PREEXPOSURE SENSITIZES RATS TO THE REWARDING PROPERTIES OF AMPHETAMINE AS MEASURED BY A PROGRESSIVE RATIO PARADIGM

by

Adrianna Mendrek

B.A., Concordia University, 1994

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF ARTS

in

THE FACULTY OF GRADUATE STUDIES

(Department of Psychology)

we accept this thesis as conforming
to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

December 1996

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of PSYCHOLOGY

The University of British Columbia
Vancouver, Canada

Date Dec. 11 / 1996

DE-6 (2/88)

ABSTRACT

Preexposure Sensitizes Rats to the Rewarding Properties of Amphetamine as Measured by a Progressive Ratio Paradigm

Two groups of male Long-Evans rats were compared to determine whether pre-exposure to amphetamine would enhance the motivation to self-administer the drug under a progressive ratio schedule of reinforcement. In the first phase of the experiment each animal received a single injection of either amphetamine or saline on alternate days for a total of ten injections. Following a 21 day withdrawal period, behavioral sensitization was confirmed by a significant increase in amphetamine-induced stereotypy in the amphetamine-pretreated group, relative to the saline-pretreated group. In the second phase of the study all rats were implanted with chronic jugular catheters and trained to self-administer amphetamine under a fixed-ratio schedule of reinforcement. The progressive ratio paradigm was then imposed for seven days; amphetamine-pretreated rats attained significantly higher breaking points than saline-pretreated animals. These data indicate that preexposure to psychoactive agents may enhance the motivation of drug self-administration, suggesting augmentation in drug addiction liability.

TABLE OF CONTENTS

LIST OF FIGURES

Page

ACKNOWLEDGMENTS

First of all, I would like to thank my thesis supervisors, Chuck Blaha and Tony Phillips, for not giving up on me and kicking me out of the lab when I developed this damn allergy to rats. Chuck, thank you for invaluable conceptual and grammatical edition of my thesis, thanks for your sense of humor and emotional support (especially the story about your grandmother). Tony, thank you for your patience, insight and intellectual guidance that helped me not only complete this project but also finally decide what I want to do with my life.

I owe my thanks to Dimitri Papageorgis for agreeing to be on my thesis committee and for providing me with a dose of healthy realism from time to time. Most importantly however, thank you for being a good and reliable film critic, so that I could avoid some of those not-so-stimulating cinematographic positions out there.

I also would like to aknowledge all members of Tony's lab for their multidimensionality, unconventionality, and friendship. It's been really wonderful knowing you and working with you.

I wouldn't be able to stay relatively sane and finish this thesis without unconditional love and support of my new husband Saeid, my wonderful parents, and my little sister Malwina.

Finally, I have to thank Fiodor Dostoyevski, Julio Cortazar, Lao Tzu, Miles Davis, Leonard Cohen, Jan Garbarek, Henri Matisse, Salvador Dali, Egon Schiele, Wim Wenders, Jim Jarmush, Krzysztof Kieslowski, and many, many others who helped me understand and enjoy life the way I do.

Introduction

The repeated, intermittent exposure to psychomotor stimulants, such as amphetamine or cocaine, results in a progressive and enduring enhancement in many stimulant induced behaviors, a phenomenon known as behavioral sensitization (Robinson & Becker, 1986). The development of behavioral sensitization appears to arise from increased synaptic transmission in the mesolimbic dopamine system (Kalivas & Stewart, 1991; Robinson & Becker, 1986) which has cell bodies in the ventral tegmental area (VTA) and axon terminals in the nucleus accumbens septi (NAS) and other limbic structures (Swanson, 1982). Because the activity in this neurotransmitter system has been strongly associated with the rewarding properties of many psychoactive drugs (Wise, 1987), of particular interest to addiction specialists is the possibility that repeated administration of psychomotor stimulants may sensitize the user to the incentive motivational properties of these drugs. This hypothesis has been explored in the present thesis.

Models of Addiction. An early explanation of drug use and abuse entailed a physical dependence model of drug addiction. Based on the principles of negative reinforcement, proponents of the physical dependence model argue that excessive drug use is motivated by withdrawal symptoms that occur when an individual stops taking the drug. Thus, addiction develops not because of a positive state that a particular drug induces, but because of a negative state that it alleviates.

Although intuitively appealing, this theory of drug dependence does not adequately account for the acquisition of drug abuse, it does not explain why relief

of withdrawal distress is only minimally effective in the treatment of drug addicts (Hunt & Oderoff, 1962; Wilson, Elms, & Thomson, 1975), or why there is a lack of correlation between withdrawal symptoms and drug-seeking behavior (Jaffe, 1992). In fact, the subjective reports of drug craving are usually highest immediately after drug administration, when the feeling of euphoria is produced and withdrawal distress is significantly diminished (Foltin & Fichman,1991; Jaffe, Cascella, Kumor, & Sherer, 1989). Moreover, experiments with laboratory animals have demonstrated that rats self-administer morphine at doses that are insufficient to cause physical dependence (Schuster, 1970), and that low doses of morphine injected directly into the VTA are habit forming but do not produce physical dependence, whereas injections into the periaqueductal gray (PAG) of non-dependent rats produce withdrawal symptoms but are not habit forming (Bozarth & Wise, 1984).

An alternative view of addiction stresses the potential role of euphoria and positive affect induced by drugs of abuse in the acquisition of self-administration, and has focused on dopamine systems that have been postulated to play a major role in a variety of positive reinforcement phenomena (Blackburn, Pfaus, & Phillips, 1992; Di Chiara & Imperato, 1988). Functional dopamine terminals in the nucleus accumbens are particularly important for the expression of cocaine (Roberts, Koob, Klanoff, & Fibiger, 1980) and amphetamine (Lyness, Friedle, & Moore, 1979) reinforcement, whereas opiates appear to have reinforcing actions at the level of dopamine cell bodies in the VTA (Bozarth & Wise, 1984). There is also evidence that increases in synaptic dopamine levels are associated with the administration of other potentially addictive drugs, such as ethanol (Di Chiara & Imperato, 1985), nicotine (Mereu, Yoon, Boi, Gessa, Naes, & Westfall, 1987),

phencyclidine (Gerhardt, Pang, & Rose, 1987) and caffeine (Govoni et al., 1984). These and many other studies implicating the involvement of brain dopamine systems in incentive motivation serve as a basis for the hypothesis that the mesolimbic dopamine system mediates the subjective pleasure produced by drugs of abuse (Wise, 1980).

The positive reinforcement view provides an attractive explanation of addiction, but it is not without problems. It has been shown for instance, that drug self-administration can be maintained in the absence of a subjective hedonic state, suggesting that pleasure is not necessary for the maintenance of drug abuse (Lamb et al., 1991). Another example which illustrates this problem quite clearly is the fact that some drugs considered to be highly addictive, such as nicotine, do not induce significant euphoria, and drugs like opiates can actually produce strong dysphoric states with their initial use (Robinson & Berridge, 1993). Furthermore, there is now evidence that the mesolimbic dopamine system may in reality mediate incentive salience and not pleasure. Treit and Berridge (1990), employing a "taste reactivity paradigm" (Grill & Norgren, 1978), demonstrated that dopamine antagonists can decrease the incentive value of saccharin without altering its sensory pleasure. In contrast, dopamine agonists do not increase the sensory pleasure of tastes but can increase their incentive value.

In addition to these data, the observation that, with repeated use, drugs are usually wanted more-and-more while at the same time they are liked less-and-less, have led to the hypothesis that the neural system implicated in "wanting" incentives may be dissociable from the ones that are responsible for "liking" incentives (Robinson & Berridge, 1993). This supposition has formed the basis of the incentive-sensitization theory of addiction (Robinson & Berridge, 1993).

According to this theory, addiction results from progressive and persistent changes in a neural system that mediates drug craving. It is proposed that incentive motivation, which is equivalent to the concept of reward, involves three psychological processes: (1) the activation of pleasure by a particular event, (2) the association of pleasure with a mental representation of the event that evoked the pleasure and, (3) the attribution of incentive salience to subsequent perceptions and representations of the associated events. Moreover, pleasure, associative learning, and incentive salience are considered to act together but are mediated by different neural systems. Only the incentive salience (wanting/craving) undergoes sensitization via repeated activation of the mesolimbic dopamine system by the drug. In this way, the normal, functional balance between these systems may be disrupted in the addict whose obsessive drug craving overpowers him to the point where acquiring the drug becomes more important than his health, family, and friends. In order to appreciate fully the implications of the incentive-sensitization theory of addiction it is essential to comprehend the phenomenon of behavioral sensitization and its underlying mechanisms.

