**Investigations of the Neural Substrates and Behavioural Correlates of
Reinforcing Substances**

by

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A dissertation submitted to
the Faculty of Graduate Studies and Research
in partial fulfilment of the requirements of the degree of
Doctor of Philosophy

Department of Psychology

Carleton University
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Abstract

Previous investigations have shown that experimental manipulations of either the dopamine (DA) or serotonin (5HT) neurotransmitter systems alter the reinforcing effects of psychostimulants. The present series of studies evaluated the effects of neurotoxic lesions of forebrain DA and 5HT systems on the behavioural effects of psychostimulant drugs in two fundamentally different experimental paradigms. First, the traditional intravenous self-administration paradigm was used to confirm that 5,7-DHT-induced depletion of forebrain 5HT produce an apparent increase in the reinforcing effects of cocaine. It was also shown that 5HT depletions appear to increase the motivation of rats to respond for food reinforcement, demonstrating that the effects of 5HT depletions may not be specific to cocaine.

Another series of studies used a novel version of the radial arm maze (RAM) paradigm to examine the behavioural effects of several reinforcing agents. The first RAM study demonstrated that amphetamine, heroin and nicotine, all produced a perseveration in the behaviour of rats exploring the maze. In contrast, psychoactive substances that do not have a high abuse potential (haloperidol and scopolamine) did not produce similar systematic changes in behaviour. These findings, together with a previous demonstration by Loh and Beck (1989) that ethanol and diazepam also increased behavioural perseveration in the RAM, show that a diverse group of reinforcing substances all promote similar changes in behaviour in this paradigm.

The third study was conducted to determine if neurotoxic lesions of DA systems in either the nucleus accumbens septi (NAS) or caudate nucleus would attenuate amphetamine-induced perseveration in the RAM. In contrast to our expectations, lesions of the NAS increased behavioural perseveration, while DA lesions of the caudate nucleus had no apparent effect.

The final study examined the effects of global forebrain 5HT depletions on the behaviour of rats exploring the RAM following injections of either amphetamine or cocaine. The results of this study were inconclusive.

These studies used both a traditional and novel paradigm to investigate common aspects of reinforcing substances. Findings from the self-administration paradigm indicated that 5HT systems may have a more general role in the mediation of the reinforcement process, while findings from the RAM studies showed that a wide range of reinforcing substances can promote similar changes in animal behaviour.

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Review of the Literature: Psychostimulant Reinforcement

Introduction

The focus of the present research was to examine the role of specific dopamine (DA) and serotonin (5HT) systems in psychostimulant reinforcement. The effects of specific lesions to DA and 5HT systems were examined using two very different experimental approaches: the intravenous (i.v.) self-administration paradigm and a modification of the radial arm maze (RAM) paradigm. The i.v. self-administration methodology is commonly used to study drug seeking behaviour in laboratory animals and it has proven to be a valuable tool for the investigation of the neural mechanisms that may mediate the addictive properties of drugs. In the following sections, findings from studies incorporating the self-administration paradigm are outlined and problems with the interpretation of the results of such studies are identified. A more sophisticated variation of the self-administration paradigm that permits some clarification of the problems is then described. The RAM paradigm is then introduced as a novel way of examining the behavioural effects of a wide variety of addictive substances. Experiments will be described that examine the effects of neurotoxic lesions to DA and 5-HT systems in both of these experimental paradigms.

Intravenous Drug Self-Administration Technique: General Principles

Intravenous drug self-administration is a technique that has been commonly used to examine the neural basis of reinforcement. Animals are implanted with a chronically indwelling jugular cannula and then placed in an operant chamber. The delivery of an

intravenous (i.v.) injection is made contingent upon a lever response, or some other predefined operant response. Traditionally self-administration studies have incorporated fixed ratio (FR) schedules of reinforcement. The FR schedule requires an animal to make a fixed number of operant responses in order to obtain an intravenous infusion of a drug. The ratio of responses required to obtain an infusion of drug is predetermined at the onset of the study and remains the same throughout the testing session.

The self-administration paradigm has been used to demonstrate the reinforcing properties of drugs known to have abuse potential in humans. For example, psychostimulants such as amphetamine, cocaine, methamphetamine, and methylphenidate are readily self-administered by both rats and monkeys (Collins et al. 1984; Denuveau, et al. 1969; Pickens and Harris, 1968; Pickens et al. 1967; Pickens, and Thompson 1968, Yokel and Pickens, 1973; Wilson et al. 1971).

