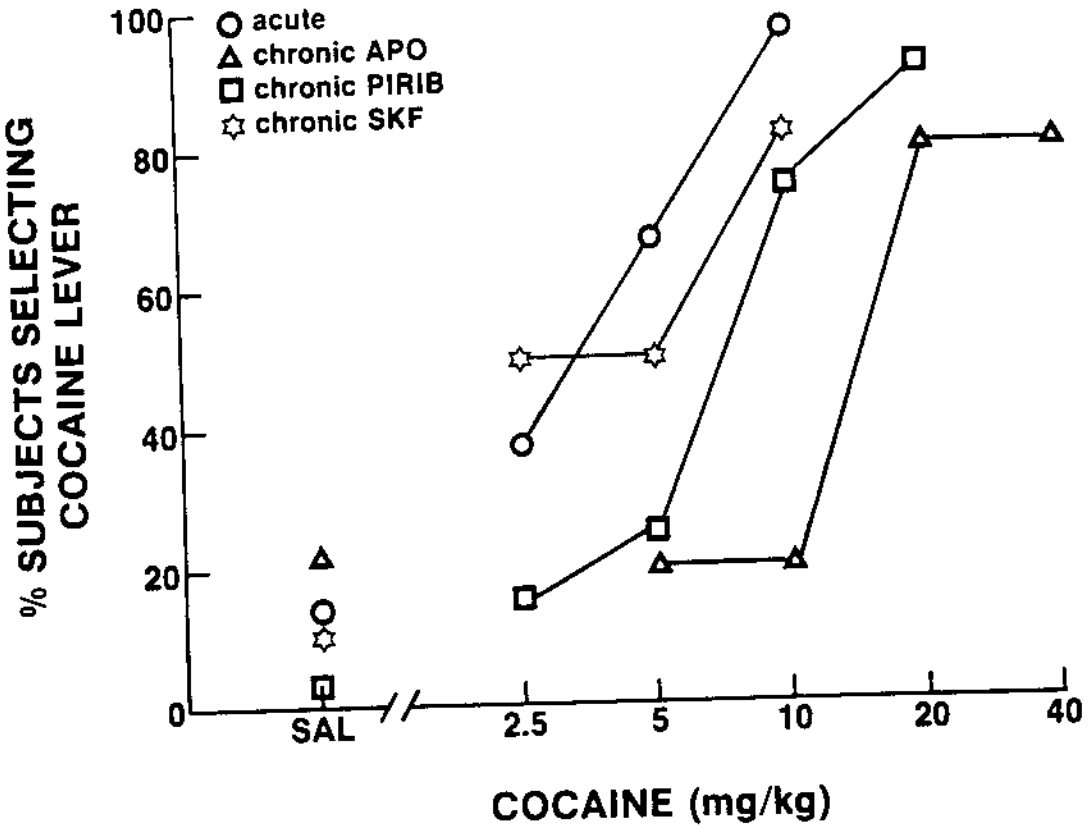


Figure 22. Chronic apomorphine, piribedil, but not SKF-38393 produce tolerance for the stimulus properties of cocaine. Cocaine was generalized to cocaine before (O) and days 7,8, and 9 of apomorphine (△), 2.5 mg/kg/8-hr, piribedil (□), 20 mg/kg, or SKF-38393 (⬡), 20 mg/kg. N = 8 for tests with apomorphine and piribedil. N = 4-5 for tests with SKF-38393.

test session. Following chronic administration of 20.0 mg/kg/8hr cocaine for 6 days, a significant shift to the right of the dose-effect curve was produced (Figure 24). Maximum generalization of cocaine occurred with 160 ug cocaine per side or 320 ug cocaine total. Doses higher than 160 ug cocaine per side resulted in behavioral toxicity.

Experiment 13: Specific brain sites mediating the discriminative stimulus properties of cocaine.

Cannnulae placements in the specific brain sites were histologically verified (Figure 25). In the first test session, intracerebral administration of artificial cerebrospinal fluid and/or the restraining procedure produced greater cocaine-appropriate lever responding compared to previous tests with saline. However, by the third consecutive habituation session with artificial CSF, this effect was abolished (Figure 26).

Cocaine injected in the nucleus accumbens produced dose-dependent selection of the cocaine-appropriate lever, with 88% of subjects selecting the cocaine lever after 10 ug cocaine injections per each side. In contrast, cocaine (5-40 ug) injected into either the caudate-putamen, or prefrontal cortex produced only partial cocaine-appropriate lever responding (Figure 27). Doses higher than 40 ug cocaine were behaviorally disruptive, and resulted in no lever selection during the 10 min. test session.

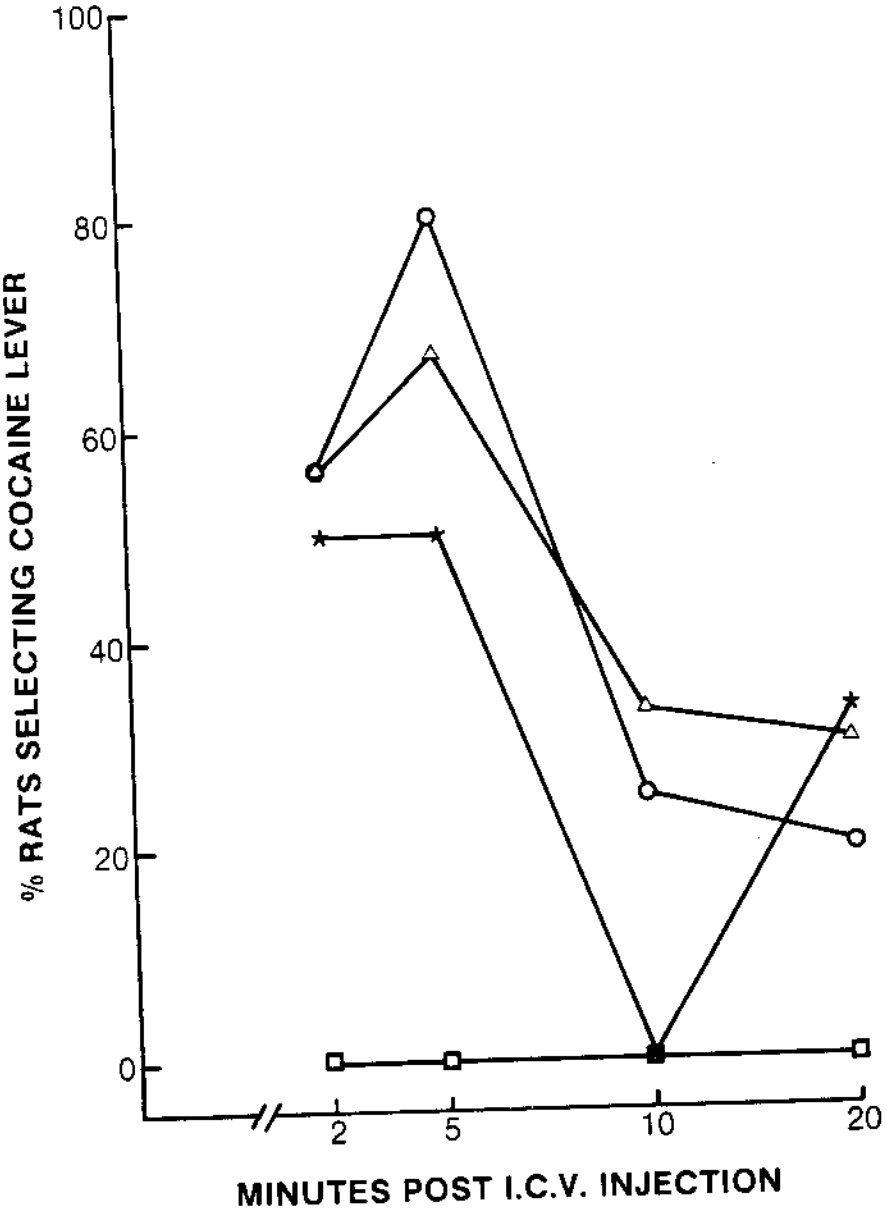


Figure 23. Time course for generalization to the cocaine stimulus for i.c.v administration of cocaine trained by peripheral administration. Abscissa: minutes post i.c.v. injection. Ordinate: percentage of subjects completing 10 responses on the cocaine lever. Dose-effect data were determined for 80 ug (O), 40 ug (△), 20 ug (✶), and 10 ug (□) injections of cocaine. Eight rats tested at all points.

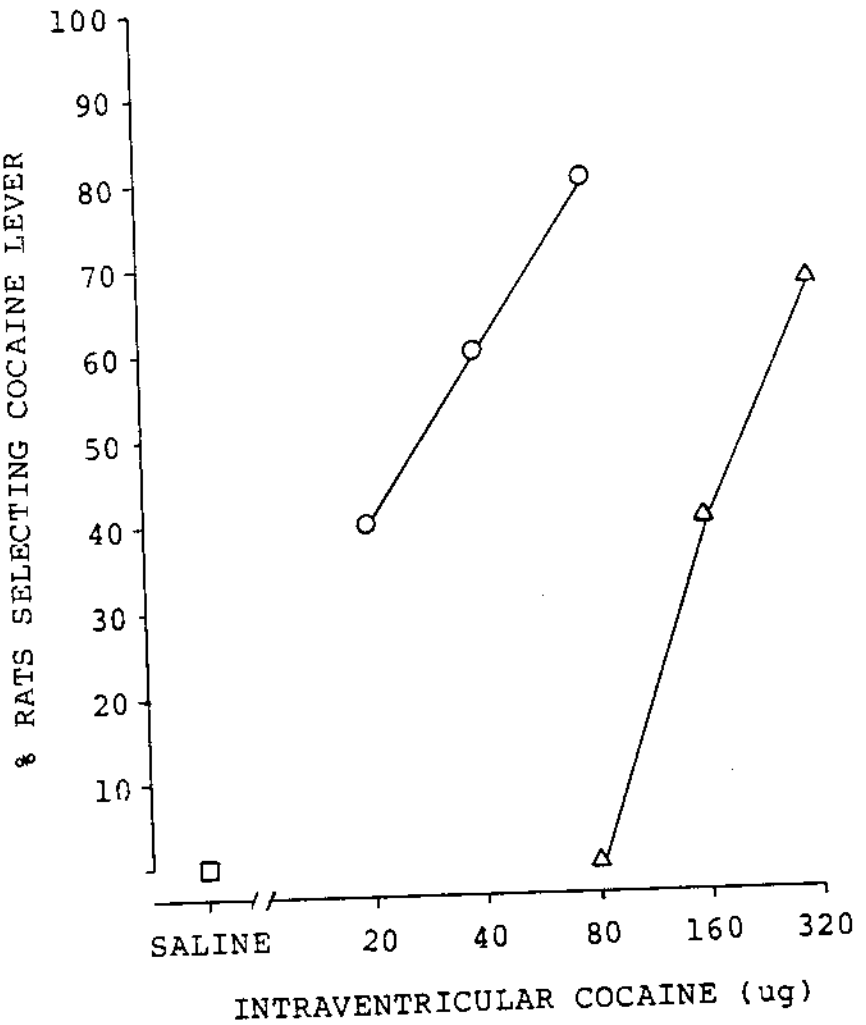


Figure 24. Dose-effect data for the detection of cocaine administered i.c.v. while trained by peripheral injection of 10 mg/kg cocaine. Testing occurred 5 min. post i.c.v. injection. Data were determined (O) before and after (△) chronic administration of 20 mg/kg/8hr cocaine for 6 days. Eight rats tested at all points with at least 5 making a lever selection.