Behavioral Sensitization. Behavioral sensitization refers to the gradual and long-lasting enhancement in many stimulant-induced behaviors produced by the repeated intermittent treatment with psychomotor stimulants, such as amphetamine or cocaine. Thus, locomotor activity and stereotyped behaviors, observed in laboratory animals after psychostimulant administration, show a more rapid onset, an increased intensity, and a longer duration with each successive injection of the drug (Robinson & Becker, 1986). Unlike drug tolerance, which can be understood readily as a homeostatic adaptive process, behavioral sensitization has no obvious function or rationale. Not only does it contradict homeostasis, but it is believed to

contribute to the development of various forms of psychopathology (Robinson & Becker, 1986). People who chronically use amphetamine may develop some symptoms that are virtually indistinguishable from those exhibited by paranoid schizophrenics (Snyder, 1973). Sensitization to psychomotor stimulants, including stress, has been implicated in depression as well (Willner, Muscat, & Papp, 1992). Perhaps the most obvious importance of behavioral sensitization however, has to do with its hypothesized involvement in addiction (Robinson & Berridge, 1993) and has led to increased interest in characterizing the nature of this phenomenon.

The manifestation of psychomotor stimulant-induced behaviors depends heavily on several factors including the dose and administration regimen of the drug. With respect to dose, an acute systemic injection of a low-to-moderate dose of amphetamine or cocaine produces an increase in locomotor activity, rearing, and rotational behavior in rats. With higher doses this hyperactivity is replaced by a stereotypy phase characterized by repetitive head movements, sniffing, licking or biting (Robinson,1984). The repeated intermittent treatment with low doses of amphetamine produces a progressive enhancement in the intensity and duration of locomotor activity whereas, with moderate doses, stereotypy emerges despite the fact that initial injections usually produce only locomotion (Segal & Kuczenski, 1994). With respect to treatment regimens, animals are typically given several injections of the drug followed by a withdrawal period and subsequent administration of a challenge dose. The treatment should be intermittent since continuous amphetamine administration is neurotoxic and may produce brain damage (Seiden, Fischman, & Schuster, 1975; Ellison & Eison, 1983). Moreover, the closer together in time the injections are given, the more likely tolerance is to develop instead of sensitization (Post, 1980). Thus, in order to produce robust

behavioral sensitization to psychomotor stimulants, it is important that the treatment interval is sufficiently long and that subjects are withdrawn for at least a few days before a challenge dose is given. For example, Kolta and colleagues reported greater behavioral sensitization 15 or 30 days after withdrawal from repeated amphetamine administration than after only three days of withdrawal (Kolta, Shrere, De Souza, & Utretsky, 1985). Indeed, one of the most striking features of sensitization is its persistence. In one study the animals treated with escalating intermittent doses of amphetamine remained behaviorally hypersensitive to drug challenges for at least one year post-treatment (Paulson, Camp, & Robinson, 1991). A second variable to consider while designing a study aimed at induction of behavioral sensitization is the total number of drug injections that the experimenter should use. This variable however, is not as critical as the intermittency requirement since behavioral sensitization has been reported to occur after a single injection of amphetamine (Segal & Schuckit, 1983).

An important aspect of behavioral sensitization is its generalizability. Besides psychomotor stimulants, many other drugs and experimental manipulations, including restrain-induced stress, footshock, and conditioned fear, can produce behavioral sensitization (Kalivas & Duffy, 1989). Interestingly, the repeated treatment with a given drug not only produces sensitization to that drug, but may also produce cross-sensitization to other drugs in the same or different classes (e.g., amphetamine vs. cocaine and opioids; Kalivas & Stewart, 1991). In addition, many drugs and environmental stressors cross-sensitize to one another. For example, preexposure to stressors enhances amphetamine-induced behaviors and preexposure to amphetamine augments the subsequent reaction to stressors (Antelman, Eichler, Black, & Kocan, 1980). The occurrence of cross-sensitization

is easily comprehensible given that the same neural mechanism may underlie both drug- and stressor-induced behavioral sensitization.

The acute psychomotor stimulant effects of amphetamine and cocaine can be blocked by selective dopamine receptor antagonists (Costall, Naylor, Cannon, & Lee, 1977), and hence have been associated with the actions of these drugs on the mesolimbic dopamine system, and the nigrostriatal system that has dopamine perikarya in the substantia nigra and terminals in the striatum. Selective lesions of dopamine terminals in the NAS and in dorsal striatum attenuate markedly both amphetamine-induced locomotion and stereotypy (Creese & Iversen, 1975). Furthermore, it is well documented that most drugs of abuse and stressors that are capable of producing behavioral sensitization also augment dopamine transmission in the brain (Robinson & Berridge, 1993). Accordingly, the present study has focused on alterations in central dopaminergic systems as potential mediators in the development of behavioral sensitization.

Converging neurochemical and behavioral studies have identified changes in the mesolimbic dopamine system that seem to be strongly correlated with the development of behavioral sensitization. Studies utilizing *in vivo* microdialysis have shown enhanced dopamine efflux in the NAS and striatum to a challenge injection of amphetamine or cocaine after repeated injections of the drugs (Parson & Justice, 1993; Robinson, Jurson, Bennett, & Bentgen, 1988). These studies point to the existence of "neurochemical" sensitization. Moreover, it has been demonstrated that cross-sensitization between stressors and cocaine is accompanied by augmented dopamine release in the NAS (Sorg & Kalivas, 1991).

In addition to changes in dopamine neurotransmission, there is evidence indicating the involvement of increased postsynaptic responsiveness to synaptically

released dopamine. Several reports have suggested that postsynaptic dopamine D1 receptor stimulation may play an important role in the induction of sensitization. First, the co-administration of the selective D1 receptor antagonist SCH 23390, but not the selective D2 receptor antagonist sulpride, blocks the development of amphetamine-induced behavioral sensitization (Vezina & Stewart, 1988). Second, NAS neurons exhibit enhanced electrophysiological responses to the selective D1 receptor agonist SKF 38393, but not to the D2 receptor agonist quinpirole, following repeated treatment with cocaine (Henry & White, 1991). Finally, chronic treatment with SKF 38393 has been shown to enhance the behavioral responses to the mixed D1/D2 receptor agonist apomorphine, as well as to SKF 38393 itself, suggesting that activation of dopamine D1 receptors alone may be sufficient for induction of behavioral sensitization (Brown & Chase, 1988).

Despite these rather convincing pharmacological data implicating postsynaptic dopamine D1 receptors as a critical factor in the induction of sensitization, paradoxically no changes in the level of D1-coupled Gs proteins have been observed after repeated administration with cocaine (Striplin & Kalivas, 1993). Instead, it has been demonstrated that chronic cocaine treatment decreases the level of the functional G protein subunit Gi alpha in the NAS one hour to two weeks after the last injection of cocaine (Striplin & Kalivas, 1993). This finding supports the possibility that the dopamine D2 receptor which is thought to exert its actions via Gi coupling to specific ion channels and/or inhibition of adenylate cyclase (Stoofe & Kababian, 1984) may be related to the enduring nature of behavioral sensitization. Whereas dopamine D2 receptors can inhibit adenylate cyclase, D1 receptor effects are mediated by a Gs-coupled activation of adenylate cyclase. Therefore, cocaine-induced decreases in levels of Gi alpha in NAS

neurons, without changes in Gs alpha, might produce a decreased inhibitory influence of other neurotransmitters on adenylate cyclase and increased excitatory influence of D1 receptor activation on the enzyme. This, in turn, could lead to functional supersensitivity to D1 receptor activation as observed recently in electrophysiological experiments (Nestler, 1993).

Regardless of the precise mechanism, it is clear that augmented transmission in the mesolimbic dopamine system is at least partly responsible for the development of behavioral sensitization. This neuronal system is believed to mediate the rewarding properties of many addictive drugs (Wise & Bozarth, 1987). Collectively, recent behavioral and neurochemical evidence has given rise to the possibility that not only drug-induced behavior may undergo sensitization, but also incentive motivation to respond for these drugs.

Sensitization of Incentive Motivation. A number of recent studies, using either a conditioned place preference (CPP) paradigm or drug self-administration procedures, suggest that prior exposure to drugs of abuse may produce sensitization to the incentive motivational effects of these drugs. Lett (1989) has found that the pretreatment with either amphetamine, morphine, or cocaine enhanced the rewarding effects of these drugs as measured by CPP. Cross-sensitization has been also reported using this paradigm; preexposure to amphetamine increased the rewarding effects of morphine, and preexposure to morphine increased the rewarding effects of amphetamine and cocaine (Lett, 1989).

Most evidence endorsing the concept of sensitization to incentive motivational effects of addictive drugs come from drug self-administration studies. In one of the earliest experiments of this kind rhesus monkeys were trained to press a lever to obtain an intravenous infusion of methamphetamine (Woolverton, Cervo,

& Johanson, 1984). A low dose of methamphetamine supported lever pressing after chronic pretreatment with this drug, but not before. Thus, the preexposure to drug lowered the threshold dose of methamphetamine that maintained lever pressing, implying an increased sensitivity to the rewarding effects of the drug (Woolverton et al., 1984). In a similar study, rats that underwent repeated intermittent treatment with cocaine acquired cocaine self-administration behavior at doses of the drug that did not sustain self-administration in drug naive animals (Horger, Shelton, & Schenk, 1990). Piazza and colleagues have found that preexposure to amphetamine or to stressful experience facilitates subsequent acquisition of amphetamine self-administration. In their study repeated treatment either with amphetamine or with tail-pinch produced increased locomotion as well as greater amphetamine intake during the acquisition phase of self-administration, as compared to control animals (Piazza, Deminiere, Le Moal, & Simon, 1990). In a different experiment rats pretreated with amphetamine or nicotine demonstrated elevated rates of responding for intravenous cocaine during the acquisition phase, but all groups eventually reached similar asymptotic levels of responding (Horger, Giles, and Schenk, 1992).