An inverse dose-response relationship is commonly observed between the unit dose (i.e., dose per infusion) and the rate that animals self-administer a particular drug. Below a threshold dose, drugs are not self-administered at all. However, if the unit dose increases above the threshold, the response rate will increase to a maximum level. Studies examining psychostimulant self-administration have repeatedly shown that the rate of drug intake is exquisitely sensitive to changes in the unit dose. Increasing the unit dose reduces the rate at which injections are self-administered. This relationship between dose and rate is widely described as being on the descending limb of an inverted U-shaped function

(Koob and Goeders, 1989). An example of the inverted U-shaped function is illustrated in Figure 1.

Once the dose range that supports self-administration behaviour has been established, it is possible to determine if experimental manipulations alter the reinforcing efficacy of the drug. Manipulations that alter the dose-response relationship have been interpreted to reflect a change in the reinforcing efficacy of the drug. For example, if an experimental manipulation produces a shift in the dose-response curve to the right, the shift would indicate that a greater unit dose is required to observe the same rate of drug self-administration. Therefore a shift may be considered to represent a change in the reinforcing potency of the drug being self-administered.

Changes in the reinforcing potency of a drug are often measured by evaluating changes in the rate of infusion at one specific unit dose. If the unit dose of reinforcing substance is kept constant, a change in rate of self-administration is often interpreted to represent a shift in the dose-response curve. However, studies have demonstrated that some experimental manipulations that alter the reinforcing efficacy of a substance may not be represented in a change in the dose-response curve (Roberts 1989). For example, studies have shown that the reinforcing properties of cocaine and amphetamine can be altered without affecting the rate of drug self-administration (see section "The Progressive Ratio Reinforcement Schedule").

Figure 1. Example of a dose-response curve for a rat self-administering amphetamine. Data adapted from Yokel and Pickens 1973.

The general methodology of the self-administration paradigm has been adopted from the operant literature. However, it is important to note that measurement and interpretation of the operant response differs between paradigms. Operant research often incorporates experimental designs that require animals to make a large number of responses, or to learn complex reinforcement schedules, to obtain reinforcement. In contrast, self-administration studies often use simple reinforcement schedules that do not require a large number of responses. The FR1 schedule is often used in self-administration studies. Traditional operant studies examine the rate at which an animal responds prior to *each* reinforcement (running rate). Self-administration studies examine the rate at which an animal obtains injections of the reinforcing agent; this is in contrast with the measurement of rate that an animal makes operant responses to obtain each reinforcement. Running rate is usually not measured in self-administration studies (Arnold and Roberts in press; Richardson and Roberts 1996).

Self-administration studies have proven to be of enormous benefit in the identification of neurotransmitter systems and localization critical sites that mediate the reinforcing properties of a variety of substances. The self-administration paradigm has a number of inherent advantages that have made it a method of choice for such investigations. However, the interpretation of changes in the reinforcing efficacy of substance resulting from experimental manipulations is problematic. See section "Problem with the Interpretation of Rate of Self-administration When Using Fixed Ratio Reinforcement Schedules" for further details.

Advantages of the Intravenous Drug Self-Administration Procedure

There are a number of inherent advantages to using the intravenous drug self-administration procedure to assess the reinforcing properties of substances. First, the animal must make a discrete operant response in order to obtain an infusion of drug. The operant response is easily quantified and does not require a subjective assessment by the researcher. Second, clear dose-response relationships have been observed for many substances that support self-administration (Koob and Goeders 1989). In particular, studies examining the dose-response relationship of amphetamines (Pickens and Harris 1968, Yokel and Pickens 1973;1974) and cocaine (Pickens and Thompson 1968) using fixed ratio reinforcement schedules have demonstrated a strong relationship between the unit dose of the drug and the rate of drug self-administration

Finally, the intravenous self-administration paradigm is considered to be a useful model of human drug taking behaviour. Many psychostimulants, such as cocaine or amphetamine, that are self-administered by animals also have a high abuse potential in humans (Collins et al. 1984; Pickens and Harris 1968). The self-administration paradigm has been recognized to be a useful screening tool for the prediction of the abuse liability of substances in humans (Collins et al. 1984).