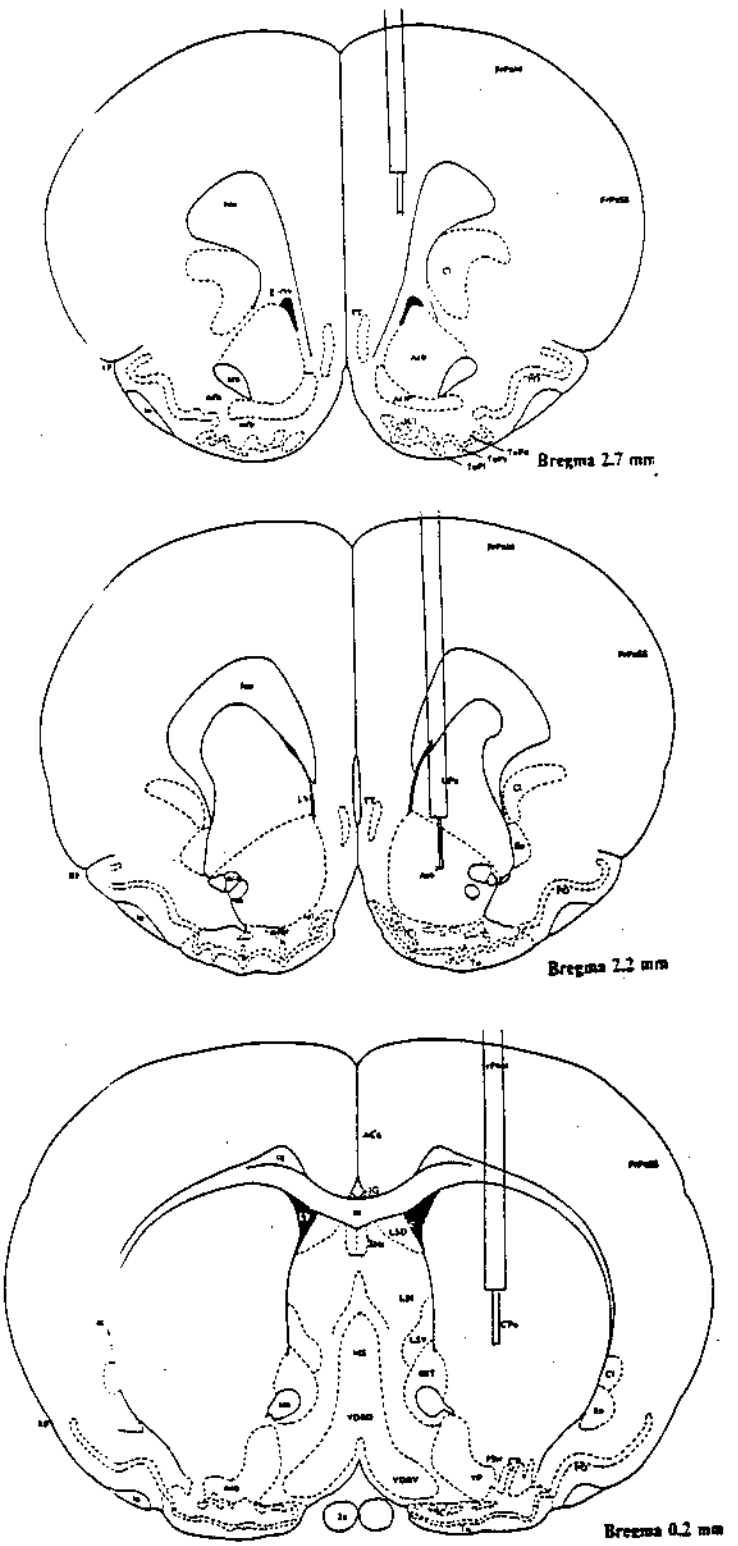


Figure 25. Examples of injection sites from animals receiving cocaine into either the prefrontal cortex (upper section), the nucleus accumbens (middle section), or the caudate-putamen (lower section).

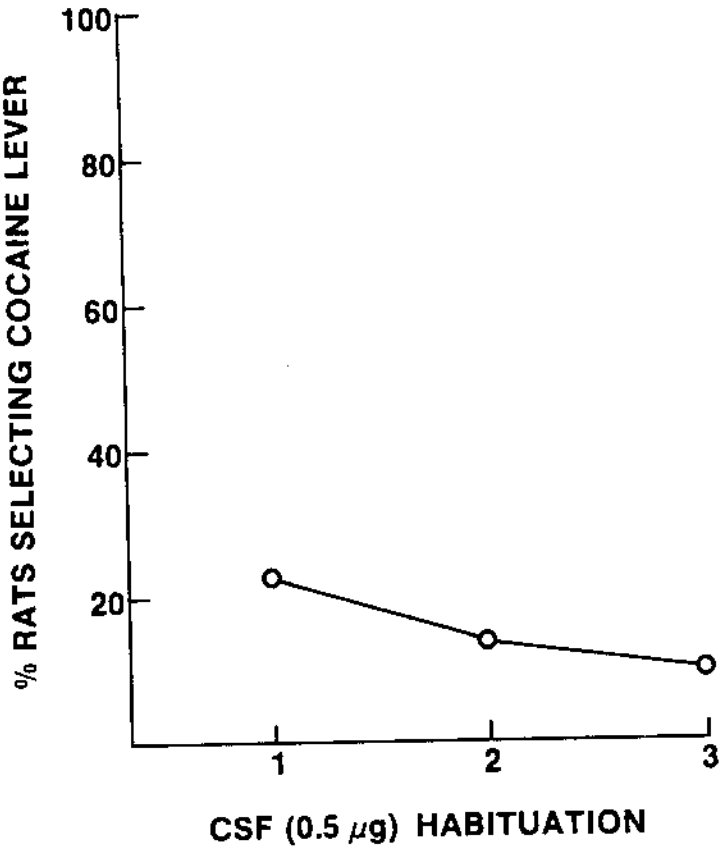


Figure 26. Habituation to intracerebral administration of cerebrospinal fluid and/or the restraint procedure. Abscissa: number of habituation sessions. Ordinate: percentage of subjects selecting the cocaine-appropriate lever after intracerebral administration of .5 ul of CSF per side. N = 22.

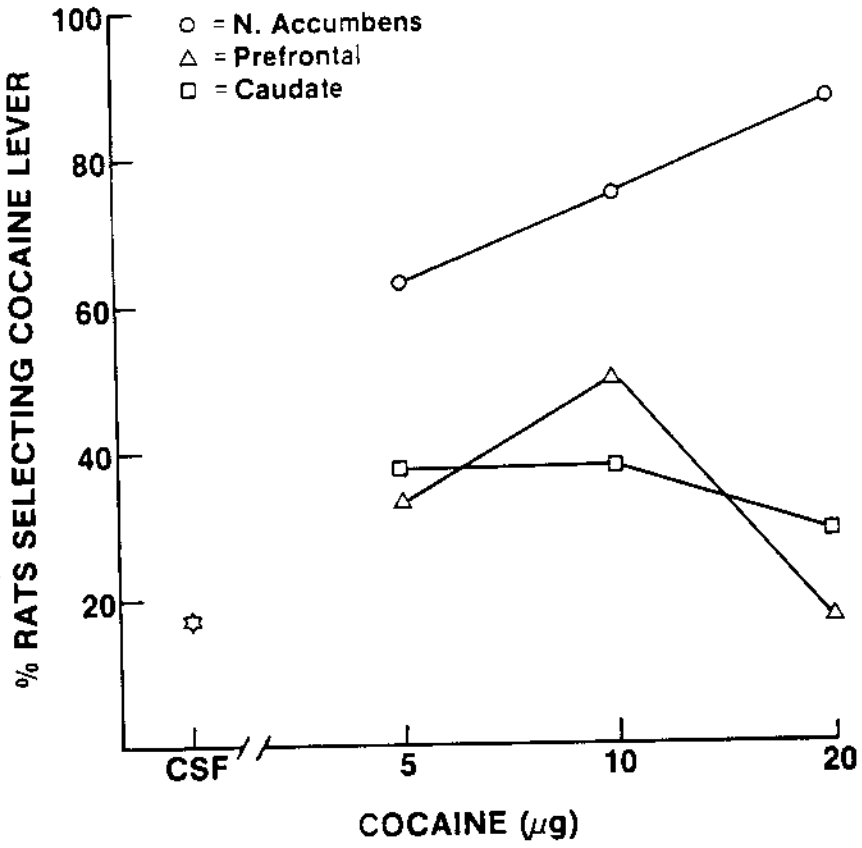


Figure 27. Dose-effect data for the detection of cocaine administered into specific brain sites while trained by peripheral injection of 10 mg/kg cocaine. Testing occurred 5 min. post injection. Data were determined for subjects selecting the cocaine-appropriate lever after microinjections into the nucleus accumbens (○), prefrontal cortex (△), and caudate-putamen (□). Eight rats tested at all points with at least 5 making a lever selection.

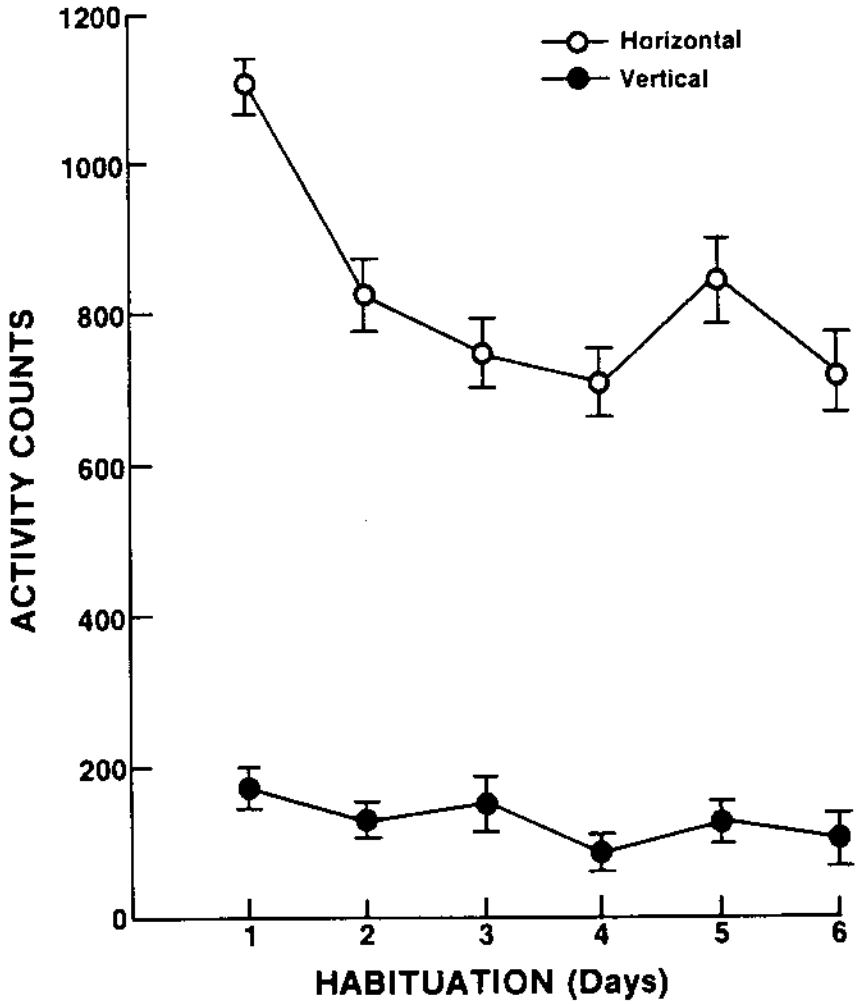


Figure 28. Habituation to the locomotor activity chambers following 6 days of saline administration. Abscissa: habituation days (20 min. session). Ordinate: cumulative activity counts. The (O) represents horizontal activity, the (●) represents vertical activity.

Experiment 14: Effects of chronic administration of cocaine on locomotor activity.

The animals were habituated to the locomotor activity chambers for 6 sessions. Horizontal and vertical activity was significantly reduced, as analyzed by one-way analysis of variance, following 6 days of habituation ($F = 4.379$, 3.509, $P < .01$, respectively)(Figure 28). Cocaine (5-40 mg/kg) produced a significant dose-dependent increase in locomtor activity in horizontal, but not vertical movements. Vertical and horizontal activity were tabulated in 10 min. bins so that the first ten minutes would correspond to the 10 min. test session in the operant chambers.

Following chronic administration of cocaine, 20 mg/kg/8-hr for 6 days, this treatment did not produce a significant shift of the dose-response curves for both horizontal and vertical activity in either the 10 or 20 min. bins (for statistical analysis using ANOVA, see appendix B)(Figures 29-32). Similarly, chronic administration of saline did not produce a significant shift of the dose-response curves for both horizontal and vertical activity in either the 10 or 20 min. bins (for statistical analysis using ANOVA, See Appendix)(Figures 33-36.).

Experiment 15. Effects of chronic administration of cocaine on stereotypy.