Although these studies provide compelling evidence for the hypothesis that repeated exposure to drugs of abuse may result in sensitization of their rewarding properties, they are not without problems. First, in the experiments described above, the drug self-administration testing phase took place in the same environment where behavioral sensitization had been previously induced. Accordingly, the drug was explicitly paired with the same environmental context where self-administration had to be acquired. This could result potentially in a conditioned locomotion that might have served as a confounding variable. Second,

sensitizing effects of drug pretreatment have been shown only during the acquisition of the drug self-administration habit. Once the behavior was established, the difference between preexposed and non-preexposed groups disappeared. Finally, these experiments have been criticized (Li, Depoortere, & Emmett-Oglesby, 1994) on the basis that the sensitization of drug self-administration is demonstrated only when very low doses of amphetamine or cocaine are available for injections, implying that the effect is dose-limited and cannot generalize over a wider range of psychomotor stimulant doses.

In order to address these issues, Emmett-Oglesby and colleagues performed a series of experiments and demonstrated that a chronic regimen with relatively high doses of cocaine increased the rate of cocaine intake in rats trained to self-administer cocaine under a fixed a ratio (FR) schedule of reinforcement (Emmett-Oglesby & Lane, 1992). In contrast, these treatments decreased breaking point values in rats trained to self-administer cocaine under a progressive ratio (PR) schedule of reinforcement (Li et al., 1994). Overall, their data suggested that tolerance to the reinforcing effects might develop, rather than sensitization.

Present Investigation. The present experiment has been designed to investigate the possibility that motivation to self-administer amphetamine undergoes sensitization as a result of prior repeated intermittent exposure to the drug. Although several recent studies have already addressed this hypothesis, the results obtained have been equivocal (Horger et al., 1990; 1992; Piazza et al., 1990; Li et al., 1994). Thus, in the present study we have adopted a strategy to eliminate most of the methodological problems encountered in these previous investigations of drug self-administration and sensitization.

Rather than utilizing a FR schedule of reinforcement, the present study employs a PR schedule of reinforcement because of several advantages inherent with its use to study motivation. In a PR schedule of reinforcement the number of responses required for each successive drug infusion is systematically increased until the subject eventually fails to receive the reinforcer within a set criterion period of time. The last performance ratio value successfully completed is defined as the "breaking point". This value reflects the maximal effort that the subject expends in order to receive a single drug infusion, thus serving as a reliable measure of incentive motivation and drug craving (Markou, Weiss, Gold, Caine, Schulteis, & Koob, 1993).

Most studies of intravenous drug self-administration, including reports of drug reward sensitization (see e.g., Horger et al., 1990, 1992; Piazza et al., 1990), have been limited to the FR schedules of reinforcement. The only dependent variable in these experiments is the rate of self-administration which is very sensitive to changes with a given unit infusion dose (Pickens & Thompson, 1968). Past some minimal threshold level, small doses of addictive drugs produce high rates of lever pressing, while higher doses produce correspondingly lower rates of responding. Thus, hourly drug intake remains constant despite imposed changes in a dose per injection. The initiation of drug seeking behavior appears to be tightly correlated with the level of drug in the blood; in the case of amphetamine rats initiate a lever press each time the drug level falls to an apparent threshold level of approximately 0.2 mg/ml of blood (Yokel & Pickens, 1974). As such, an animal may self-titer blood levels of the drug.

The interpretation of changes in the rate of drug self-administration under an FR schedule of reinforcement can be problematic, as well. On one hand, data

acquired in pharmacological studies of drug intravenous self-administration imply that a relative increase in the rate of drug intake reflects a decline in the rewarding value of a given drug. This notion is supported by findings showing that systemic administration of low doses of a dopamine receptor antagonist produces an increased rate of lever-pressing for infusions of intravenous cocaine, an effect that is comparable to the increased rate of responding following a reduction in a unit dose of the drug (Yokel & Wise, 1975; 1976). On the other hand, neurotoxic lesions of dopamine cells in the VTA result in a reduction in cocaine and amphetamine self-administration, an effect attributed to a decrement in the rewarding effects of these drugs (Lyness, Friedle, & Moore, 1979). Therefore, the opposing results obtained in FR schedules of reinforcement, namely an increase in drug intake in pharmacological studies and decrease in rate of self-administration in lesion studies, have been used to support the same conclusion. This difficulty can be avoided when a PR paradigm is employed since motivation to self-administer drugs of abuse is thought to be related directly to the magnitude of the breaking point (Roberts & Richardson, 1992).

Another difficulty associated with the use of FR schedules of reinforcement stems from the fact that the rate of self-administration does not change necessarily when the incentive motivational value of the drug changes. In some cases a dissociation between the rate of drug intake in FR paradigms and breaking points in PR procedures has been reported. For example, following bilateral 6-hydroxydopamine lesions of the NAS, rats self-administer apomorphine at stable pre-lesioned rates, whereas they exhibit higher breaking points with each successive day, suggesting the development of DA receptor supersensitivity on NAS neurons (Roberts, 1989). In a similar fashion 5,7-dihydroxytryptamine lesions of

serotonergic neurons in the forebrain produce no changes in the rate of cocaine intake, while a dramatic augmentation in breaking points has been observed (Loh & Roberts, 1990). In two recent experiments intracerebral injections of the dopamine D1 receptor antagonist SCH 23390, either into the amygdala or the striatum, produced an increase in the rate of cocaine self-administration under an FR schedule of reinforcement but had no effect on breaking point levels under a PR paradigm. In contrast, injections into NAS and medial prefrontal cortex (mPFC) produced an enhancement in the rate of responding for cocaine infusions and a decrement in the breaking point values (McGregor & Roberts, 1993; 1995). These latter results are consistent with the incentive-sensitization theory of addiction (Robinson & Berridge, 1993) and show clearly that FR and PR schedules of reinforcement measure two different aspects of drug reward, namely the subjective euphoric effects of the drug (liking) and the incentive salience of the drug (wanting), respectively. Because the present thesis is based partly on the idea that the incentive salience, and not the subjective pleasure induced by drug consumption, undergoes sensitization, and because PR procedures seem to be inherently suited for the analysis of drug motivational behaviors, for the purpose of our study we employed a PR schedule of reinforcement.

In addition to the schedule of drug self-administration, another factor that has contributed to results obtained in earlier studies of drug reward sensitization has been the pretreatment regimen. In all previous reports of the sensitization of incentive motivation to self-administer amphetamine or cocaine, the repeated intermittent preexposure to psychomotor stimulants or to various environmental stressors has been used (Horger et al., 1990, 1992; Piazza et al., 1990). By comparison, researchers who have argued that the reinforcing effects of cocaine

undergo tolerance rather than sensitization, have typically used chronic continuous drug treatment regimens (Emmett-Oglesby & Lane, 1992; Li et al., 1994). This apparent discrepancy is not surprising considering that long-term chronic exposure to amphetamine has neurotoxic effects (Seiden et al., 1975; Ellison & Eison, 1983) and that intermittent injections, as opposed to continuous infusions of the psychomotor stimulant, are necessary to produce optimal behavioral sensitization (Robinson & Becker, 1986). Because the present study is based on findings that the same neural mechanism may be implicated in the development of behavioral sensitization and in the drug rewarding effects, we employed a drug treatment schedule that has been shown to produce robust behavioral sensitization (Paulson & Robinson, 1995).

Previous reports of sensitization of incentive motivation have been criticized for the utilization of relatively low doses of amphetamine or cocaine in the intravenous self-administration paradigm. It has been argued that with higher doses, tolerance rather than sensitization to the incentive motivational effects of psychomotor stimulants becomes readily apparent (Li et al., 1994). Accordingly, the present study employed a relatively high dose of amphetamine that has been shown previously to reliably sustain self-administration in rats (Di Ciano et al., 1995). Finally, to circumvent the problem of conditioned locomotion, the induction and testing of behavioral sensitization took place in an environment that was different from that where amphetamine self-administration was subsequently examined.

With all these methodological considerations taken into account we hypothesized that the repeated intermittent exposure to amphetamine would produce

sensitization of both motor behavior and incentive motivation to self-administer amphetamine under a PR schedule of reinforcement.

Method

Subjects

Twenty-two male Long-Evans rats (Charles River, Quebec) weighing 300-350 g at the beginning of the experiment were used. Rats were housed individually in stainless-steel wire cages prior to surgery and in plastic cages with Sanicel lining after surgery in a temperature-controlled animal colony, with lights on between 07:00 and 19:00 h. The animals were handled daily for five days before the start of the experiment. Food and water were freely available except during testing.