**Investigations of Psychostimulant Reinforcement with the
Self-Administration Paradigm and Fixed Ratio Reinforcement Schedules:
Pharmacological Manipulations**

Pharmacological manipulations of noradrenergic and dopaminergic systems

The intravenous drug self-administration technique has been used to identify neurotransmitter systems that have a specific involvement in the mediation of the reinforcing properties of psychostimulants. Much of the initial work on the identification of neural substrates of psychostimulant reinforcement concentrated upon manipulations of the noradrenaline (NE) and dopamine (DA) neurotransmitter systems.

Early studies indicated that it was unlikely that NE systems play a significant role in the mediation of the reinforcing properties of psychostimulants. For example, Risner and Jones (1976) demonstrated that pretreatment with methoxamine, an α -adrenergic agonist, did not alter the rate of responding for intravenous infusions of amphetamine in dogs. Yokel and Wise (1975; 1976) did find a reduction in responding in rats self-administering amphetamine when pretreated with the α -noradrenergic antagonist phentolamine or the β -noradrenergic blocker *l*-propranolol. However, the reduction in responding was interpreted as a non-specific behavioural effect, rather than an a result of an alteration in the reinforcing effects of amphetamine. Finally, pretreatment of animals with phenoxybenzamine, which also has α -noradrenergic blocking properties, did not alter amphetamine self-administration (Yokel and Wise 1975; 1976).

It is now well established that direct DA receptor agonists have reinforcing properties. Early studies demonstrated the direct DA receptor agonist apomorphine is self-administered by rats (Baxter et al. 1974; 1976; Davis and Smith 1977). Since then many different D1 and D2 specific DA agonists, including pipradrol, piribedil, propylbutyldopamine, bromocriptine SKF 82958, SKF 77434 and SKF 38393 have been shown to be self-administered by rats and monkeys (Self and Stein 1992; Weed and Woolverton 1995; Wilson et al. 1971; Woolverton et al. 1984).

These studies indicate that the direct stimulation DA receptors is important in psychostimulant reinforcement. Other research has demonstrated that the reinforcing effects of DA agonists can be altered by DA receptor antagonists. For example, the rate of amphetamine self-administration can be altered by pretreatment of animals with DA receptor antagonists such as chlorpromazine (Risner and Jones 1976; Wilson and Schuster 1972), pimozide (Risner and Jones 1976; Yokel and Wise 1975), butaclamol (Yokel and Wise 1976) or haloperidol (Davis and Smith 1974; 1975).

Cocaine and amphetamine self-administration is similarly affected by DA system antagonists. Chlorpromazine (Roberts and Vickers 1984; Wilson and Schuster 1972), pimozide (de Wit and Wise 1977; Roberts and Vickers 1984), alpha-flupenthixol (Roberts, and Vickers 1984; Ettenberg et al. 1982), perphenazine (Johanson et al. 1976), haloperidol, sulpiride, metoclopramide, and clozapine (Roberts, and Vickers, 1984) have all been shown to alter the rate of drug self-administration. All but one of these substances

showed a positive relationship between the dose of the DA antagonist and the rate of cocaine infusion. The atypical neuroleptic clozapine showed the opposite relationship. Increasing doses of clozapine produced a reduction in the rate of cocaine intake. Unfortunately the fixed ratio self-administration paradigm could not discern the nature of this anomalous finding. More sophisticated reinforcement schedules were to shed light on this relationship (see below).

These studies have demonstrated that increases in the unit dose of a reinforcing agent commonly results in a decrease in the rate of self-administration. Conversely, reductions in the unit dose of a reinforcing substance results in increases in self-administration rate (Deneau et al. 1969; Wilson et al. 1971; Yokel and Pickens 1974). When animals are pretreated with relatively low doses of DA system antagonists it is typical to find an increase in the rate of self-administration. For example, Wise and coworkers have demonstrated that partial DA receptor blockade will increase the rate of both amphetamine and cocaine self-administration (De Wit and Wise 1977; Yokel and Wise 1975; 1976). This increase in rate has been interpreted to be a result of the DA antagonists producing a partial blockade of the DA receptor which in turn leads to a compensatory increase in the drug intake of the animal to "reach the same level of drug satiety" (Yokel and Wise 1975, p. 548). At relatively high doses of DA antagonists, it is assumed that DA receptor blockage cannot be overcome by compensatory increases in the rate of psychostimulant self-administration. This is evidenced by a pattern of extinction-like responding similar to situations in which the reinforcing substance is removed or

replaced with a saline solution.