Rats displayed a dose-dependent increase in stereotypy scores following administration of cocaine. During the

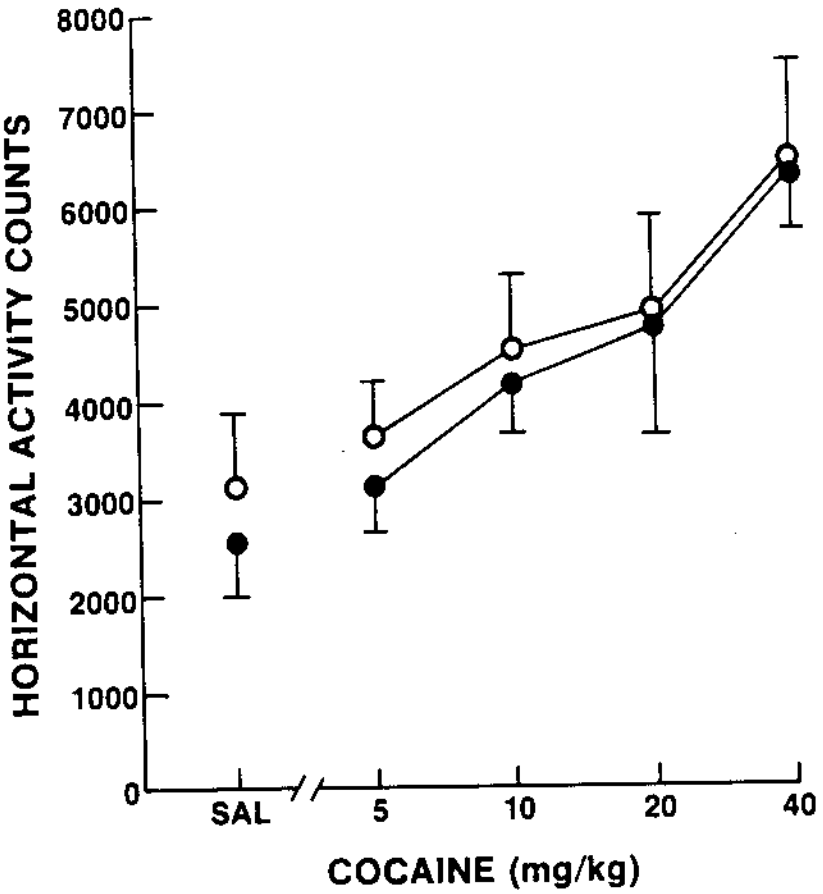


Figure 29. Horizontal locomotor activity does not change following chronic administration of cocaine. Abscissa: dose of cocaine. Ordinate: horizontal activity counts for the first 10 minute session. The (O) represents acute dose effect curve for cocaine, the (●) represents the dose-effect curve following administration of cocaine, 20 mg/kg/8-hr, for 6 days.

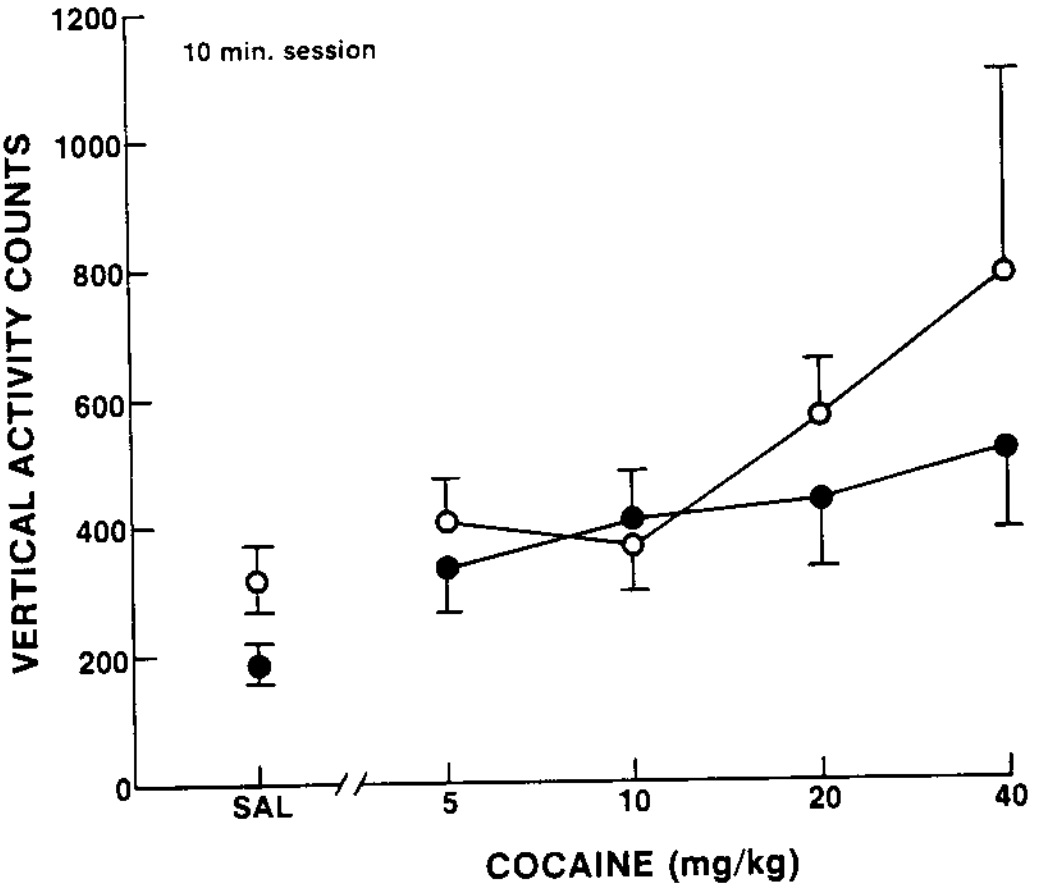


Figure 30. Vertical locomotor activity does not change following chronic administration of cocaine. Abscissa: dose of cocaine. Ordinate: vertical activity counts for the first 10 minute session. The (O) represents acute dose effect curve for cocaine, the (●) represents the dose-effect curve following administration of cocaine, 20 mg/kg/8-hr, for 6 days.

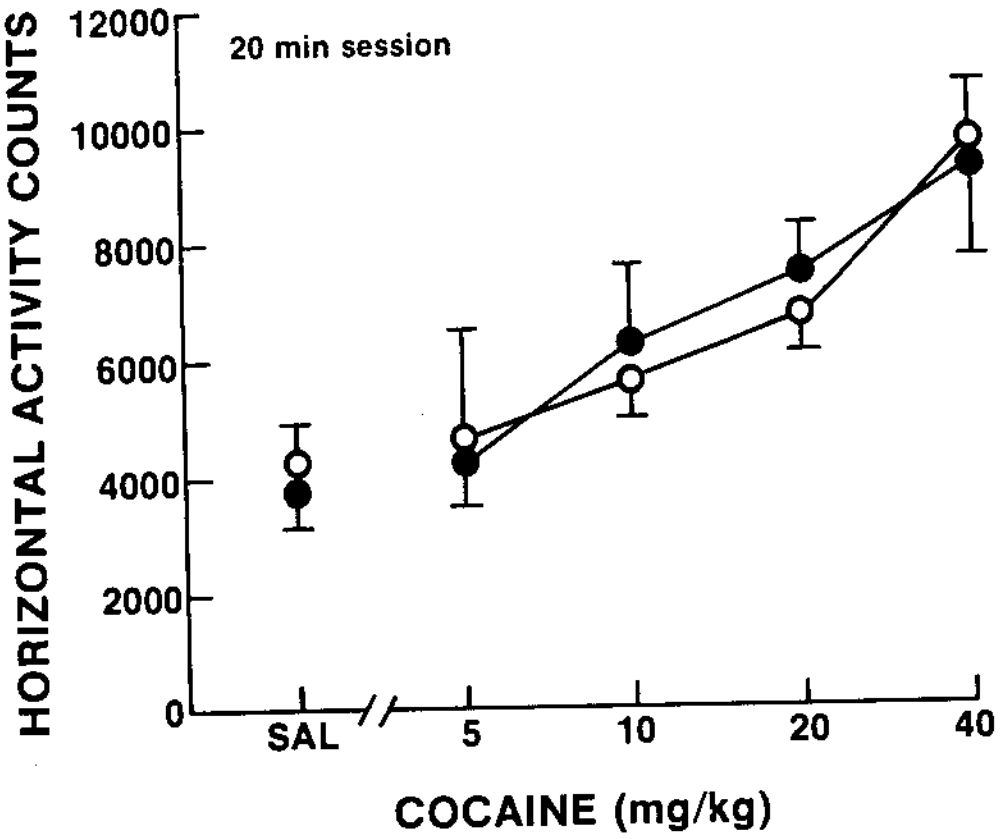


Figure 31. Horizontal locomotor activity does not change following chronic administration of cocaine. Abscissa: dose of cocaine. Ordinate: horizontal activity counts for the 20 minute session. The (O) represents acute dose effect curve for cocaine, the (●) represents the dose-effect curve following administration of cocaine, 20 mg/kg/8-hr, for 6 days.

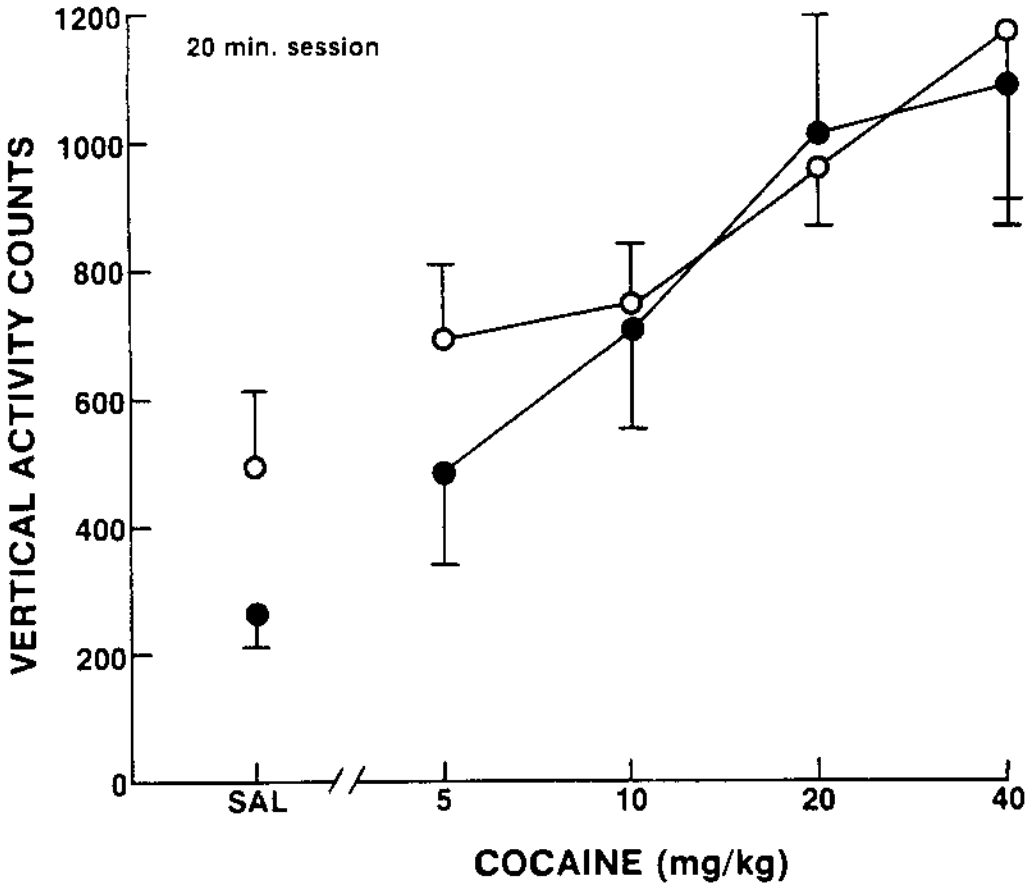


Figure 32. Vertical locomotor activity does not change following chronic administration of cocaine. Abscissa: dose of cocaine. Ordinate: vertical activity counts for the 20 minute session. The (O) represents acute dose effect curve for cocaine, the (●) represents the dose-effect curve following administration of cocaine, 20 mg/kg/8-hr, for 6 days.