Apparatus

Activity testing chambers. Four bilevel Plexiglas boxes (51 X 60.5 X 15 cm) served as activity testing chambers. A platform 30.5 cm in length centered and set 28 cm above the floor divided the chamber into two levels. Animals were able to move freely from one level to the other because of a set of ramps with Plexiglas strips to provide footholds, and a narrow landing at each end of the interior of the box. The floor of each level was lined with Sanicel and covered with a metal grid.

Self-administration chambers. Self-administration testing was conducted in six Plexiglas chambers (32 X 32 X 41 cm) enclosed in sound- and light-attenuating black wooden boxes. Each chamber was equipped with a stainless-steel operant lever (7 cm X 3 cm) and a white cue light (28 V 170 mA; Spectra) located directly

above the lever. The floor of the chamber was lined with Sanicel and covered with a metal grid. Tygon tubing, fixed to the end of the animal's intravenous catheter, extended from the head-mounted connector through the wooden box up to a mechanical infusion pump (Sage Apparatus, pump model 341 equipped with 20 ml syringe) mounted on the top of the wooden box. Drug delivery and data collection were controlled by MANX computer system (Gilbert & Rice, 1979).

Procedure

The animals were tested in the light phase of the day-night cycle. The details are discussed in three phases: behavioral sensitization, surgery, and amphetamine self-administration.

Behavioral sensitization. Animals were divided into two groups. The experimental group received intraperitoneal (i.p.) injections of d-amphetamine sulfate (2.0 mg/kg salt weight), whereas the control group received saline vehicle (0.9% w/v). The injections were administered in the home cages once every other day for a total of ten injections. Because the expression of behavioral sensitization is usually more pronounced a few weeks rather than a few days following the intermittent treatment with psychomotor stimulants, in the present study animals were given three weeks withdrawal period. On day 21 of the withdrawal period animals were transported to the testing room, weighed, and placed in the bilevel chambers. After one hour of habituation to the chambers all rats were injected with a challenge dose of amphetamine (2.0 mg/kg, i.p.). Their behavior was videotaped for subsequent detailed analysis performed by the experimenter unaware of the rats' group designation. To minimize the influence of remaining odors from

preceding groups before each session the activity boxes were cleaned with a dilute Windex solution.

Both locomotor activity and stereotyped behaviors were assessed for two hours following the challenge injection of amphetamine. To quantify locomotion a score of one was assigned for crossing either the top or bottom floor of the bilevel chamber (horizontal activity), for changing levels from the floor to one of the two landings located in between the levels (vertical activity), and one score for rearing. Activity counts were then added and averaged at ten-minutes intervals. Stereotypy was rated for one minute periods at ten-minutes intervals. The rating scale employed in the present experiment was developed by MacLennan and Maier (1983): a score of 'zero' was assigned to inactive animal (no movement); 'one' for intermittent activity; 'two' for continuous activity; 'three' for intermittent stereotypy (e.g., stereotyped sniffing, rearing, or repetitive head movements); 'four' for continuous stereotypy over the testing area; 'five' for continuous stereotypy over a restricted area; 'six' for pronounced, continuous stereotypy in a restricted area (e.g., stereotyped biting, licking, or gnawing).

Surgery. Two days after activity testing, rats were implanted with chronic indwelling intravenous catheters. Immediately prior to surgery all instruments were cold sterilized with 0.15% alkylbenzyldimethylammonium chloride (EMI industries) for about twenty minutes, followed by 70% ethanol for five minutes. Animals were given garamycin (8 mg i.m.) and ampicillin (50 mg i.m.), and then were anesthetized with separate injections of ketamine hydrochloride (100 mg/kg i.p.; MTC Pharmaceuticals, Cambridge, Ontario) and xylazine (7mg/kg i.p., Rompun, Etobicoke, Ontario). A Silastic catheter was inserted into the right jugular vein and its distal end was guided subcutaneously to an exposed portion of

the skull and secured in place with dental acrylic. Two rats, one from the control and one from the experimental group, died during the surgery. Each day following surgery and later, before and after the rat was placed in the intravenous self-administration chamber, the catheters were flushed with sterile saline solution containing 10 unit/ml heparin.

Amphetamine self-administration. Seven days following surgery animals began training under a fixed-ratio 2 (FR2) schedule of reinforcement with amphetamine sulfate, (salt weight 0.075 mg/0.1 ml injection; 0.2 mg/ml per infusion), serving as a drug reinforcer. All self-administration sessions were initiated with a 'free' priming injection of amphetamine at the dose available throughout the session. The house lights remained on during the sessions except after each drug infusion, when the lights flashed for five seconds, followed by a 30 second time-out during which the lights were turned off and responding on the lever had no programmed consequences. The FR2 sessions lasted either until nine drug infusions were self-administered (a total of ten injections including the priming dose) or until five hours had elapsed. Only those animals that reached the criterion of ten infusions of amphetamine during two days of training under FR2 participated in the second stage of self-administration phase: testing under progressive ratio (PR) schedule of reinforcement. Three subjects, two amphetamine-preexposed (experimental) and one saline-preexposed (control), did not attain this criterion. The experiment was designed in such a way to ensure that all rats were capable of acquiring the drug self-administration habit.

Daily PR sessions were similar to FR2 sessions in terms of priming infusion, length of the session, and house lights. Here, however, a progressively greater number of lever presses was required for each successive reinforcer. The

progression in the number of requested responses (ratio) was a version of the exponential equation described by Roberts and Richardson (1992): Ratio = 5 * exp(infusion number * 0.2) - 5. This scale was developed to permit self-administration behavior to extinguish in each animal each test day. It has been subsequently modified by Depoortere, Li, Lane, and Emmett-Oglesby (1993) to produce a more rapid increase in the size of the ratio required by replacing the first six values: 1, 2, 4, 6, 9,12, with only three values: 3, 6, 10, and then continuing with the progression given by the equation: 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 603, 737, 901, 1102, etc. There was a one-hour time limit to complete the ratio for a particular reinforcer and the failure to do so terminated the session. Testing with amphetamine continued for seven days and was followed by two days of extinction during which responding under the PR schedule resulted in the delivery of saline infusion.

Drugs

d-Amphetamine sulfate was obtained from Smith-Kline Beecham, Oakville, Ontario. For intraperitoneal injections the drug was dissolved as the salt weight in 0.9% sterile physiological saline and for intravenous self-administration it was mixed fresh daily in 1 unit/ml heparin solution. Heparin was purchased as a concentrated solution and was diluted in 0.9% w/v sterile physiological saline. All antibiotics and anesthetics were purchased as sterile solution from local distributors.