Ritz and coworkers (1987) examined the association between reinforcing efficacy of cocaine and cocaine analogs, and the receptor binding potencies of the substances. The authors demonstrated a strong correlation between the relative behavioural potencies of the reinforcing substances, determined by self-administration studies, with the ability of those substances to inhibit the binding of a marker for the DA transporter ([³H] mazindol). A statistically significant correlation ($r^2 = 0.94$) was found between the reinforcing potency of the substance and the concentration of substance required to inhibit binding of the marker. In contrast, no statistically significant relationship was found between the NE transport inhibition properties and the reinforcing properties of the drugs tested. A small negative correlation was found between the inhibition of 5HT uptake and the reinforcing effects of the drug tested. These findings were taken to suggest that the primary site of action for cocaine reinforcement is the at the dopamine transporter.

Together, these studies provide substantial evidence that DA systems play a key role in the mediation of psychostimulant reinforcement. Both direct stimulation of DA receptors or the indirect augmentation of DA system function appear to have reinforcing effects.

Psychostimulant reinforcement and pharmacological manipulations of serotonergic systems

A large number of investigations have used pharmacological manipulations to examine the role of DA systems in the mediation of psychostimulant reinforcement; fewer studies have explored the role of serotonergic systems. Results from psychostimulant self-administration studies that have examined the effects of manipulations of 5HT systems are somewhat ambiguous. For example, investigations that have augmented serotonergic function by either increasing brain 5HT levels through dietary supplements of tryptophan (Carroll et al. 1990a; Smith et al. 1986), pretreatment with the 5HT re-uptake blocker fluoxetine (Carroll et al. 1990b; Leccese and Lyness 1980; Porrino et al. 1989; Yu et al. 1986) injections of L-tryptophan, or pretreatment with the 5HT receptor agonist quipazine 8-OH-DPAT (Leccese and Lyness 1980; Peltier and Schenk, 1993) have all found a reduction in amphetamine self administration. Lyness and Moore (1983) demonstrated that pretreating rats with the 5HT antagonist metergoline increased the rate of amphetamine self-administration. These studies would suggest that augmenting 5HT function will reduce amphetamine self-administration while attenuation of 5HT function will increase amphetamine self-administration. However, Porrino and associates (1989) found a decrease in amphetamine self-administration if rats were pretreated with the 5HT₂ receptor antagonist cianaserin (Porrino et al. 1989). In order to be consistent with the other studies, an increase in amphetamine self-administration would have been expected. Together these studies provide some evidence of a negative relationship between serotonergic system function and the rate of amphetamine self-administration. However

the finding that the 5HT antagonist cinanserin will also reduce the rate of amphetamine self-administration (Porrino et al. 1989) complicates the issue.

Investigations of the effects of pharmacological manipulations of 5HT function on the rate of cocaine self-administration have also been conducted. Pretreatment of animals with 5HT antagonists such as cinanserin or GR38032F, the latter of which is specific to 5HT₃ receptor, does not appear to effect that rate of cocaine self-administration (Peltier and Schenk 1991; Porrino et al. 1989). However, pretreatment of animals with the 5HT re-uptake blocker fluoxetine, the 5HT_{1A} receptor agonist 8-OH-DPAT and dietary tryptophan supplements all appear to reduce the rate at which animals self-administer cocaine under fixed ratio reinforcement schedules (Carroll et al. 1990a, b; Peltier and Schenk 1993; Porrino et al. 1989). Therefore, it would appear that attenuation of 5HT system function does affect cocaine self-administration, while augmenting of 5HT function will reduce cocaine self-administration.

In summary, studies incorporating pharmacological manipulations have demonstrated that DA but not NE systems are important in the mediation of psychostimulant reinforcement. Investigations of 5HT systems indicate that manipulations of the neurotransmitter systems can also alter the reinforcing properties of psychostimulants. However, the relationship between 5HT system function and psychostimulant reinforcement can not be clearly determined from these studies.