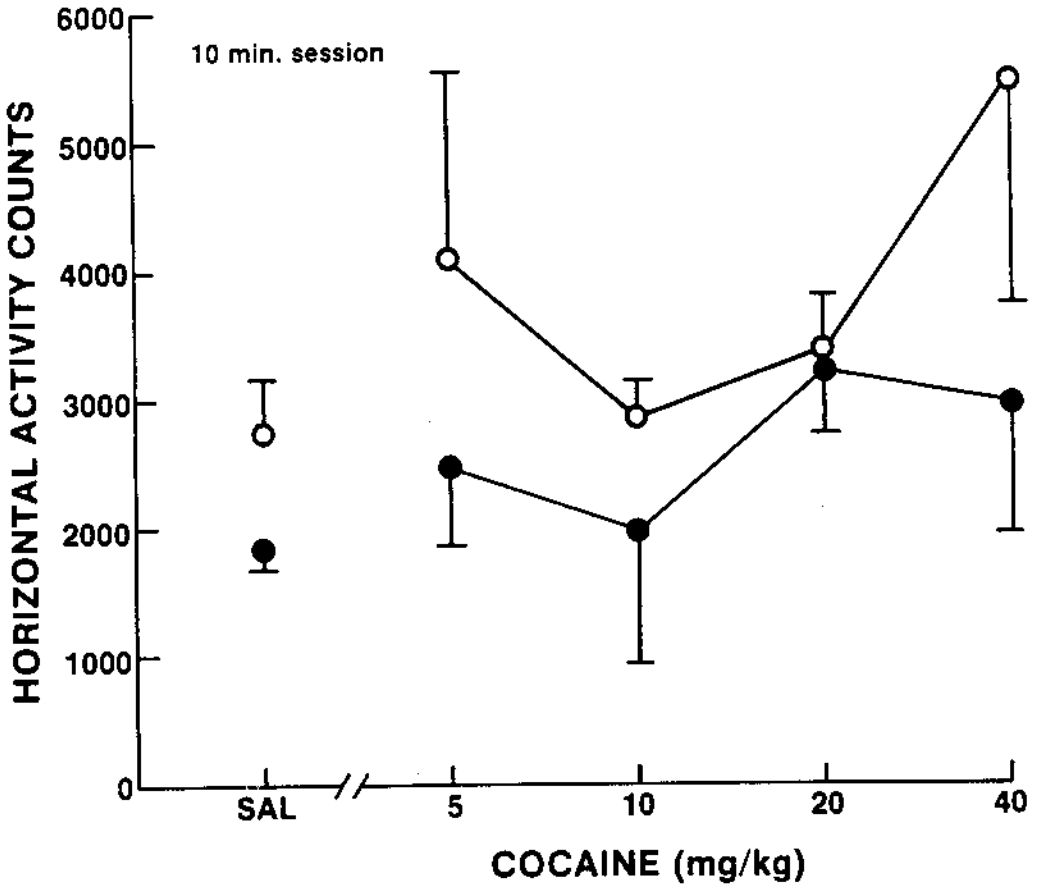


Figure 33. Horizontal locomotor activity does not change following chronic administration of saline. Abscissa: dose of cocaine. Ordinate: horizontal activity counts for the first 10 minute session. The (O) represents acute dose effect curve for cocaine, the (●) represents the dose-effect curve following administration of saline, 1 ml/kg/8-hr, for 6 days.

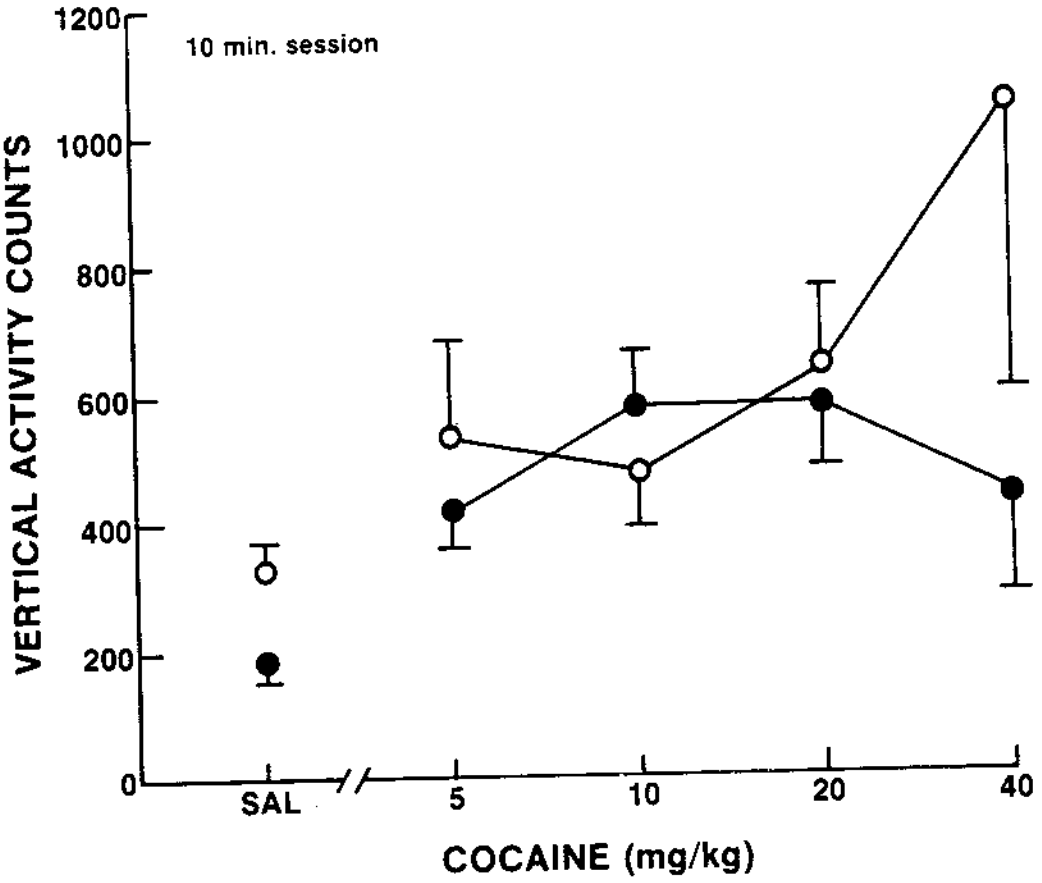


Figure 34. Vertical locomotor activity does not change following chronic administration of saline. Abscissa: dose of cocaine. Ordinate: vertical activity counts for the first 10 minute session. The (O) represents acute dose effect curve for cocaine, the (●) represents the dose-effect curve following administration of saline, 1 ml/kg/8-hr, for 6 days.

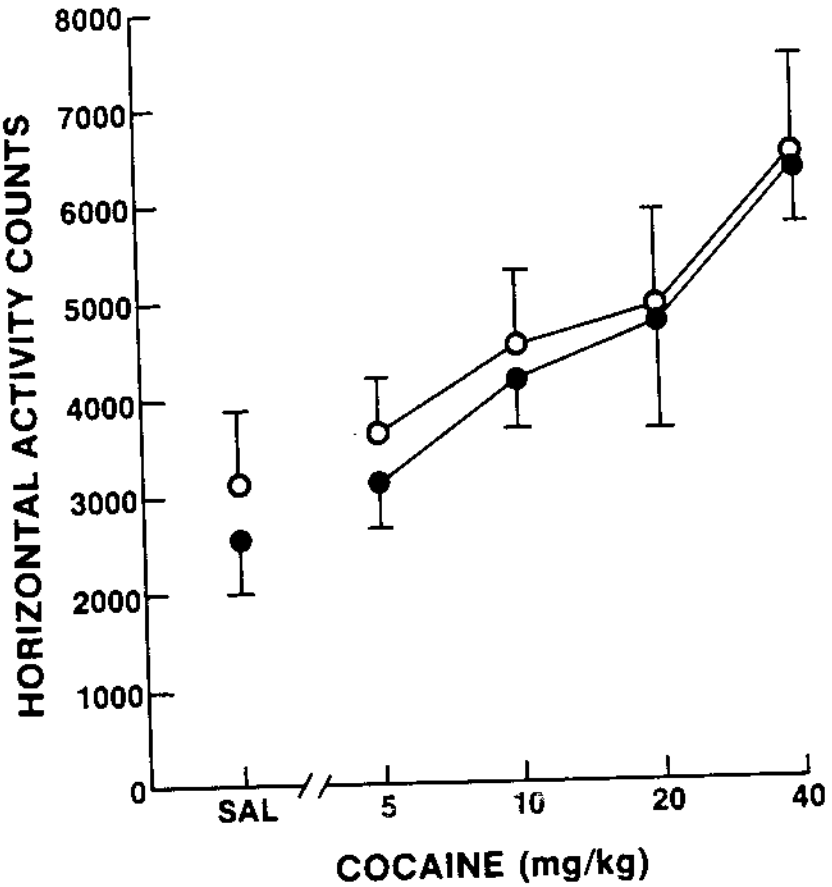


Figure 35. Horizontal locomotor activity does not change following chronic administration of saline. Abscissa: dose of cocaine. Ordinate: horizontal activity counts for the 20 minute session. The (O) represents acute dose effect curve for cocaine, the (●) represents the dose-effect curve following administration of saline, 1 ml/kg/8-hr, for 6 days.

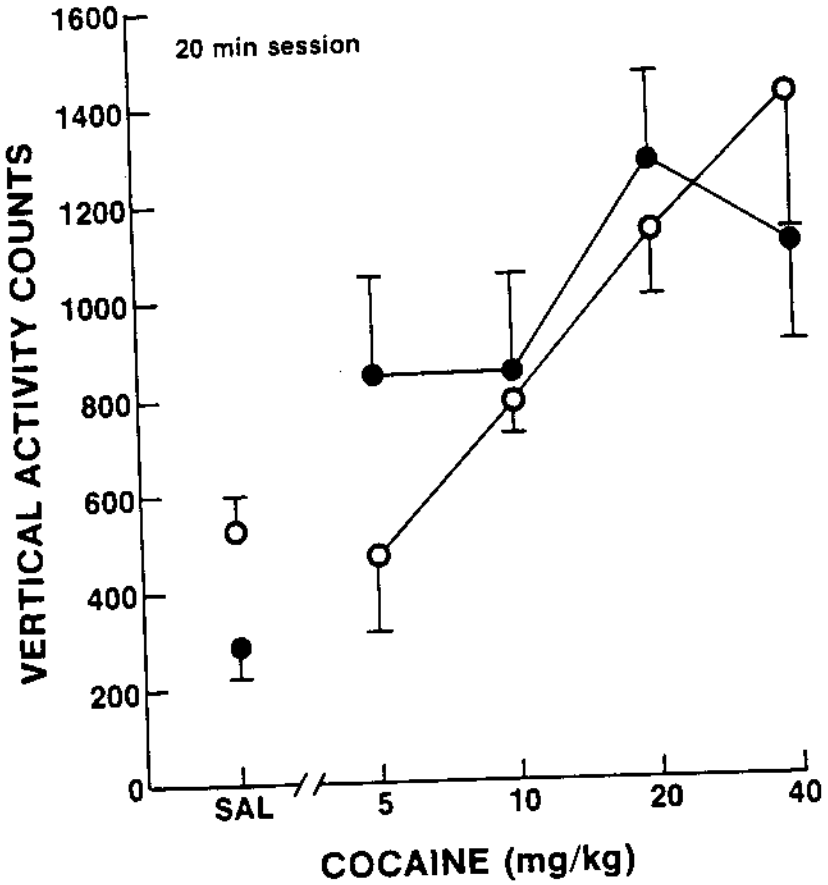


Figure 36. Vertical locomotor activity does not change following chronic administration of saline. Abscissa: dose of cocaine. Ordinate: vertical activity counts for the 20 minute session. The (O) represents acute dose effect curve for cocaine, the (●) represents the dose-effect curve following administration of saline 1 ml/kg/8-hr for 6 days.

period of cocaine administration, the animals appeared normal and did not manifest convulsions at any of the doses tested. Following 6 days of chronic administration of cocaine, 20 mg/kg/8-hr, the dose-response curve for stereotypic behavioral was not significantly different (for statistical analysis using ANOVA, Appendix)(Figure 37). However, there was a significant decrease at one dose of cocaine, 20 mg/kg ($F = 30$; $p > .01$).