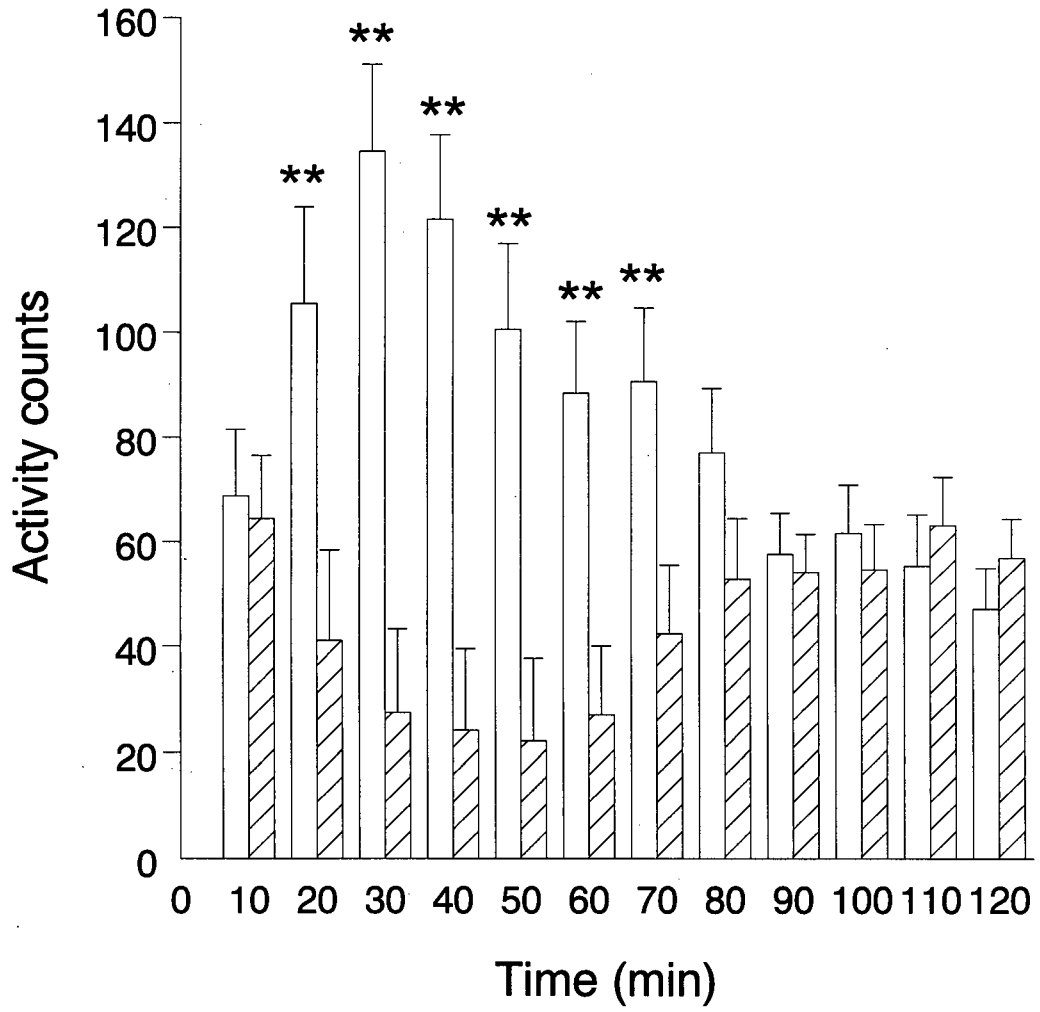
Data Analysis

The locomotion and stereotypy data were analyzed separately using a two-way analysis of variance (ANOVA) with repeated measures; Pretreatment Condition served as a between group factor, whereas Time was a within group factor. Spjotroll and Staline (1973) multiple comparisons for groups with unequal n's were used for *post hoc* analysis. For the analysis of self-administration under the PR paradigm the number of infusions obtained, rather than the final ratio completed, was used as a dependent variable because the final ratios were derived from an escalating exponential function and thus violated ANOVA's assumption of the homogeneity of variance. The number of reinforcers, on the other hand, was a natural logarithmic function of the ratio value and was therefore amenable to parametric analysis (Roberts & Richardson, 1992). Thus, similar to behavioral sensitization, self-administration data were subjected to a two-way ANOVA analysis(Pretreatment Condition X Day) and to Spjotroll and Staline (1973) *post hoc* multiple comparisons.

Results

The mean activity counts in response to challenge injections of amphetamine (2.0 mg/kg i.p.) are shown in Figure 1. Locomotion increased in saline-pretreated animals but decreased in the amphetamine-pretreated group. A two-way ANOVA on the locomotor activity scores yielded a significant main effect of the Pretreatment Condition ($F_{(1,15)} = 8.1$; $p < 0.05$) and a significant interaction between Pretreatment Condition and Time ($F_{(11,165)} = 10.9$; $p < 0.01$). Subsequent *post hoc* comparisons revealed that the saline-pretreated rats were

Figure 1. The effects of amphetamine challenge injections (2.0 mg/kg i.p.) on locomotion in rats that had received 10 previous injections of either amphetamine (striped bars) or saline (open bars). The bars represent the mean (+S.E.M.) locomotor counts during the two hours following amphetamine administration. The stars indicate a significant difference ($p < 0.01 = **$) between the two groups at a given time interval.


160
140
120
100
80
60
40
20
0
Activity counts
**
**
**
**
**
**
0 10 20 30 40 50 60 70 80 90 100 110 120
Time (min)

significantly more active at 20, 30, 40, 50, 60, and 70 minutes postinjection ($p < 0.01$).

Figure 2 illustrates the effects of the amphetamine challenge on the stereotyped behaviors in both saline- and amphetamine-pretreated animals. While the control group received relatively low and stable ratings of stereotyped behaviors during two hours of testing, the experimental group exhibited intense stereotypy, reaching a maximum mean score of 4.9, that lasted throughout the session. There was a statistically significant interaction between Pretreatment Condition and Time ($F_{(11,165)} = 4.8$; $p < 0.01$), and Spjotroll and Staline multiple comparison procedures showed that the two groups differed significantly across time ($p < 0.05$), except for the first five and last fifteen minutes.

The two groups of rats did not differ in terms of time that they needed in order to reach the criterion of ten infusions over two days of training under the FR2 schedule of reinforcement ($F_{(1,15)} = 1.3$, n.s.; Fig.3). Nevertheless, the analysis of amphetamine self-administration under the PR schedule revealed a main effect of Pretreatment Condition ($F_{(1,15)} = 4.9$; $p < 0.05$), a main effect of Testing Day ($F_{(8,120)} = 17.1$; $p < 0.01$), and a significant interaction between these two factors ($F_{(8,120)} = 3.6$; $p < 0.01$). Amphetamine-pretreated animals exhibited higher breaking points (mean range from 10.9 to 14.2 of amphetamine reinforcers corresponding to 77-145 bar presses for the last amphetamine infusion) than saline-preexposed rats (mean range from 5.8 to 8.1 of amphetamine reinforcers, corresponding to 25-40 bar presses for the last amphetamine infusion) on second, third, fourth, fifth, sixth, and seventh day ($p < 0.05$), but there was no statistically significant difference between the groups during the first session of amphetamine self-administration under PR schedule of reinforcement or in the two extinction

trials when instead of amphetamine animals were allowed to self-administer saline. (Fig. 4).

Figure 2. The effects of amphetamine challenge injections (2.0 mg/kg i.p.) on stereotypy in rats that had received 10 previous injections of either amphetamine (striped bars) or saline (open bars). The bars represent the mean (+S.E.M.) stereotypy scores over 12 one-min intervals following amphetamine administration. The stars indicate a significant difference ($p<0.05$=*; $p<0.01$=**) between the two groups at a given time interval.

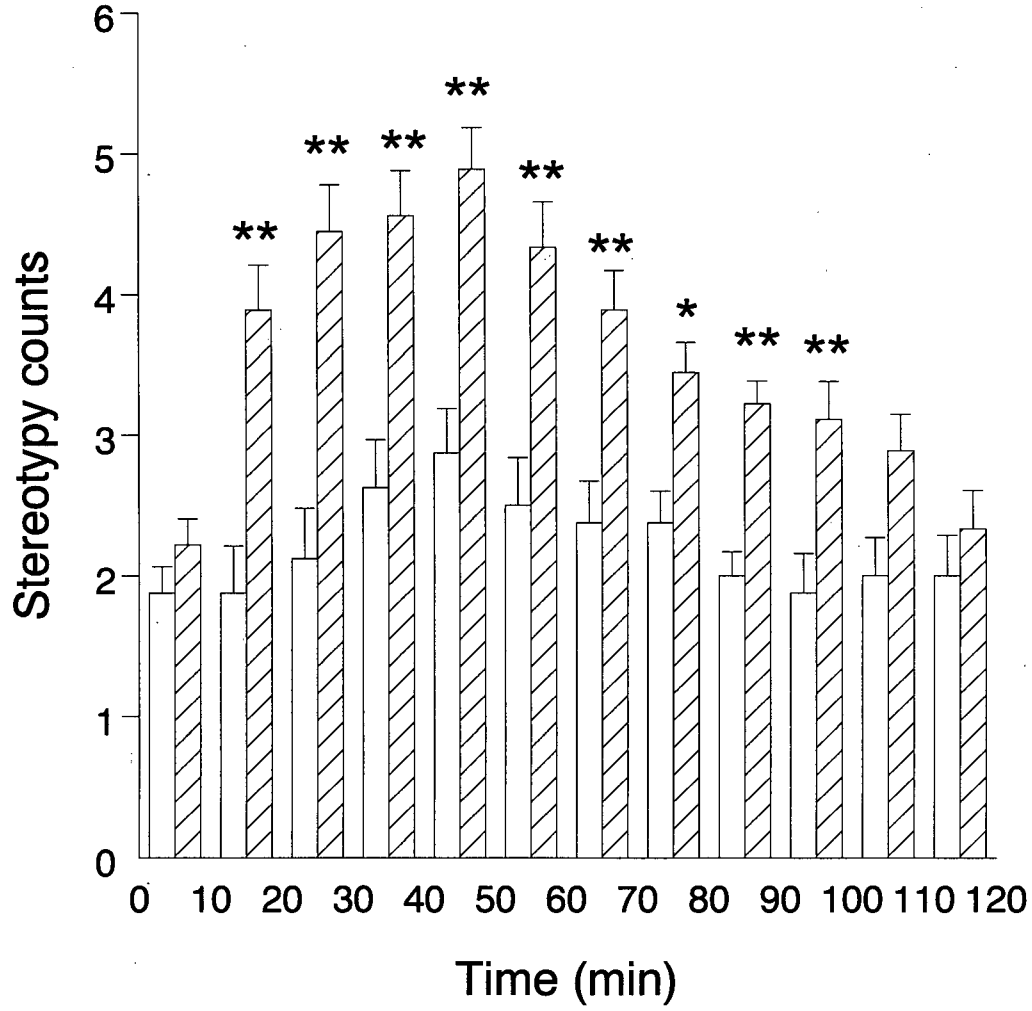

Stereotypy counts
0
1
2
3
4
5
6
0 10 20 30 40 50 60 70 80 90 100 110 120
Time (min)
**
**
**
**
**
**
*
**
**

Figure 3. The mean (+S.E.M.) time to reach the criterion of 10 amphetamine infusions (0.2 mg/kg/infusion) under FR2 schedule of reinforcement in rats that had been previously either sensitized to amphetamine stimulating effects (amphetamine-preexposed group; striped bars) or not exposed to the drug (saline-preexposed group; open bars).