**Investigations of Psychostimulant Reinforcement with the
Self-Administration Paradigm and Fixed Ratio Reinforcement Schedules:
Neurotoxic and Surgical Lesion Studies**

Lesions of Noradrenergic and Dopaminergic Neurotransmitter Systems

The role of specific dopaminergic systems in the mediation of reinforcing properties of cocaine was first demonstrated by Roberts and coworkers (1977). Their investigations showed that substantial lesions of dopaminergic nerve terminals in the nucleus accumbens septi (NAS) produced a marked reduction in the rate of cocaine intake. In contrast the rate of apomorphine self-administration, a direct DA receptor agonist, was unaffected. Of interest was the finding that depletions that reduced DA in NAS by less than 70% resulted in a transient reduction in cocaine self-administration whereby the rate of responding returned to baseline levels within three days of the lesion. In contrast, lesions that reduced the DA content in the NAS by more than a 70% produced a long term reduction in responding for cocaine (Roberts et al. 1977).

Together these findings demonstrate that the integrity of dopaminergic nerve terminals in the NAS is important in the mediation of the reinforcing effects of cocaine. However, it would appear that the reinforcing effects of cocaine can be sustained by a subset of the entire population of DA terminals. Apomorphine self-administration was not affected by the DA lesions indicating that stimulation of DA receptors in the NAS continued to have reinforcing effects following the compromise of the nerve terminals in

the region. Other investigations have demonstrated similar findings (Pettit et al. 1984).

Roberts and co-workers (1977) also examined the role of NE systems in cocaine reinforcement. Lesions of dorsal and ventral NA bundles that resulted in substantial reductions in NE content in hippocampal-cortical regions (96% reduction) and hypothalamic regions (72% reduction) did not affect the rate of cocaine self-administration. The substantial NE depletions did not affect responding for cocaine. When the animals were pretreated with pimozide, or the unit dose of cocaine was reduced by 50%, a statistically significant increase in the rate of cocaine self-administration was demonstrated. These findings suggest that significant depletions of NE systems do not affect responding for cocaine; NE depleted animals respond in a similar fashion as unlesioned animals in response to alterations in the unit dose of cocaine or a pimozide challenge. Finally, DA specific lesions of the NAS did not effect responding for food reinforcement, with the exception of a short term reduction in the rate of food intake during the first three days following surgery (Roberts et al. 1977).

In a subsequent study, Roberts and associates (1980) demonstrated that pretreatment of animals with pargyline, a drug that promotes the effects of 6-OHDA, either alone or with desmethylinipramine (DMI), which acts to protect NE neurons from the toxic effects of 6-OHDA, resulted in a near complete cessation of responding for cocaine. The rate of recovery from the 6-OHDA lesions was also examined. The number of days required for the animals to return to 50% of pre-operative cocaine intake (index of

recovery) was correlated with the degree of DA depletion in NAS and striatum. The index of recovery was statistically significantly correlated with the DA depletions in the NAS ($r = .075$), while correlations with striatal DA were not statistically significant (Roberts et al. 1980).

Lyness and associates (1979) examined the effects of DA specific lesions in the NAS on amphetamine self-administration. The investigators incorporated the use of bilateral injections of the 6-OHDA to the NAS, as did Roberts and coworkers (1977). Rats had either been previously trained to self-administer amphetamine, or had no prior experience with the drug. Rats that had previously received amphetamine injections demonstrated an almost complete cessation of amphetamine self-administration. Rats with no prior exposure to amphetamine did not initiate drug self-administration, even when tested for a period of 19 days post surgery. Together these studies provide strong evidence that DA neurons innervating the NAS play a critical role in the mediation of the reinforcing effects of cocaine and amphetamine.

The role of the ventral tegmental area (VTA) in the mediation of cocaine reinforcement was examined by Roberts and Koob, (1982). The authors demonstrated that if 6-OHDA is used to create DA specific lesions of the VTA marked reductions in cocaine intake are observed. Responding for apomorphine was unaffected. When the analysis was restricted to the group of rats that received the 6-OHDA lesions, comparison of the DA content in the NAS with changes in cocaine intake demonstrated only a

moderate correlation ($r = 0.51$). Some animals that were shown to have extensive DA depletions in the NAS maintained rates of cocaine self-administration that approximated pre-lesion rates. Findings from this study support the argument that DA neurons originating in the VTA are important in the mediation of the reinforcing effects of cocaine. However, the relatively low correlations between DA depletion in the NAS and changes in cocaine intake cannot rule out the possibility that other systems may be involved in the effect.