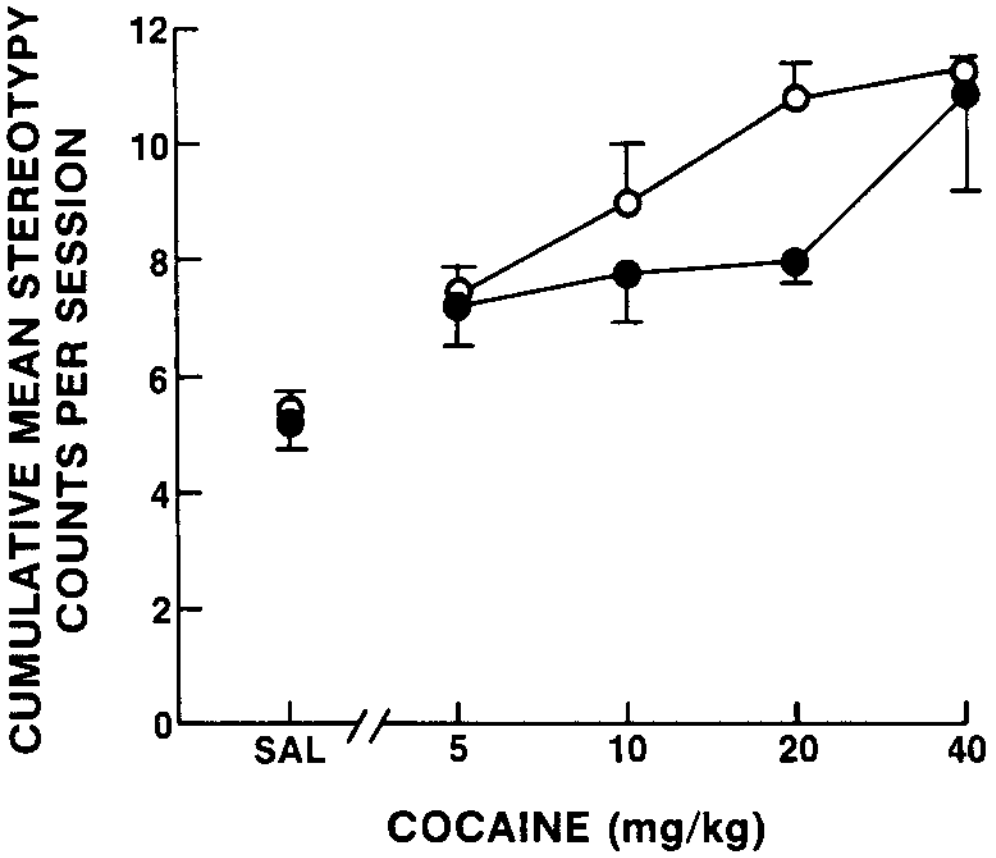


Figure 37. The effect of chronic administration of cocaine on stereotypic behavior. Abscissa: dose of cocaine. Ordinate: mean stereotypy counts per session. The data represents stereotypic counts per 20 minute session before (O) and after (●) chronic administration of cocaine, 20 mg/kg/8-hr for 6 days.

CHAPTER IV

DISCUSSION

The present results indicate that the training phase for drug discrimination experiments can be shortened significantly by increasing the number of training sessions conducted per day. In Experiment 1, days of training were reduced to approximately 75% of those required with daily training of either cocaine simply by conducting an additional training session on half of the training days. Therefore, this methodology shortens the time required for rats to obtain the discrimination by increasing the number of sessions per unit time, but does not appear to increase learning per session.

The number of reinforcers per session was important for obtaining optimum discriminability. There was no difference in time to obtain the cocaine discrimination using either 25 or 50 food reinforcers per session when trained daily. However, when an extra training session following a saline session was used, the group receiving 50 reinforcers per session acquired the discrimination approximately 30% faster than the group receiving 25 reinforcers per session.

From previous investigations it has been demonstrated that rats can be trained to discriminate cocaine by 50

sessions of training (Wood, Lal, and Emmett-Oglesby, 1984; Wood and Emmett-Oglesby, 1986). However, others have reported that rats can acquire cocaine discrimination in approximately 20 sessions (Colpaert, Niemegeers, and Janssen, 1976; D'Mello and Stolerman, 1977). The reason for this discrepancy is unclear. One possible explanation is that while the training procedure is relatively consistent between different laboratories, the initial shaping procedure varies. Therefore, it is suggested that the initial shaping procedure may play an important role in acquisition of discrimination learning. Further investigations of the limits to which these and other factors can be varied without decrement in acquisition rate will be of considerable practical utility for drug discrimination methodology, and of theoretical interest for the psychology of learning.

Tolerance developed for the discriminative stimulus properties of cocaine when 20.0 or 10.0 mg/kg/8-hr were administered for 1 week, but not when 5.0 mg/kg/8-hr were administered for as long as 2 weeks. Tolerance for the discriminative stimulus properties of cocaine has been reported (Wood et al., 1984; McKenna and Ho, 1977) when rats were injected with cocaine, 20.0 mg/kg/8-hr, for 7 days. However, it has been argued that tolerance cannot develop when a dose less than or equal to the training dose is administered chronically in a discrimination procedure

(Hutchings et al., 1978; Colpaert, 1978a). The present data demonstrate tolerance when the chronically administered dose is equivalent to the training dose. Furthermore, the degree of tolerance appears to be comparable whether cocaine, 10.0 or 20.0 mg/kg, is administered chronically; and because the effects of 1 and 2 weeks of the 20.0 mg/kg regimen were nearly identical, these findings raise the possibility that there is a maximum degree of tolerance that can be produced when cocaine is administered every 8 hrs. Although these data may also suggest that tolerance is a result of accumulation of drug with repeated dosing, the reported half-life of cocaine is 20-30 minutes (Misra, 1976); thus this finding is not compatible with the hypothesis that tolerance to the discriminative stimulus properties of cocaine is a result of drug accumulation.

Rats recovered sensitivity to the training stimulus after termination of chronic cocaine without retraining. The reacquisition of base-line sensitivity demonstrates the persistence of stimulus control exerted by the training stimulus. Subjects were not trained for 30 days, including 12 days of chronic cocaine injection, and saline lever selection was reinforced during testing. Despite these treatments, cocaine was increasingly generalized to the cocaine lever after termination of chronic injection. Thus, reinforcement of the saline lever choice during recovery tests did not bias rats to continue selecting this lever.

These results can be most parsimoniously explained as demonstrating that rats were under stimulus control throughout the procedure; therefore test results accurately reflect the extent to which the test stimulus resembles the stimulus that was trained before chronic drug administration.

There has been an ideological controversy concerning whether tolerance develops to cocaine in the discrimination procedure. It has been suggested that tolerance does not develop to the cocaine stimulus because in one study, the discriminability of cocaine did not fade after several months of training (Colpaert et al., 1976). However, in that study, training was conducted 5 days a week with only a maximum exposure of 2-3 cocaine doses per week. According to the tolerance theory described by Kalant et al. (1971), tolerance occurs when a drug is administered repeatedly and in high doses. One explanation for failure of the Colpaert et al. study to observe tolerance may be related to a lack of exposure to sufficiently high doses or frequent doses of cocaine.

Colpaert, Niemegeers and Janssen (1978) have also suggested that "tolerance" in the drug discrimination paradigm is an artifact. Tolerance has been shown to develop for the discriminative stimulus properties of d-amphetamine (Barrett and Leith, 1981), $(\Delta)^9$-tetrahydrocannabinol (Jarbe and Henriksson, 1973; Hirschhorn and

Rosecrans, 1974), morphine (Hirschhorn and Rosecrans, 1974; Shannon and Holtzman, 1976; Miksic and Lal, 1977; Colpaert et al., 1978b; Witkin, Dykstra, and Carter, 1982), barbital (York and Winter, 1975), and fentanyl (Colpaert et al., 1976a). However, their conclusion was based on the observation that tolerance failed to develop in a number of studies when training was continued during the phase of chronic injection (Bueno and Carlini, 1972; Colpaert et al., 1976a; 1976b; Hirschhorn and Rosecrans, 1974). Colpaert et al. suggested that terminating training and injecting high doses of the training drug may teach subjects to attend to the higher magnitude of these doses. However, this learning hypothesis has not been supported by data reported in Experiment 4, or by Schechter's study (1986). In those studies, when chronic drug administration was terminated, sensitivity to the training stimulus spontaneously recovered. This result is consistent with a tolerance hypothesis, but it is inconsistent with a learning hypothesis. In addition, Overton (1984) has pointed out that chronic administration of high doses in a procedure such as Colpaert et al. (1978) used could result in a stimulus that diminishes progressively, and training during the development of this tolerance teaches subjects to detect lower magnitudes of the stimulus. Thus, tolerance may have occurred in the Colpaert et al. study, but it would have gone undetected. In support of this hypothesis, several

studies have shown that subjects can learn to detect progressively lower intensity of drug stimuli using "fading" procedures (Overton, 1979; Stolerman and D'Mello, 1986; Stolerman, Garcha, Pratt, and Kumar, 1984).).

Previous studies of tolerance for the behaviorally disruptive effects of cocaine have found that tolerance occurs only if the behavior tested was performed repeatedly while under the influence of the drug (Woolverton, Kandel, and Schuster, 1978). This phenomenon has been described as tolerance contingent on performing a behavioral task (Carlton and Wolgin, 1971; Campbell and Seiden, 1973), and it can be contrasted with the present findings of rapidly developing tolerance for the discriminative stimulus properties of cocaine based on multiple daily administrations of drug, which occurred without practice on the behavioral task. In agreement with the predictions of behavioral tolerance, rats tolerant to the discriminative stimulus properties of cocaine are not tolerant to its behaviorally disrupting effects as measured by suppression of bar-press responding (Wood et al., 1984). Therefore, tolerance for the discriminative stimulus properties of cocaine is most likely a form of pharmacodynamic tolerance for interoceptive stimulus.

Several behavioral investigators have suggested that tolerance is a learning process, since it involves a change in the perception of a stimulus (Dews, 1978; Le Blanc and

Cabell, 1977). Many models of tolerance have been developed by these investigators, the most prominent being the reinforcement density hypothesis and the classical conditioning (habituation) hypothesis. The reinforcement density hypothesis is based on a study by Schuster, Dockens, and Woods (1966), who demonstrated that tolerance develops to behavioral effects if the initial drug effect caused loss of reinforcement or reward. This hypothesis was further developed by Corfield-Sumner and Stolerman (1978) and Demellweek and Goudie (1983), who produced a comprehensive review of tolerance on a variety of drugs and operant conditions. However, Goudie and Demellweek (1986) have pointed out that although this hypothesis may involve many drugs and operant conditions, it is not inclusive for all drugs or conditions. In the drug discrimination procedure, there is no loss of food reinforcement following injection with the training or lower doses of cocaine, and while tolerance developed to the discriminative stimulus properties of cocaine, no tolerance developed to the rate suppressing effects of the drug (Wood et al., 1984). While the reinforcement density hypothesis may describe behavioral tolerance, the lack of tolerance to the behaviorally disruptive effects of cocaine may indicate that the subjective and behavioral aspects of cocaine can be differentiated in the drug discrimination paradigm.

A second major hypothesis on the development of

tolerance is the classical conditioning model. In the early 1960's, it was observed that environmental conditions were important for the development of tolerance to opiates. For example, tolerance to morphine-induced analgesia occurred more rapidly in the same environment than when the subjects were placed in a different environment (Adams, Yeh, Woods and Mitchell, 1969). Siegel (1975, 1977) suggested that this type of tolerance was due to a Pavlovian (1926) or classical conditioning phenomenon. In the classical conditioning model a drug serves as an unconditioned stimulus (UCS) that produces an unconditioned response (UCR)(i.e. for cocaine, euphoria). Siegel (1977) further suggested that cues paired with drug administration (i.e. injection ritual) elicit conditioned responses (CR) that are compensatory, in nature. Conceptually, drug discrimination has been described as an UCS. Tolerance to and spontaneous recovery (extinction) from pharmacological effects of a drug have been described in Pavlovian terms and are similar to the development of tolerance observed for the discriminative stimulus properties of cocaine. It is suggested that the interpretation for tolerance in a discrimination paradigm can be constructed in a Pavlovian framework.

With respect to drug discrimination studies, McKenna and Ho (1977) demonstrated that chronic administration of saline did not cause a shift of the cocaine dose-effect curve to the right, prompting the present study of the

effect of chronic administration of morphine. A defining feature of tolerance is that tolerance and cross-tolerance arise only within a class of drugs (Kalant et al., 1971); therefore, chronic administration of a drug of a different class should have little influence on the perception of the cocaine stimulus. This hypothesis was confirmed in that morphine treatment did not modify the base-line sensitivity to the cocaine stimulus. The morphine dosing regimen resulted in clear signs of physical dependence when naloxone was administered; thus narcotic dependence did not modify the neurochemical mechanisms involved in the detection of the cocaine stimulus.

d-Amphetamine substituted for cocaine in a dose-dependent fashion, and it was approximately 8 times more potent than cocaine. Diethylpropion, phenmetrazine, phentermine and methylphenidate also substituted for the discriminative stimulus properties of cocaine; however, fenfluramine did not substitute for the cocaine stimulus. These findings agree with previous studies showing that amphetamine-type drugs will substitute for the stimulus produced by cocaine (D'Mello and Stolerman, 1977; Emmett-Oglesby et al., 1983; Huang and Ho, 1974; Ho et al., 1976; Ho and McKenna, 1978; Wood et al. 1984). These data concur with previous studies which demonstrate that amphetamine-type drugs produce discriminative stimuli similar to that produced by cocaine (D'Mello and Stolerman, 1977; Colpaert

et al. 1978; Wood et al. 1984, Wood and Emmett-Oglesby, 1986).

These data also concur with those reported by Schuster and Johanson (1985), in which these anorectic drugs were tested across monkeys, pigeons, and rats for their ability to substitute for the stimulus properties of d-amphetamine. Similarly to Experiment 5, fenfluramine could be differentiated from other anorectic drugs as having no amphetamine-like properties.