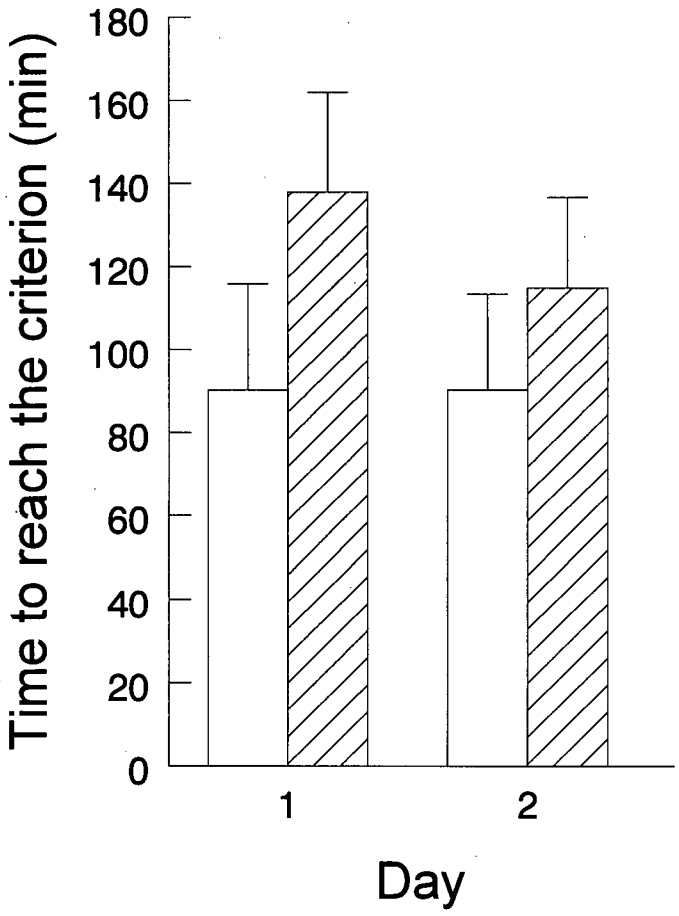
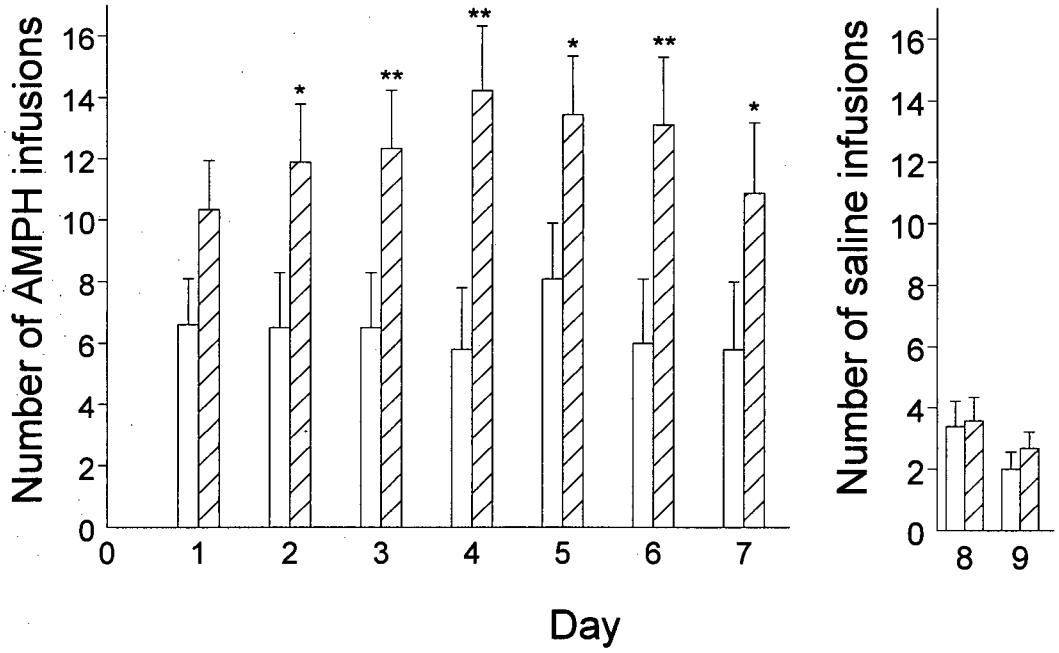

Time to reach the criterion (min)
180
160
140
120
100
80
60
40
20
0
1
2
Day

Figure 4. The mean breaking point (+S.E.M.) as defined by the number of obtained infusions over seven days of amphetamine (0.2 mg/kg/infusion) self-administration testing sessions and two days of extinction in rats that had been previously preexposed either to amphetamine (striped bars) or to saline (open bars). The stars indicate a significant difference ($p < 0.05 = *$; $p < 0.01 = **$) between the two groups at a given time interval.


Number of AMPH infusions
16
14
12
10
8
6
4
2
0
0
1
2
3
4
5
6
7
Day
*
**
**
**
*
*
Number of saline infusions
16
14
12
10
8
6
4
2
0
8
9

Discussion

The present results are the first report of the effects of sensitization on amphetamine self-administration under a PR schedule of reinforcement and support the hypothesis that repeated intermittent treatment with amphetamine results in sensitization of both motor behavior and incentive motivation. In the first phase of the study, rats were repeatedly exposed to amphetamine and behavioral sensitization of motor responses was confirmed after three weeks of withdrawal by a significant increase in amphetamine-induced stereotypy in the amphetamine-pretreated group of rats as compared to a saline-pretreated group. In the second phase of the study, sensitization of incentive motivation was indicated when amphetamine-pretreated rats, relative to saline-pretreated animals, attained significantly higher breaking points under a PR schedule of reinforcement.

Behavioral Sensitization. At first, the sensitization of motor behavior may appear paradoxical given that the overall locomotor activity was decreased in the amphetamine-pretreated group of rats in comparison to the saline-pretreated group. However, it is important to note that the responses that emerge after repeated amphetamine administration differ both in quantity and in quality as a function of dose. Sensitized animals express either enhanced locomotion or a displacement of locomotor activity via increased stereotypy (Segal & Kuczenski, 1994). The qualitatively different behavioral profile for these two groups is consistent with the effects of different doses of amphetamine on behavior. It is well known that lower doses of psychomotor stimulants promote prolonged periods of increased locomotion, whereas higher doses produce stereotyped behaviors (Kuczenski & Segal, 1989). Accordingly, augmented stereotypy and a decline in locomotor

activity, exhibited by the animals pretreated with amphetamine in the present experiment, parallel the type of changes that occur as a function of increasing doses of amphetamine, and thus reflect behavioral sensitization (Segal & Kuczenski).

Sensitization of Incentive Motivation. The most important finding of the present study is the fact that the preexposure to amphetamine resulted in enhanced motivation to respond for the drug as demonstrated by the elevated breaking points exhibited by the amphetamine-pretreated rats relative to the saline-pretreated animals. These results are consistent with some earlier results attributed to sensitization of drug rewarding effects (e.g., Horger et al., 1990; Piazza et al., 1990) which showed that repeated intermittent treatment with psychomotor stimulants produced higher rates of responding during the acquisition phase of drug self-administration in comparison to control animals. These experiments however, were limited to the use of FR schedules of reinforcement. The increased rate of amphetamine or cocaine self-administration, attributed in these studies to sensitization of drug rewarding efficacy, has been often interpreted as representing a diminution rather than an enhancement of the rewarding value of a given drug (Yokel & Wise, 1975). Thus, in an attempt to provide unequivocal evidence of drug reward sensitization, researchers have used extremely low doses of psychomotor stimulants available for self-administration as low doses induce either high rates of lever-pressing or no responding at all. Under these conditions, animals that reliably self-administer a low dose of a drug are considered to be sensitized and can be distinguished from rats for which a given dose is subthreshold (Horger et al., 1992). Nevertheless, the use of subthreshold doses has given rise to other criticisms: when higher doses are available for injection there is no significant effect of chronic pretreatment with psychomotor stimulants on the time

to acquire a steady rate of self-administration responding (Li et al., 1994). This observation is somewhat contrary to the present findings since we employed a relatively high dose of amphetamine and the results show clearly that amphetamine-pretreated animals were willing to pay a much higher behavioral price in the form of elevated breaking points to maintain drug reinforcement than their saline-pretreated counterparts.

Interestingly, even though an enhanced motivation was evident during self-administration of amphetamine under PR schedule of reinforcement, there was no difference between the two groups of rats during two extinction sessions when saline was available for self-administration. This effect seems to reflect specifically sensitization of incentive motivation to ingest amphetamine and not sensitization of motivation in general.