In a similar study, Le Moal and coworkers examined the effects of radio frequency lesions to the VTA (A-10 region) on the acquisition and maintenance of amphetamine self-administration in rats. Surprisingly, the authors reported that the lesioned animals self-administered greater amounts of amphetamine and acquired self-administration behaviour faster. It would be expected that the lesions would diminish that rat's propensity to self-administer amphetamine. These findings could be accounted for by a number of explanations: the radio frequency lesions did not produce sufficient destruction of the DA cell bodies and a sufficient number of DA neurons remained intact to sustain amphetamine self-administration; the non-specific nature of the lesions resulted in damage to other neuronal systems that possibly attenuated the reinforcing effects of amphetamine, or the reinforcing effects of cocaine and amphetamine are, in some manner, mediated differently. It was also demonstrated that the lesions produced a time-limited hyper-sensitivity to the behavioural effects of amphetamine that could have resulted in an increased probability of lever responding that is not contingent upon reinforcement (Le Moal et al. 1979).

In an attempt to clarify their previous findings, Le Moal and associates (Deminière et al. 1984) examined the effects of 6-OHDA lesions in the A10 region of the VTA on the amphetamine self-administration. The authors incorporated low unit doses of amphetamine in their investigations. The rate at which the animals acquired the self-administration task and rate at which animals obtained amphetamine infusions, once self-administration behaviour had stabilized, was examined. Animals receiving the DA system lesions demonstrated a greater rate of acquisition of amphetamine self-administration behaviour as well as a greater rate of drug intake. The DA system lesions produced a substantial reduction in the DA content of NAS and prefrontal cortex. However, the reductions in DA were not significantly correlated with changes in cocaine intake. The authors (Deminière et al. 1984) did not offer an explanation for these findings, but reaffirmed a role for DA system in that VTA as being important in the acquisition and mediation of amphetamine reinforcement and suggested that the disruption of the DA cell bodies in the VTA may produce an imbalance in function of DA and non-DA projections. The possibility of this hypothesis was examined, although somewhat indirectly, by examining the effects of DA specific lesions to the amygdala (Deminière et al. 1988). Based upon the previous finding that DA specific lesions of the amygdala resulted in an increase in DA turnover in the NAS (Louilot et al. 1985), the investigation of the role of the amygdala in amphetamine self-administration was undertaken. Dopamine specific lesions of the amygdala were shown to increase the rate of amphetamine self-administration and facilitated the acquisition of amphetamine self-administration behaviour at doses lower than those that support self-administration in unlesioned animals

(Deminière et al. 1988). These results suggest that DA systems in the amygdala play a role in the mediation of amphetamine self-administration.

It is possible that dopaminergic neurons originating in the VTA that course through the NAS but terminate in regions anterior to the NAS may have a role in the mediation of the reinforcing effects of cocaine. Zito et al. (1985) examined this possibility by using kainic acid to destroy cell bodies in the NAS. In a preliminary experiment, infusions of the kainic acid into the NAS were shown to produce a statistically significant reduction in DA content of the NAS, prefrontal cortex and the olfactory tubercle. If DA content of these regions was reduced by more than 60% when compared to a control lesion group, a reduction in DA content of the amygdala was also observed. However, this reduction failed to achieve statistical significance. The NE content of all of the regions examined was reduced to 70-80 % of the control group as well. In the next two experiments, rats were trained to self-administer either cocaine or apomorphine and then were given intra-accumbens kainic acid infusions. Following the lesions, rats either ceased responding for either reinforcing substance altogether or self-administered the drugs at greatly reduced rates. A statistically significant correlation ($r = 0.76$) was observed between reduction in pre-lesion cocaine intake and the ranking of degree of destruction of the NAS. In a final study, the effects of kainic acid lesions of the NAS on responding for heroin were examined. It was shown that the lesions also produced a reduction in heroin self administration that was also correlated with the degree of NAS damage ($r = 0.67$).

Together these studies demonstrated that the destruction of cell bodies in the NAS disrupts responding for cocaine, apomorphine and heroin. Further, the degree of disruption in responding appears to correlate with the degree of damage to the NAS. This suggests that the NAS plays a role in mediation of the reinforcing properties of both direct and indirect DA system agonists.