In recent reports, the experimental paradigm of studying the discriminative stimulus properties of drugs has been extended to human subjects. For example, it has been demonstrated that humans can be trained reliably to discriminate amphetamine from placebo (Chait, Uhlenhuth, and Johanson, 1984; Chait, Uhlenhuth, and Johanson, 1985; Chait, Uhlenhuth, and Johanson, 1986). In tests involving substitution of anorectic drugs for the amphetamine stimulus, phenmetrazine was not differentiated from amphetamine (Chait et al., 1986); however, fenfluramine (Chait et al., 1986) was different compared to the amphetamine stimulus. In the above studies, subjective effects were also assessed with standard questionaires, and these results parallelled those of the discriminative paradigm. These data support the hypothesis that the discrimitive stimulus and subjective effects are closely interrelated, and that changes in the subjective status may

be a major factor for drug discrimination responding.

Tolerance to cocaine neither increased nor decreased cocaine-lever selection when subjects were tested with fenfluramine. Fenfluramine has been reported to be nonstimulating in humans, and it also appears to act by a different neurological mechanism than amphetamine (Dykes, 1984). These data are consistent with the present finding of the lack of substitution and cross-tolerance to cocaine. Thus the present data support the hypotheses that 1) the disciminative stimulus properties of cocaine are specific, and that 2) the relative efficacy of other drugs in substituting for the cocaine stimulus depends on the intensity of the test drug's stimulus as compared to the cocaine stimulus.

Tolerance to cocaine conferred cross-tolerance to diethylpropion, methylphenidate, phenmetrazine, and phentermine. During cocaine tolerance, the curves for substitution of methylphenidate, phenmetrazine, and phentermine for the cocaine training stimulus shifted approximately 2-fold to the right, which is the same magnitude as the shift for the detection of cocaine. Because the discriminative stimulus properties of cocaine and amphetamine-type drugs have been mechanistically linked to the property of enhancement of central nervous system dopaminergic neurotransmission (D'Mello and Stolerman, 1977; Jarbe, 1984; McKenna and Ho, 1980), it is suggested that

drugs sharing this neurochemical action will show similar tolerance and cross-tolerance profiles.

Although diethylpropion is an amphetamine-related drug, the curve for the substitution of diethylpropion was shifted to a much greater extent. The magnitude of this cross-tolerance was such that diethylpropion did not completely substitute for the cocaine training stimulus. Doses above 5 mg/kg of diethylpropion resulted in behavioral disruption such that no lever selection was made during the 10 minute test period. The reason for the profound cross-tolerance from cocaine to diethylpropion is unknown. However, in the United States the Drug Enforcement Agency has classified cocaine, methylphenidate, and phenmetrazine as Schedule II drugs, indicating that they have known high abuse liability. In the present experiment, the degree of shift of the dose-effect curves during tolerance was comparable for these drugs. Phentermine, although classified as a Schedule IV drug (less abuse potential than Schedule II drugs), has also been implicated as having high abuse potential (Carabillo, 1978). Thus the present data demonstrate that amphetamine-type drugs with high abuse potential have a comparable pattern with respect to the development of cross-tolerance to their stimulus properties. Diethylpropion is also a Schedule IV substance, and it is generally considered to have less abuse potential then the drugs described above (Cohen, 1980; Hoekenga et al., 1978); interestingly, it

showed a different profile in the test for cross-tolerance to the cocaine stimulus. Thus, these findings suggest that tolerance in the drug discrimination procedure may have potential for establishing a comprehensive evaluation of dependence liability of CNS stimulants.

Chronic administration of d-amphetamine produced tolerance for d-amphetamine and cross-tolerance for cocaine. Chronic administration of d-amphetamine produced a greater shift to the right of the cocaine dose-effect curve than chronic administration of cocaine. No dosing regimen of cocaine shifted the dose-effect curve for the detection of the cocaine stimulus more than 2-fold. In contrast, chronic d-amphetamine resulted in an approximate 4-fold shift to the right of the dose-effect curves for both d-amphetamine and cocaine. The duration and timing of amphetamine administration were identical with those for cocaine: so these are unlikely to be important variables in producing this effect. Similarly, the dose of amphetamine that was selected for chronic administration was operationally equilavent to the 20 mg/kg dose of cocaine; in both cases, it was twice the dose that produced maximum generalization. Perhaps one variable that might account for these differences is the difference in duration of action of cocaine and d-amphetamine. In rats, the elimination half-lives of cocaine and amphetamine are approximately 20 min. (Misra, 1976) and 50 min. (Kuhn and Schanberg, 1978; Zenick,

Lasley, Greenland, Caruso, Succop, Price, and Michaelson, 1982), respectively. Thus, the longer duration of action of d-amphetamine might contribute to its tolerance characteristics. These data further support the hypothesis that a common mechanism mediates tolerance for both drugs (Wood et al., 1984). Because the discriminative stimulus properties of cocaine and d-amphetamine have been mechanistically linked to the property of enhancement of central nervous system dopaminergic neurotransmission (D'Mello and Stolerman, 1977; Jarbe, 1984; McKenna and Ho, 1980), it is suggested that drugs sharing this neurochemical action and substituting for cocaine will show similar tolerance and cross-tolerance profiles.

There is substantial evidence that brain dopamine is the mediator of the cocaine discriminative stimulus. For example, dopamine receptor agonists such as apomorphine substitute for the cocaine stimulus (Colpaert et al., 1978a; McKenna and Ho, 1980); and dopamine receptor blocking agents antagonize the cocaine cue (Jarbe, 1984; McKenna and Ho, 1980; Colpaert et al, 1978b; Colpaert et al., 1978c). However, recently, there has been a distinction between two types of functional dopamine receptors in the brain based on its ability to stimulate adenylate cyclase activity. D1-type dopamine receptors stimulate adenylate cyclase activity (Stoof and Kebabian, 1984), and D2-type dopamine receptors inhibit adenylate cyclase (Onali, Olianas, and Gessa, 1984).

Furthermore, these two receptors can be physically separated by steric exclusion (Dumbrille-Ross, Niznik, and Seeman, 1985).

In the present experiment, apomorphine, a mixed D1 and D2 receptor agonist and piribedil, a selective D2 receptor agonist, substituted for the discriminative stimulus properties of cocaine. However, SKF-38393, a selective D1 receptor agonist, only partially substituted for cocaine. These data suggest that the discriminative stimulus properties of cocaine are mediated by D2 receptors, and supports the hypothesis that dopamine is the primary mediator of the cocaine stimulus. However, there have been no studies investigating the neurochemical mediators of tolerance to the discriminative stimulus properties of cocaine.

If the tolerance to cocaine is mediated by brain dopamine then one would expect three basic findings. First, chronic administration of cocaine should decrease sensitivity for the detection of a dopaminergic agonist. Second, chronic administration of a dopaminergic agonist should decrease the detection of cocaine. Third, pretreatment of a dopamine receptor antagonist before chronic cocaine should block the development of tolerance.

Chronic administration of cocaine produced tolerance to the discriminative stimulus properties of cocaine and cross-tolerance to the discriminative stimulus properties of

apomorphine and piribedil. However, cross-tolerance to SKF-38393, did not develop. These data are significant because they suggest that D2 receptors are involved in mediating tolerance to the discriminative stimulus properties of cocaine. One interesting observation from these results was that tolerance to cocaine abolished the substitution of apomorphine for the cocaine stimulus such that no dose of apomorphine resulted in cocaine-appropriate lever responding. The magnitude of the shift of the apomorphine dose-effect curve could not be established since doses higher than 5.0 mg/kg apomorphine resulted in no lever selection during the 10 minute test session.

Although mechanisms mediating tolerance to the effects of cocaine are not established, it does not appear that pharmacokinetic mechanisms account for the tolerance observed in this procedure. For example, chronic administration of cocaine produces negligible effects on its rate of elimination, or its distribution in the brain (Miscra, 1975). Thus, the tolerance that was observed in the present experiment is more compatible with a pharmacodynamic interpretation of tolerance. Results from the present experiment demonstrated a decrease in potency for both the substitution of apomorphine following chronic administration of cocaine and generalization to cocaine following chronic administration of apomorphine. Since apomorphine and piribedil directly stimulate post-

synaptic dopamine receptors (Kebabian and Calne, 1979), these results suggest that stimulation of post-synaptic dopaminergic receptors is a critical event in the production of tolerance to the cocaine stimulus.

Further evidence supporting the hypothesis that tolerance to the discriminative stimulus properties of cocaine is a centrally mediated pharmacodynamic phenomenon, is that chronic administration of apomorphine and piribedil shifted the dose-effect curve for generalization to the cocaine stimulus greater than two-fold to the right. However, chronic administration of SKF-38393 did not shift the cocaine dose effect curve. Again, these data are consistent with the hypothesis that tolerance to the discriminative stimulus properties of cocaine is mediated by a mechanism involving D2 receptors.

Chronic administration of haloperidol (D1 and D2) and sulpiride (D2) prior to cocaine administration blocked the development of tolerance. One interesting observation is that haloperidol blocked the training dose only 20% and sulpiride blocked the training dose only 30%. These data indicate that dopamine may not be exclusively involved in the cocaine cue. While the dopamine hypothesis concerning the cocaine cue is generally accepted, the role of other neurotransmitters such as phenylethylamine (Colpaert et al., 1980), GABA (Gayle, 1984), and endogenous proteins (Colpaert et., al, 1983) may also be responsible for mediating the

cocaine stimulus.

Increased sensitivity to the cocaine stimulus following treatment with the dopamine receptor antagonists, haloperidol and sulpiride, is further evidence that the discriminative stimulus properties of cocaine are mediated by a dopaminergic mechanism. Furthermore, when animals were tested on saline following chronic haloperidol and sulpiride, they responded as though administered a small dose of cocaine. This indicates that even in the absence of challenge with dopamine agonists, dopamine-related behavioral supersensitivity can be demonstrated with the drug discrimination paradigm. These data agree with other behavioral studies which have reported increased dopamine receptor supersensitivity following chronic administration of various neuroleptics in response to a dopamine challenge (Tarsey and Baldessarini, 1974). However, since the drug discrimination paradigm is a behavioral assay system which is not influenced by rate of responding, enhanced cocaine discrimination following chronic haloperidol or sulpiride treatment would suggest changes in dopaminergic function in systems other than those directly involved in the control of motor behavior.

These data also agree with a study conducted by Barrett and Steranka (1983) which demonstrated that chronic administration of haloperidol will increase sensitivity to amphetamine when amphetamine was trained as a discriminative

stimulus in rats. They also reported that when tested on saline, subjects increased selection of the amphetamine lever following chronic haloperidol, but decreased selection of the amphetamine lever following chronic amphetamine. These changes provide evidence for both enhanced (following chronic haloperidol) and diminished (following chronic amphetamine) dopaminergic function.

In order to empirically test the hypothesis that the discriminative stimulus properties of cocaine are centrally mediated, rats trained by i.p. injection of cocaine were administered microinjections of cocaine in the lateral ventricles and tested for cocaine-lever selection. In Experiment 12, cocaine administered intracerebroventricularly (i.c.v.) was generalized to the discriminative stimulus properties of cocaine trained by peripheral administration. However, when measured at the corresponding peak times, cocaine administered i.c.v. was approximately 40 times more potent than by i.p. administration. The most reasonable account for this observation is that cocaine administered i.c.v. is limited only by local diffusion for interaction with receptors in various brain areas, whereas, with i.p. administration of cocaine, the amount of drug reaching brain receptors is dependent on the absorption, distribution, and metabolism of cocaine peripherally.

Data from the present experiment suggest that the discriminative stimulus properties of cocaine are centrally

mediated. These results are comparable to those in which d-amphetamine administered in the lateral ventricles (Richards et al., 1973) and nucleus accumbens (Nielsen and Scheel-Kruger, 1986) generalized to the amphetamine stimulus in rats trained by peripheral administration. With respect to cocaine, two previous studies (Ho and Silverman, 1978; Richards et al., 1973) have failed to demonstrate that cocaine injected i.c.v. will generalize to the cocaine stimulus. The reason for these previous failures is unclear. However, as demonstated by the time course for generalization to the cocaine stimulus for i.c.v. administration, it may be possible that the rapid duration or offset of the cocaine stimulus is responsible for the lack of generalization in the previous experiments since in our experiments we tested the rats 5 min. post i.c.v. injection compared to 15 min. in the other studies.