Sensitization vs. Tolerance. Despite the fact that the present results complement earlier findings (e.g., Horger et al., 1990; Piazza et al., 1990), they also differ significantly from a recent report showing tolerance to the reinforcing effects of cocaine under a PR schedule of reinforcement (Li et al., 1994). Specifically, chronic treatment with cocaine (18 mg/kg, given once every eight hours for seven days) produced a subsequent decrement in the breaking points under a PR paradigm. Interestingly, this effect abated following a five day recovery from chronic drug administration. On the basis of these results Li and colleagues criticized previous findings and argued strongly that the rewarding properties of cocaine undergo tolerance rather than sensitization. It should be emphasized however, that these researchers failed to take into consideration the complex time course of sensitization-related changes in brain and behavior. It is now well documented that sensitization is a time-dependent process. Drug

injections must be given intermittently in order to avoid the development of tolerance (Post, 1980). Moreover, animals pretreated with escalating doses of amphetamine exhibit drug sensitization in the form of enhanced behavioral responses and amphetamine-stimulated dopamine efflux in the NAS and dorsolateral caudate nucleus after 28, but not three or seven, days of drug withdrawal (Paulson & Robinson, 1995). Therefore, it is not surprising that tolerance to the reinforcing effects of cocaine developed with a regimen of high drug doses administered closely together in time, and with the absence of an extended withdrawal period (Li et al., 1994).

The complexity of sensitization is readily apparent in the present study. Although enhanced motivation to self-administer amphetamine was evident four weeks after last drug exposure, it did not increase during subsequent seven days of testing of either amphetamine- or saline-pretreated animals. In case of amphetamine-pretreated rats, this result could be attributed to a "ceiling" effect: it is possible that these rats were already maximally sensitized. Alternative explanations could account for the lack of gradual increase in motivation to self-administer amphetamine in both groups of rats: perhaps the time permitted between testing trials was not sufficient to produce this effect.

Liking vs. Wanting. As noted above, drug reward has been recently conceptualized as consisting of two distinct components: subjective pleasure induced by a given drug (liking) and its incentive salience (wanting) (Robinson & Berridge, 1993). There is now evidence that a PR schedule of reinforcement measures incentive salience while an FR schedule is more sensitive to the hedonic, pleasure-inducing properties of addictive drugs. It has been demonstrated that dopamine D1 receptor blocker SCH 23390, injected directly into the NAS or the

amygdala can induce a dose-dependent increase in the rate of cocaine self-administration under an FR schedule of reinforcement. In comparison, under a PR schedule of reinforcement, D1 receptor blockade in the NAS can reduce breaking points, whereas blockade in the amygdala has no effect on the breaking points (McGregor & Roberts, 1993). In a subsequent study, SCH 23390 injected into either the striatum or the mPFC produced a dose-dependent increase in the rate of cocaine self-administration using an FR procedure. Interestingly, similar injections of dopamine D1 receptor antagonists had no effect on the breaking points under a PR schedule of reinforcement when injected into the striatum, but significantly reduced the breaking points when injected into the mPFC (McGregor & Roberts, 1995). Thus, it has been concluded that the two schedules of reinforcement measure different aspects of psychomotor stimulant self-administration. On one hand, the rate of drug intake, as measured by an FR paradigm, seems to be particularly sensitive to factors that interfere with the interoceptive stimulus qualities of a given drug, and hence reflects the subjective experience of that drug. On the other hand, the breaking point under a PR procedure, may be conceptualized as a function of the perceived incentive value of the anticipated drug infusion and thus measures drug craving or incentive salience (McGregor & Roberts, 1995). In the light of these studies, the present data can be interpreted as an indication that preexposure to psychomotor stimulants, such as amphetamine, may increase the drug craving without necessarily affecting the subjective euphoric actions of the drugs, especially since in the present study there were no differences between amphetamine- and saline-pretreated rats in time to reach the criterion of ten amphetamine infusions under the FR2 schedule of reinforcement, whereas performance under the PR was dramatically different between the groups.

Summary and Conclusions. The present study demonstrated for the first time sensitization of amphetamine self-administration under a PR schedule of reinforcement, suggesting that the attribution of incentive salience to psychomotor stimulants undergoes sensitization. The neural mechanism responsible for this effect remains to be specified. As noted above, the mesolimbic dopamine system, implicated in the development of behavioral sensitization is also involved in drug rewarding efficacy (Robinson & Berridge, 1993). Accordingly, enhanced mesolimbic dopamine transmission could be responsible for both development of sensitization of motor behaviors and increased motivation to self-administer addictive drugs. However, recent findings of a dissociation between behavioral and incentive motivational sensitization indicate that the locomotor activating effects of psychomotor stimulants and their reinforcing properties might be mediated by separate, independent neuronal systems. In one study, the locomotor activating effects of cocaine were enhanced following amphetamine, but not nicotine, pretreatment (Schenk, Snow, & Horger, 1991), whereas in other studies both amphetamine- and nicotine-pretreated rats demonstrated elevated rates of cocaine self-administration during the acquisition phase (Horger et al., 1992). Moreover, amphetamine preexposure has been shown to induce behavioral sensitization as measured by motor activity while failing to alter the rewarding efficacy of drugs as measured by an intracranial self-stimulation paradigm (Wise & Munn, 1993). In the present experiment we obtained evidence for both sensitization of motor behavior and incentive motivation, but it is conceivable that with some novel regimens of drug preexposure, or with different doses of amphetamine available for self-administration, we may have observed drug reinforcement sensitization without behavioral sensitization and vice versa.

Overall, the present thesis supports the hypothesis that occasional exposure to drugs of abuse may induce sensitization to the incentive-motivational properties of these drugs and thus may have a profound influence on the development of human addictive behavior. Moreover, drug reinforcement cross-sensitization may also occur under these conditions and would be consistent with previous studies showing behavioral cross-sensitization between drugs of the same class (e.g. amphetamine and cocaine), and between drugs of different classes (e.g. stimulants and opioids; Kalivas & Stewart, 1991) as well as environmental stressors (Antelman et al., 1980).

References

Antelman, S. M., Eichler, A. J., Black, C. A., & Kocan, D. (1980). Interchangeability of stress and amphetamine in sensitization. Science, 207, 329-331.

Blackburn, J. R., Pfaus, J. G., & Phillips, A. G. (1992). Dopamine functions in appetitive and defensive behaviours. Progress in Neurobiology, 39, 247-279.

Bozarth, M. A., & Wise, R. A. (1984). Anatomically distinct opiate receptor fields mediate reward and physical dependence. Science, 224, 516-517.

Braun, A. R., & Chase, T. N. (1988). Behavioral effects of chronic exposure to selective D-1 and D-2 dopamine receptor agonists. European Journal of Pharmacology, 147, 441-151.

Costall, B., Naylor, R. J., Cannon, J. G., & Lee, T. (1977). Differentiation of the dopamine mechanisms mediating stereotyped behavior and hyperactivity in the nucleus accumbens and caudate-putamen. Journal of Pharmacy and Pharmacology, 29, 337-342.

Creese, I., & Iversen, S. D. (1975). The pharmacological and anatomical substrates of the amphetamine response in the rat. Brain Research, 83, 419-436.

Di Chiara, G., & Imperato, A. (1985). Ethanol preferentially stimulates dopamine release in the nucleus accumbens of freely moving rats. European Journal of Pharmacology, 115, 131-132.

Di Chiara, G., & Imperato, A. (1988). Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proceedings of the National Academy of Science of the United States of America, 85, 5274-5278.

Di Ciano, P., Coury, A., Depoortere, R. Y., Egilmez, Y., Lane, J. D., Emmet-Oglesby, M. W., Lepiane, F. G., Phillips, A. G., & Blaha, C. D. (1995). Comparison of changes in extracellular dopamine concentrations in the nucleus accumbens during intravenous self-administration of cocaine or d-amphetamine. Behavioural Pharmacology, 6, 311-322.

Emmett-Oglesby, M. W., & Lane, J. D. (1992). Tolerance to the reinforcing effects of cocaine. Behavioural Pharmacology, 3, 193-200.

Ellison, G. D., & Eison, M. S. (1983). Continuous amphetamine intoxication: an animal model of the acute psychotic episode. Psychological Medicine, 13, 751-762.

Foltin, R. W., & Fichman, M. W. (1991). Assessment of abuse liability of stimulant drugs in humans: a methodological survey. Drug and Alcohol Dependence, 28, 3-48.

Gerhardt, G. A., Pang, K., & Rose, G. M. (1987). In vivo electrical demonstration of the presynaptic actions of phencyclidine in rat caudate nucleus. Journal of Pharmacology and Experimental Therapeutics, 241, 714-721.

Glickman, S. E., & Schiff, B. B. (1967). A biological theory of reinforcement. Psychological Review, 74, 81-109.

Govani, S., Retkov, V. V., Montefusco, O., Missale, C., Bettaini, F., Sparo, P. F., & Trabucchi, M. (1984). Differential effects of caffeine on dihydroxyphenylacetic concentrations in various rat brain regions. Journal of Pharmacy and Pharmacology, 36, 458-460.

Grill, H. J., & Norgren, R., (1978). The taste reactivity test. I. Mimetic responses to gustatory stimuli in neurologically normal rats. Brain Research, 143, 263-279.

Henry, D. J. & White, F. J. (1991). Repeated cocaine administration causes persistent enhancement of D1 dopamine receptor sensitivity within the rat nucleus accumbens. Journal of Pharmacology and Experimental Therapeutics, 258, 882-890.