A number of studies have suggested that the prefrontal cortex may also have an involvement in the mediation of psychostimulant reinforcement. Goeders and Smith (1983) reported that drug naive rats would learn to perform lever responses in order to receive small amounts of cocaine infused directly into the prefrontal cortex. When the intracerebral infusions of cocaine included the DA receptor antagonist sulpiride, self-administration behaviour was attenuated. Further, direct infusions of cocaine into the NAS or VTA regions did not support self-administration behaviour. In a later study, the authors examined the effects of 6-OHDA lesions of the prefrontal cortex on rats self-administering cocaine to the region. The neurotoxic lesions reduced the level of responding for cocaine to levels observed when rats were given the vehicle solution alone. However, when DA was substituted for cocaine, rats would maintain self-administration behaviour which was also compromised by the inclusion of sulpiride in the injected solution (Goeders and Smith, 1986). These studies suggest that the prefrontal cortex may also play a role in the mediation of cocaine reinforcement, or in any event, the infusion of cocaine into the prefrontal cortex has reinforcing properties.

The role of the prefrontal cortex in the mediation of the reinforcing properties of intravenous infusions of cocaine has also been examined. Martin-Iverson and colleagues examined the effects of DA specific lesions of the prefrontal cortex in rats self-administering cocaine. While no substantial changes in psychostimulant self-administration was observed, a significant increase in the rate of DA turnover (DOPAC/DA ratio) in the NAS and striatal regions was demonstrated (Martin-Iverson et al. 1986). Schenk and co-workers (1991) replicated Martin-Iverson's findings and also reported that the prefrontal cortex lesion promoted the acquisition of cocaine self-administration at unit doses of the drug lower than would support the acquisition of self-administration behaviour in unlesioned animals. These findings suggest that the prefrontal cortex may also be involved in the mediation of the reinforcing effects of cocaine and DA systems in the prefrontal cortex may, in some manner, influence the rate of DA turnover in the NAS (Martin-Iverson et al. 1986; Schenk, 1991).

Studies that have examined the effects of lesions of mesolimbic DA systems have found an attenuation in responding for psychostimulants when sufficiently large DA lesions are accomplished. Lesser depletions of DA systems do not appear to alter the rate of drug self-administration or attenuate the rate marginally. However, findings from studies of pharmacological manipulations of DA systems would predict that less than complete lesions of DA systems should augment responding for psychostimulants rather than produce an attenuation of the behaviour. The consistent interpretation of these findings is difficult, the issue of interpretation of what a change in rate of responding

represents (see below) complicates the issue even further and fosters the need to look to different experimental paradigms to clarify these issues.

Lesions of serotonergic neurotransmitter systems

In comparison to studies of DA systems, fewer studies have examined the effects of lesions to 5HT systems on psychostimulant self-administration on fixed ratio schedules of reinforcement. Lyness et al. (1980) first examined the issue in a study that assessed the effects of intraventricular infusions of the 5HT specific neurotoxin 5,7-dihydroxy-tryptamine (5,7-DHT) on the acquisition of intravenous amphetamine self-administration behaviour. A comparison of the rate of acquisition of amphetamine self-administration showed no difference in the rate in comparison to the control group. However, rats receiving the intraventricular infusions of the neurotoxin consistently obtained more infusions of amphetamine per testing session when compared with a group of rats that received the sham lesions. Subsequent neurochemical analysis of the dissected rat brains showed that the 5,7-DHT infusions produced substantial reductions in the 5HT content of the hypothalamus (40%), hippocampus (24%), striatum (10%) and cortex (17%). The authors also reported that infusions of 5,7-DHT into the NAS did not alter the rate of acquisition of amphetamine self-administration behaviour or the rate at which rats obtained amphetamine infusions (Lyness et al. 1980).

The increased rate of amphetamine self-administration in the 5HT lesioned animals was of particular interest. It was reported that some rats would self-administer lethal

quantities. It was also reported that the increased rate of self-administration was not an artifact on any drug induced stereotypical behavioural patterns (Lyness et al. 1980). The finding that rats would self-administer lethal doses of amphetamine was surprising since many other studies have demonstrated that rats are extremely sensitive to the unit dose per injection of psychostimulants and will actively alter their rate of self-administration accordingly. Further, animals self-administer toxic doses of psychostimulants only when given continuous access to the drug (Deneau et al. 1969; Johanson et al. 1976). Presumably, rats would also adjust their rate of self-administration if the concentration of the psychostimulant reached near toxic levels. Thus lesions of 5HT systems may compromise the ability of rats to regulate their intake of drug.