In subjects trained to detect a peripheral injection of 10.0 mg/kg cocaine and made tolerant by peripheral injection, tolerance also occurred to cocaine administered i.c.v. Moreover, the tolerance that was produced was of a comparable magnitude for both ventricular and peripheral administration of cocaine. Thus, these data are incompatible with a pharmacokinetic explanation of tolerance, which is consistent with the observation that chronic administration of cocaine produces negligible effects on its rate of metabolism, elimination, or distribution in the brain or

plasma (Misra, 1976). The data are, however, compatible with the hypothesis that tolerance to the discriminative stimulus properties of cocaine is a centrally mediated pharmacodynamic phenomenon. As additional support for this hypothesis, chronic administration of the dopamine receptor agonists apomorphine and piribedil, shifted the dose-effect curve for generalization to the cocaine stimulus two-fold to the right. Thus tolerance to the discriminative stimulus properties of cocaine may be mediated by a mechanism involving dopamine receptors.

There have been no studies investigating whether the discriminative stimulus properties of cocaine are mediated at site-specific brain areas. Recently, Nielsen and Scheel-Kruger (1986) reported that amphetamine administered in the nucleus accumbens generalized to the discriminative stimulus properties of amphetamine trained by peripheral (i.p.) administration. Compatible with the results in the Nielsen study, cocaine injected in the the nucleus accumbens produced a discriminative stimulus similar to that produced by peripheral administration. Cocaine administered in either the prefrontal cortex or caudate-putamen did not produce cocaine-like responding. These data suggest that the nucleus accumbens may play an important role in mediating cocaine discrimination. Furthermore, the lack of a cocaine-like response following intracerebral administration of cocaine in the prefrontal cortex or caudate-putamen

indicates that diffusion of cocaine from the nucleus accumbens into other brain areas does not account for cocaine-like responding following cocaine injection in the nucleus accumbens.

Neuroanatomical pathways have shown several dopaminergic pathways within the central nervous system. The mesolimbic system cell bodies (A10) originate in the ventral tegmentum within the decussation of the superior cerebellar peduncle with axons extending rostrally to the nucleus accumbens, olfactory tubercle, and striatum (Kelley, Domesick, and Nauta, 1982). A second pathway, the mesocortical system, contains cell bodies that originate in the ventral tegmental area and extend to the frontal cortex (Lindvall and Bjorklund, 1974). A third pathway, the combined axons from cells in A8 and A9 located in the ventrolateral tegmentum and zona compacta of the substantia nigra, forms the nigrostriatal pathway which innervate the caudate-putamen area.

Various behavioral effects have been associated with the specific dopaminergic pathways. For example, the mesocortical system has been implicated in cocaine self-administration since it is reinforced in the prefrontal cortex, but not in the nucleus accumbens, striatum, or caudate-putamen area (Dworkin, Goeders and Smith, 1986). The nigrostriatal system has been implicated in mediating locomotor and stereotyped behavior (Moore and Kelly, 1978;

Moore, 1978), whereas the mesolimbic system has been implicated in drug effects related to psychosis (Carrand White, 1983; Neill and Justice, 1981) and highly integrated forms of behavior such as reinforcement and learning (Robbins and Everett, 1982; Robbins and Koob, 1980; Solomon and Staton, 1982).

While it may be oversimplistic to categorize highly intergrated forms of behavior to specific brain areas, the present data suggest that the mesolimbic dopamine pathway may mediate cocaine drug discrimination. It is recognized that other sites cannot be excluded since only a few were tested. However, it may even be possible that drug discrimination in general may be related to specific brain areas, since cocaine, amphetamine (Nielsen and Scheel-Kruger, 1986), and LSD (Nielsen and Scheel-Kruger, 1986) injected in the nucleus accumbens will produce a stimulus similar to that of systemically administered drug.

The effects of repeated administration of cocaine on spontaneous motor activity and stereotypy have been extensively studied. However, these studies have produced conflicting results. For example, tolerance has been reported for locomtor activity (Roy, Bhattacharyya, Pradhan, and Pradhan, 1978; Sahakian, Robbins, Morgan, and Iversen, 1975) and stereotypy (Post, Kopanda, and Black, 1976). Other studies using similar paradigms have either failed to find tolerance or enhanced sensitivity for locomotor

activity (Ho, Taylor, Estevez, Englert, and McKenna, 1977; Post and Rose, 1976) and stereotypy (Post, Kopanda, and Black, 1978; Pradhan, Roy, and Pradhan, 1978; Stripling and Ellinwood, 1977). In one study, repeated administration of cocaine, 15 mg/kg/12-hr, produced enhanced stereotypy and locomotor activity by 9 days of chronic administration, but produced tolerance by 15-30 days (Roy, Bhattacharyya, Pradhan, and Pradhan, 1978). Thus, it appears that the amount and duration of cocaine administration play an important role whether tolerance or sensitivity develops following chronic cocaine administration.

In Experiment 14 and 15, cocaine produced a dose-dependent increase in horizontal and vertical locomotor activity and stereotypy. However, chronic administration of cocaine, 20 mg/kg/8-hr for 7 days, did not produce either tolerance or sensitivity for locomotor activity or stereotypy. With respect to cocaine discrimination studies, this chronic drug regimen produced tolerance to the discriminative stimulus properties of cocaine (for reviews, see Introduction). The importance of these data are that tolerance develops to one behavioral paradigm (drug discrimination), but not to other behavioral paradigms (locomotor activity and stereotypy) maintaining a similar chronic cocaine regimen. While all three behavioral events have been associated with alterations of dopamine transmission, it appears that different mechanisms mediate

these events.

In conclusion, the data from the above experiments suggest that tolerance to the discriminative stimulus properties produced by cocaine is mediated by the dopaminergic system which is independent of other behavioral parameters. Furthermore, the stimulus properties of cocaine and amphetamine-type compounds are similar. The data suggest that the discriminative stimulus properties of these compounds are mediated via alteration of dopaminergic transmission in the mesolimbic pathway. Since the drug discrimation paradigm has been proposed as an *in vivo* assessment of the subjective effects of drugs which parallel the subjective effects experienced in man (Schuster and Balster, 1984), the drug discrimination paradigm may offer a powerful methodology for investigating pharmacodynamic tolerance.

Virtually nothing is known about pharmacodynamic tolerance for the subjective effects of cocaine or amphetamine-type drugs in humans. In one of the few controlled clinical trials investigating tolerance for cocaine, the euphoric effects of a 32-mg dose of cocaine were diminished if the dose was given 1 hr after a previous dose (Fischman and Schuster, 1982). This observation suggests that tolerance for the subjective effects of cocaine may be more prevalent and easily obtained than commonly believed. In this regard, drug discrimination

learning in animals has been proposed as an assay for *in vivo* assessment of "subjectively" experienced effects of drugs in subhuman subjects (Emmett-Oglesby et al, 1984; Lal and Emmett-Oglesby, 1983; Jarbe, 1984). Therefore, this paradigm may offer a powerful methodology for investigating neural mechanisms mediating tolerance to subjective drug effects following chronic administration of drugs of abuse.

APPENDIX

(OPERANT PACKAGE FOR THE NEUROSCIENCES)

SHAPING PROGRAM UTILIZING OPN

Developed by:

M.W. Emmett-Oglesby

D.G. Spencer, Jr.

and

D.E. Arnoult

Procedure file: DAY1L
Component Sequence:
1,2,3,4,R 1,10 0

Component 1
FR 1
Left levers are correct.
No correction contingency.
Component exits on timeout to Component 1.
Component lasts 60 minutes.
Exit after 50 reinforcements to next item in list.
This component delivers reinforcement to LVB 96.
Constant outputs on:
Output 110, cycle time: Constantly on.

Component 2
FR 2
Left levers are correct.
No correction contingency.
Component exits on timeout to Component 2.
Component lasts 60 minutes.
Exit after 50 reinforcements to next item in list.
This component delivers reinforcement to LVB 96.
Constant outputs on:
Output 110, cycle time: Constantly on.

Component 3
FR 5
Left levers are correct.
No correction contingency.
Component exits on timeout to Component 3.
Component lasts 60 minutes.
Exit after 50 reinforcements to next item in list.
This component delivers reinforcement to LVB 96.
Constant outputs on:
Output 110, cycle time: Constantly on.

Component 4
FR 10
Left levers are correct.
No correction contingency.
Component exits on timeout to Component 1.
Component lasts 60 minutes.
Exit after 50 reinforcements to next item in list.
This component delivers reinforcement to LVB 96.
Constant outputs on:
Output 110, cycle time: Constantly on.

Procedure file: DAY1R
Component Sequence:
1,2,3,4,R 1,10 0

Component 1
FR 1
Right levers are correct.
No correction contingency.
Component exits on timeout to Component 1.
Component lasts 60 minutes.
Exit after 50 reinforcements to next item in list.
This component delivers reinforcement to LVB 96.
Constant outputs on:
Output 110, cycle time: Constantly on.

Component 2
FR 2
Right levers are correct.
No correction contingency.
Component exits on timeout to Component 2.
Component lasts 60 minutes.
Exit after 50 reinforcements to next item in list.
This component delivers reinforcement to LVB 96.
Constant outputs on:
Output 110, cycle time: Constantly on.

Component 3
FR 5
Right levers are correct.
No correction contingency.
Component exits on timeout to Component 3.
Component lasts 60 minutes.
Exit after 50 reinforcements to next item in list.
This component delivers reinforcement to LVB 96.
Constant outputs on:
Output 110, cycle time: Constantly on.

Component 4
FR 10
Right levers are correct.
No correction contingency.
Component exits on timeout to Component 1.
Component lasts 60 minutes.
Exit after 50 reinforcements to next item in list.
This component delivers reinforcement to LVB 96.
Constant outputs on:
Output 110, cycle time: Constantly on.

Procedure file: DAY2L
Component Sequence:
1,2,3,4,R 1,10 0

Component 1
FR 1
Left levers are correct.
No correction contingency.
Component does not exit on time.
Exit after 10 reinforcements to next item in list.
This component delivers reinforcement to LVB 96.
Constant outputs on:
Output 110, cycle time: Constantly on.

Component 2
FR 2
Left levers are correct.
No correction contingency.
Component does not exit on time.
Exit after 10 reinforcements to next item on list.
This component delivers reinforcement to LVB 96.
Constant outputs on:
Output 110, cycle time: Constantly on.

Component 3
FR 5
Left levers are correct.
No correction contingency.
Component does not exit on time.
Exit after 50 reinforcements to next item in list.
This component delivers reinforcement to LVB 96.
Constant outputs on:
Output 110, cycle time: Constantly on.

Component 4
FR 10
Left levers are correct.
No correction contingency.
Component does not exit on time.
Exit after 50 reinforcements to next item in list.
This component delivers reinforcement to LVB 96.
Constant outputs on:
Output 110, cycle time: Constantly on.

Procedure file: DAY2R
Component Sequence:
1,2,3,4,R 1,10 0

Component 1
FR 1
Right levers are correct.
No correction contingency.
Component does not exit on time.
Exit after 10 reinforcements to next item in list.
This component delivers reinforcement to LVB 96.
Constant outputs on:
Output 110, cycle time: Constantly on.

Component 2
FR 2
Right levers are correct.
No correction contingency.
Component does not exit on time.
Exit after 10 reinforcements to next item on list.
This component delivers reinforcement to LVB 96.
Constant outputs on:
Output 110, cycle time: Constantly on.

Component 3
FR 5
Right levers are correct.
No correction contingency.
Component does not exit on time.
Exit after 50 reinforcements to next item in list.
This component delivers reinforcement to LVB 96.
Constant outputs on:
Output 110, cycle time: Constantly on.

Component 4
FR 10
Right levers are correct.
No correction contingency.
Component does not exit on time.
Exit after 50 reinforcements to next item in list.
This component delivers reinforcement to LVB 96.
Constant outputs on:
Output 110, cycle time: Constantly on.

Procedure file: DAY3L
Component Sequence:
1,2,3,0

Component 1
FR 2
Left levers are correct.
No correction contingency.
Component does not exit on time.
Exit after 5 reinforcements to next item on list.
This component delivers reinforcement to LVB 96.
Constant outputs on:
Output 110, cycle time: Constantly on.

Component 2
FR 5
Left levers are correct.
No correction contingency.
Component does not exit on time.
Exit after 5 reinforcements to next item in list.
This component delivers reinforcement to LVB 96.
Constant outputs on:
Output 110, cycle time: Constantly on.

Component 3
FR 10
Left levers are correct.
No correction contingency.
Component does not exit on time.
Exit after 50 reinforcements to next item in list.
This component delivers reinforcement to LVB 96.
Constant outputs on:
Output 110, cycle time: Constantly on.

Procedure file: DAY3R
Component Sequence:
1,2,3,0

Component 1
FR 2
Right levers are correct.
No correction contingency.
Component does not exit on time.
Exit after 5 reinforcements to next item on list.
This component delivers reinforcement to LVB 96.
Constant outputs on:
Output 110, cycle time: Constantly on.