Horger, B. A., Giles, M. K., & Schenk, S. (1992). Preexposure to amphetamine and nicotine predisposes rats to self-administer a low dose of cocaine. Psychopharmacology, 107, 271-276.

Horger, B. A., Shelton, K. & Schenk, S. (1990). Preexposure sensitizes rats to the rewarding effects of cocaine. Pharmacology Biochemistry & Behavior, 37, 707-711.

Hunt, G. H., & Oderoff, M. E. (1962). Follow-up study of narcotic drug addicts after hospitalization. Public Health Reports, 77, 41-54.

Jaffe, J. H. (1992). Current concepts of addiction. In C. P. O'Brien and J.H. Jaffe (Eds). The Pharmacological Basis of Therapeutics, Pergamon Press, New York, pp. 1-22.

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

Kalivas, P.W., & Duffy, P. (1989). Similar effects of daily cocaine and stress on mesocorticolimbic dopamine neurotransmission in the rat. Biological Psychiatry, 25, 913-928.

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

Kolta, M. G., Sherve. P., De Souza, V., and Uretsky, N. J. (1985). Time course of the development of the enhanced behavioral and biochemical responses to amphetamine after pretreatment with amphetamine. Neuropharmacology, 24, 823-829.

Kuczenski, R., & Segal, D. S. (1988). Psychomotor stimulant-induced sensitization: Behavioral and neurochemical correlates. In P. W. Kalivas & C. D. Barnes (Eds.), Sensitization in the nervous system (pp. 175-205). Caldwell, NJ: Telford Press.

Lamb, R. J., Preston, K. L., Schindler, C., Meisch, R. W., Davis, F., Katz, J. L., Henningfield, J. E., & Goldberg, S. R. (1991). The reinforcing and subjective effects of morphine in post-addicts: a dose response study. Journal of Pharmacology and Experimental Therapeutics, 259, 1165-1173.

Lett, B. T. (1989). Repeated exposures intensify rather than diminish the rewarding effects of amphetamine, morphine and cocaine. Psychopharmacology, 98, 357-362.

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

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

Lyness, W. H., Friedle, N. M., & Moore, K. E. (1979). Destruction of dopaminergic nerve terminals in nucleus accumbens: Effects on d-amphetamine self-administration. Pharmacology, Biochemistry & Behavior, 22, 101-106.

Markou, A., Weiss, F., Gold, L. H., Caine, B., Schulteis, G., & Koob, G. F. (1993). Animal models of drug craving. Psychopharmacology, 112, 163-182.

MacLennan, A. J., & Maier, S. F. (1983). Coping and stress-induced potentiation of stimulant stereotypy in the rat. Science, 219, 1091-1093.

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

McGregor, A., & Roberts, D. C. S. (1995). Effects of medial prefrontal cortex injections of SCH 23390 on intravenous cocaine self-administration under both a fixed and progressive ratio schedule of reinforcement. Behavioural Brain Research, 67, 75-80.

Mereu, G., Yoon, K.-W. P., Boi, V., Gessa, G.L., Naes, L., & Westfall, T.C. (1987). Preferential stimulation of ventral tegmental area dopaminergic neurons by nicotine. European Journal of Pharmacology, 141, 395-400.

Nestler E. J., Hope B. T., & Windell K. L. (1993). Drug addiction: a model for the molecular basis of neural plasticity. Neuron,11, 995-1006.

Parson, L. H., & Justice, J. B. Jr. (1993). Serotonin and dopamine sensitization in the nucleus accumbens, ventral tegmental area and dorsal raphe nucleus following repeated cocaine administration. Journal of Neurochemistry,

Paulson, P. E., Camp, D. M. , & Robinson, T. E. (1991). The time course of transient behavioral depression and persistent behavioral sensitization in relation to regional brain monoamine concentraton during amphetamine withdrawal in rats. Psychopharmacology, 103, 480-492.

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

Pickens, R., & Thompson, T. (1968). Cocaine-reinforced behavior in rat: effects of reinforcement magnitude and fixed ratio size. Journal of Pharmacology and Experimental Therapeutics, 161, 122-129.

Post, R. (1980). Intermittent versus continuous stimulasion: effect of time interval on the development fo sensitization or tolerance. Life Sciences, 26, 1275-1282.

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

Roberts, D. C. S., Koob, G. F., Klanoff, P., & Fibiger, H. C. (1980). Extinction and recovery of cocaine self-administration following 6-OHDA lesions of the nucleus accumbens. Pharmacology, Biochemistry & Behavior, 12, 781-787.

Roberts, D. C. S., & Richardson, N. R. (1992). Self-administration of psychomotor stimulants using progressive ratio schedule of reinforcement. In A. Boulton, G. Becker, and P. H. Wu (Eds.) Animal Models of Drug Addiction, The Human Press, pp. 233-269.

Robinson, T. E. (1984). Behavioral sensitization: characterization of enduring changes in rotational behavior produced by intermittent injections of amphetamine in male and female rats. Psychopharmacology, 84, 466-475.

Robinson, T. E., & Becker, J. B. (1982). Behavioral sensitization is accompanied by an enhancement in amphetamine-stimulated dopamine release from striatal tissue in vitro. European Journal of Pharmacology, 85, 253-254.

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

Robinson, T. E., & Berridge, K.C. (1993). The neural basis of drug craving: an incentive sensitization theory of addiction. Brain Research Reviews, 18, 247-291.

Robinson, T. E., Jurson, P. A., Bennett, J. A., & Bengten, K. M. (1988). Persistent sesitization of dopamine neurotransmission in ventral striatum (nucleus accumbens) produced by past experience with (+)-amphetamine: a microdialysis study in freely moving rats. Brain Research, 462, 211-222.

Schuster, C. R. (1970). Psychological approaches to opiate dependence and self-administration by laboratory animals. Federation Procedings, 29, 1-5.

Segal, D. S., & Kuczenski, R. (1994). Beahvioral pharmacology of amphetamine. In A. K. Cho and Segal, D. S. (Eds.) Amphetamine and its analogs. Academic Press, San Diego, pp. 115-150.

Segal, D. S., & Schuckit, M. A. (1983). Animal model of stimulant induced pychosis. In I. Creese (Ed.) Stimulants: Neurochemical, Behavioral and Clinical Perspective, Raven Press, New York, pp. 131-167.

Seiden, L. S., Fischman, M. W., & Schuster, C. R. (1975). Long-term methamphetamine-induced changes in brain catecholamines in tolerant rhesus monkeys. Drug and Alcohol Dependence, 1, 215-219.

Snyder, S. H. (1973). Amphetamine psychosis: A 'model' schizophrenia mediated by catecholamines. American Journal of Psychiatry, 130, 61-67.

Sorg, B. A., & Kalivas, P. W.(1991). Effects of cocaine and footshock stress on extacellular dopamine levels in the ventral striatum. Brain Research, 559, 29-36.

Stoofe J. C. & Kebabian J. W. (1984). Two dopamine receptors biochemistry, physiology, and pharmacology. Life Sciences, 35, 2281-2296.

Striplin C. D., & Kalivas P. W. (1993). Robustness of G protein changes in cocaine sensitization shown with immunoblotting. Synapse, 14, 10-15.

Swanson, L. W. (1982). The projections of the ventral tegmental area and adjacent regions: A combined fluorescent retrograde tracer and immunofluorescent study in the rat. Brain Research Bulletin, 9, 321-353.

Treit, D., & Berridge, K. C. (1990). A comparison of benzodiazepine, serotonin and dopamine agents in the taste reactivity paradigm. Pharmacology, Biochemistry & Behavior, 37, 451-456.

Vezina. P. & Stewart, J. (1990). Amphetamine administered to the ventral tegmental are but not to the nucleus accumbens sensitizes rats to systemic morphine: lack of conditioned effects. Brain Research, 516, 99-106.

Willner, P., Muscat, R., & Papp, M. (1992). Chronic mild strress-induced anhedonia: a realistic animal model of depression. Neuroscience and Biobehavioral Reviews, 16, 525-534.

Wilson, B. K., Elms, R. R., & Thompson, C. P. (1975). Outpatient versus hospital methadone detoxification. International Journal of the Addiction,10, 13-21.

Wise, R. A. (1980). Action of drugs of abuse on brain reward systems. Pharmacology, Biochemistry & Behavior, 13, 213-223.

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

Woolverton, W. L., Cervo, L., & Johanson, C. E. (1984). Effects of repeated methamphetamine administration on methamphetamine self-administration in rhesus monkeys. Pharmacology, Biochemistry & Behavior, 16, 293-302.

Yokel, R. A., & Pickens, R. (1974). Drug level of d- and l-amphetamine during intravenous self-administration. Psychopharmacologia, 34, 255-264.

Yokel, R. A., & Wise, R. A. (1975). Increased lever pressing for amphetamine after pimozide in rats: Implications for a dopamine theory of reward. Science, 187, 547-549.

Yokel, R. A., & Wise, R. A. (1976). Attenuation of intravenous amphetamine reinforcement by central dopamine blocade in rats. Psychop