Component 2
FR 5
Right levers are correct.
No correction contingency.
Component does not exit on time.
Exit after 5 reinforcements to next item in list.
This component delivers reinforcement to LVB 96.
Constant outputs on:
Output 110, cycle time: Constantly on.

Component 3
FR 10
Right levers are correct.
No correction contingency.
Component does not exit on time.
Exit after 50 reinforcements to next item in list.
This component delivers reinforcement to LVB 96.
Constant outputs on:
Output 110, cycle time: Constantly on.

Procedure file: DAY5L
Component Sequence:
1,2,0

Component 1
FR 5
Left levers are correct.
No correction contingency.
Component does not exit on time.
Exit after 5 reinforcements to next item in list.
This component delivers reinforcement to LVB 96.
Constant outputs on:
Output 110, cycle time: Constantly on.

Component 2
FR 10
Left levers are correct.
No correction contingency.
Component does not exit on time.
Exit after 50 reinforcements to next item in list.
This component delivers reinforcement to LVB 96.
Constant outputs on:
Output 110, cycle time: Constantly on.

Procedure file: DAY5R
Component Sequence:
1,2,0

Component 1
FR 5
Right levers are correct.
No correction contingency.
Component does not exit on time.
Exit after 5 reinforcements to next item in list.
This component delivers reinforcement to LVB 96.
Constant outputs on:
Output 110, cycle time: Constantly on.

Component 2
FR 10
Right levers are correct.
No correction contingency.
Component does not exit on time.
Exit after 50 reinforcements to next item in list.
This component delivers reinforcement to LVB 96.
Constant outputs on:
Output 110, cycle time: Constantly on.

APPENDIX

GANOVA 3

GENERAL UNIVARIATE AND MULTIVARIATE
ANALYSIS OF VARIANCE

GANOVA 3

GENERAL UNIVARIATE AND MULTIVARIATE
ANALYSIS OF VARIANCE

SUMMARY OF THE DESIGN
* *

2 -WAY ANOVA WITH EQUAL N AND FIXED EFFECTS

2 WITHIN-SUBJECT FACTORS	NO.OF LEVELS
(1) TREATME	2
(2) DOSE	4

* *

DATA: 6 SUBJECTS ENTERED FROM TERMINAL

HORIZONTAL LOCOMOTOR ACTIVITY, 10 MIN, CN GROUP

* *

MAIN EFFECT OF FACTOR 1 (TREATME)

UNIVARIATE TESTS

SSH= 688802.1 DF= 1 MSH= 688802.0625

SSE= 5674164 DF= 5 MSE= 1134833

F= .6069635 DF= 1 , 5 P=0.4755

* *

MAIN EFFECT OF FACTOR 2 (DOSE)

UNIVARIATE TESTS

SSH= 1.512182E+07 DF= 3 MSH= 5040606

SSE= 1.706579E+07 DF= 15 MSE= 1137719

F= 4.430448 DF= 3 , 15 P=0.0200
(VALID ONLY UNDER COMPOUND SYMMETRY)

GANOVA 3

GENERAL UNIVARIATE AND MULTIVARIATE
ANALYSIS OF VARIANCE

SUMMARY OF THE DESIGN
* *

2 -WAY ANOVA WITH EQUAL N AND FIXED EFFECTS

2 WITHIN-SUBJECT FACTORS	NO.OF LEVELS
(1) TREATME	2
(2) DOSE	4

* *

DATA: 6 SUBJECTS ENTERED FROM TERMINAL

VERTICAL LOCOMOTOR ACTIVITY, 10 MIN., CN GROUP

* *
MAIN EFFECT OF FACTOR 1 (TREATME)

UNIVARIATE TESTS

SSH= 170885.3 DF= 1 MSH= 170885.328125

SSE= 716976.7 DF= 5 MSE= 143395.4

F= 1.191708 DF= 1 , 5 P=0.3258

* *
MAIN EFFECT OF FACTOR 2 (DOSE)

UNIVARIATE TESTS

SSH= 363641.1 DF= 3 MSH= 121213.7

SSE= 1097374 DF= 15 MSE= 73158.28

F= 1.656869 DF= 3 , 15 P=0.2180
(VALID ONLY UNDER COMPOUND SYMMETRY)

GANOVA 3

GENERAL UNIVARIATE AND MULTIVARIATE
ANALYSIS OF VARIANCE

SUMMARY OF THE DESIGN
* *

2 -WAY ANOVA WITH EQUAL N AND FIXED EFFECTS

2 WITHIN-SUBJECT FACTORS	NO.OF LEVELS
(1) TREATME	2
(2) DOSE	4

* *

DATA: 6 SUBJECTS ENTERED FROM TERMINAL

HORIZONTAL LOCOMOTOR ACTIVITY, 20 MIN., CN GROUP

* *
MAIN EFFECT OF FACTOR 1 (TREATME)

UNIVARIATE TESTS

SSH= 483004.7 DF= 1 MSH= 483004.6875

SSE= 2.873617E+07 DF= 5 MSE= 5747234

F= 8.404125E-02 DF= 1 , 5 P=0.7727

* *
MAIN EFFECT OF FACTOR 2 (DOSE)

UNIVARIATE TESTS

SSH= 6.296274E+07
DF= 3 MSH= 2.098758E+07

SSE= 3.953243E+07
DF= 15 MSE= 2635495

F= 7.96343 DF= 3 , 15 P=0.0024
(VALID ONLY UNDER COMPOUND SYMMETRY)

GANOVA 3

GENERAL UNIVARIATE AND MULTIVARIATE
ANALYSIS OF VARIANCE

SUMMARY OF THE DESIGN
* *

2 -WAY ANOVA WITH EQUAL N AND FIXED EFFECTS

2 WITHIN-SUBJECT FACTORS	NO.OF LEVELS
(1) TREATME	2
(2) DOSE	4

* *

DATA: 6 SUBJECTS ENTERED FROM TERMINAL

VERTICAL LOCOMOTOR ACTIVITY, 20 MIN., CN GROUP

* *
MAIN EFFECT OF FACTOR 1 (TREATME)

UNIVARIATE TESTS

SSH= 26461.02 DF= 1 MSH= 26461.021484375

SSE= 1680433 DF= 5 MSE= 336086.6

F= 7.873275E-02 DF= 1 , 5 P=0.7785

* *
MAIN EFFECT OF FACTOR 2 (DOSE)

UNIVARIATE TESTS

SSH= 2340616 DF= 3 MSH= 780205.3

SSE= 2448514 DF= 15 MSE= 163234.3

F= 4.779666 DF= 3 , 15 P=0.0156
(VALID ONLY UNDER COMPOUND SYMMETRY)

GANOVA 3

GENERAL UNIVARIATE AND MULTIVARIATE
ANALYSIS OF VARIANCE

SUMMARY OF THE DESIGN
* *

2 -WAY ANOVA WITH EQUAL N AND FIXED EFFECTS

	WITHIN-SUBJECT FACTORS	NO.OF LEVELS
(1)	TREATME	2
(2)	DOSE	4

* *

DATA: 6 SUBJECTS ENTERED FROM TERMINAL

HORIZONTAL LOCOMOTOR ACTIVITY, 10 MIN., CONTROL GROUP

* *
MAIN EFFECT OF FACTOR 1 (TREATME)

UNIVARIATE TESTS

SSH= 860816.3 DF= 1 MSH= 860816.3125

SSE= 1.086763E+07 DF= 5 MSE= 2173530

F= .3960453 DF= 1 , 5 P=0.5607

* *
MAIN EFFECT OF FACTOR 2 (DOSE)

UNIVARIATE TESTS

SSH= 6.490334E+07 DF= 3 MSH= 2.163445E+07

SSE= 2.702212E+07 DF= 15 MSE= 1801474

F= 12.0093 DF= 3 , 15 P=0.0005
(VALID ONLY UNDER COMPOUND SYMMETRY)

GANOVA 3

GENERAL UNIVARIATE AND MULTIVARIATE
ANALYSIS OF VARIANCE

SUMMARY OF THE DESIGN
* *

2 -WAY ANOVA WITH EQUAL N AND FIXED EFFECTS

2 WITHIN-SUBJECT FACTORS	NO.OF LEVELS
(1) TREATME	2
(2) DOSE	4

* *

DATA: 6 SUBJECTS ENTERED FROM TERMINAL

VERTICAL LOCOMOTOR ACTIVITY, 10 MIN., CONTROL GROUP

* *
MAIN EFFECT OF FACTOR 1 (TREATME)

UNIVARIATE TESTS

SSH= 130625.3 DF= 1 MSH= 130625.3359375

SSE= 465326.2 DF= 5 MSE= 93065.24

F= 1.403589 DF= 1 , 5 P=0.2896

* *
MAIN EFFECT OF FACTOR 2 (DOSE)

UNIVARIATE TESTS

SSH= 445802.8 DF= 3 MSH= 148600.9

SSE= 1289891 DF= 15 MSE= 85992.72

F= 1.728064 DF= 3 , 15 P=0.2034
(VALID ONLY UNDER COMPOUND SYMMETRY)

GANOVA 3

GENERAL UNIVARIATE AND MULTIVARIATE
ANALYSIS OF VARIANCE

SUMMARY OF THE DESIGN
* *

2 -WAY ANOVA WITH EQUAL N AND FIXED EFFECTS

2 WITHIN-SUBJECT FACTORS	NO.OF LEVELS
(1) TREATME	2
(2) DOSE	4

* *

DATA: 6 SUBJECTS ENTERED FROM TERMINAL

HORIZONTAL LOCOMOTOR ACTIVITY, 20 MIN., CONTROL GROUP

* *
MAIN EFFECT OF FACTOR 1 (TREATME)

UNIVARIATE TESTS

SSH= 705432.5 DF= 1 MSH= 705432.5

SSE= 1.235647E+07 DF= 5 MSE= 2471293

F= .2854508 DF= 1 , 5 P=0.6189

* *
MAIN EFFECT OF FACTOR 2 (DOSE)

UNIVARIATE TESTS

SSH= 7.493143E+07 DF= 3 MSH= 2.497714E+07

SSE= 4.275363E+07 DF= 15 MSE= 2850242

F= 8.763164 DF= 3 , 15 P=0.0016
(VALID ONLY UNDER COMPOUND SYMMETRY)

GANOVA 3

GENERAL UNIVARIATE AND MULTIVARIATE
ANALYSIS OF VARIANCE

SUMMARY OF THE DESIGN
* *

2 -WAY ANOVA WITH EQUAL N AND FIXED EFFECTS

2 WITHIN-SUBJECT FACTORS	NO.OF LEVELS
(1) TREATME	2
(2) DOSE	4

* *

DATA: 6 SUBJECTS ENTERED FROM TERMINAL

VERTICAL LOCOMOTOR ACTIVITY, 20 MIN., CONTROL GROUP

* *
MAIN EFFECT OF FACTOR 1 (TREATME)

UNIVARIATE TESTS

SSH= 30906.75 DF= 1 MSH= 30906.75

SSE= 381102 DF= 5 MSE= 76220.4

F= .4054919 DF= 1 , 5 P=0.5562

* *
MAIN EFFECT OF FACTOR 2 (DOSE)

UNIVARIATE TESTS

SSH= 1027199 DF= 3 MSH= 342399.5

SSE= 2087636 DF= 15 MSE= 139175.7

F= 2.460196 DF= 3 , 15 P=0.1020
(VALID ONLY UNDER COMPOUND SYMMETRY)

GANOVA 3

GENERAL UNIVARIATE AND MULTIVARIATE
ANALYSIS OF VARIANCE

SUMMARY OF THE DESIGN
* *

2 -WAY ANOVA WITH EQUAL N AND FIXED EFFECTS

2 WITHIN-SUBJECT FACTORS	NO.OF LEVELS
(1) treatme	2
(2) dose	4

* *

DATA: 6 SUBJECTS ENTERED FROM TERMINAL

EFFECT OF CHRONIC COCAINE ON STEREOTYPIC BEHAVIOR
* *
MAIN EFFECT OF FACTOR 1 (treatme)

UNIVARIATE TESTS

SSH= 11.02083 DF= 1 MSH= 11.02083301544189

SSE= 16.85417 DF= 5 MSE= 3.370834

F= 3.269468 DF= 1 , 5 P=0.1290

* *
MAIN EFFECT OF FACTOR 2 (dose)

UNIVARIATE TESTS

SSH= 101.5625 DF= 3 MSH= 33.85417

SSE= 82.5625 DF= 15 MSE= 5.504167

F= 6.150644 DF= 3 , 15 P=0.0064
(VALID ONLY UNDER COMPOUND SYMMETRY)

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