The effects of massive depletions of forebrain 5HT on amphetamine self-administration were also examined in a later study by Leccese and Lyness (1984). Infusion of 5,7-DHT into the medial forebrain bundle (MFB), the primary pathway of ascending 5HT neurons innervating the forebrain, resulted in a 94% depletion of forebrain 5HT and a 88% depletion of its metabolite 5HIAA. In comparison with the control group, 5HT depleted animals self-administered statistically significantly more amphetamine. When rats were challenged with l-tryptophan or fluoxetine pretreatment, animals in the control group demonstrated a marked reduction in amphetamine self-administration while the rate of self-administration of the lesioned animals was unaffected. The effects of 5HT antagonists (methysergide and cyproheptadine) was also examined. In the control animals the 5HT antagonists produced reductions in the rate of amphetamine self-administration. These

findings were unexpected, as it would be predicted that an increase in responding for amphetamine should be observed when the function of 5HT systems was disrupted. However, the observation of particular interest was that the pretreatment of the 5HT lesioned animals with either 5HT antagonist produced an unusual pattern of responding. Animals would self-administer amphetamine at very high rates (bursts of responding) and then cease responding for significant periods of time. The high rates of amphetamine intake would lead to the animals over-dosing if not removed from the apparatus. The effects could, again, not be accounted for by an augmentation of stereotypical behavioural patterns.

In summary, the results of studies examining the effects of manipulations of 5HT systems on the psychostimulant self-administration behaviour are somewhat contradictory. Dramatic increases in responding for amphetamine are observed in rats that have received neurotoxic lesions of forebrain 5HT systems. Further, the increases in responding were observed in the lesioned animals when the animals were pretreated with 5HT antagonists methysergide and cyproheptadine. While 5HT antagonists such as methysergide or cyproheptadine will reduce amphetamine self-administration; the 5HT agonist fluoxetine and tryptophan treatments have also been shown to have the same effect. These findings suggest that 5HT systems may play an important role in the mediation of psychostimulant reinforcement, however, the precise relationship between 5HT system function and psychostimulant reinforcement cannot be established.

Problem with the Interpretation of Rate of Self-Administration When Using Fixed Ratio Schedules of Reinforcement

Studies that measure changes in the rate of drug intake with fixed ratio schedules of reinforcement appear to provide evidence of a change in drug potency. However, it is difficult to determine whether a change in rate of self-administration represents an increase or a decrease in the reinforcing effects of a drug. It has been argued that increases in the rate of self-administration can be interpreted to reflect a reduction in the reinforcing efficacy of each infusion of drug; animals are required to obtain more infusions of a substance to attain the same reinforcing effect (Yokel & Wise, 1975; 1976). This position appears to have some face validity; however, a paradox emerges when the interpretation is examined carefully. If a substance is relatively more reinforcing, it would be predicted that the probability of the operant behaviour that resulted in the reinforcement should be increased. In the context of intravenous self-administration studies using FR schedules of reinforcement, an increase in the reinforcing effects of a substance should result in an increase in the rate of self-administration of the reinforcing agent. Conversely, if a substance becomes less reinforcing a reduction in the rate of drug injection would be expected. Wilson and Schuster (1973) have convincingly argued this point, which contrasts with the interpretation of Yokel & Wise (1975; 1976) and thus identifies a substantial flaw in any interpretation of the direction of change (increase or decrease) in the reinforcing efficacy of a substance resulting from experimental manipulations. The studies reviewed above have used fixed ratio reinforcement schedules, but have not used the traditional measure of rate of responding for each reinforcement, therefore it is

impossible to establish if a change in rate of drug self-administration reflects an increase or decrease in the reinforcing efficacy.

An additional issue that cannot be resolved by examining changes in the rate of self-administration in the context of fixed ratio reinforcement schedules, is the possibility that the reinforcing efficacy of a substance can be altered with no apparent change in the rate of self-administration. See the next section for a discussion of studies that have demonstrated alterations in the reinforcing properties of substances without finding changes in the rate of drug self-administration.

The investigations reviewed in the previous section have used fixed ratio schedules of reinforcement, and have predominantly measured rate of self-administration to examine changes in the reinforcing efficacy. These studies have provided valuable insight into which neurotransmitter systems play important roles in the mediation of the reinforcing effects of psychostimulants. Changes in the rate of self-administration can still be used as an indication of alterations in the reinforcing efficacy of the substance. However, conclusively establishing the direction of the changes requires a more sophisticated experimental paradigm.

The Progressive Ratio Schedule of Reinforcement

The progressive ratio (PR) schedule of reinforcement is an alternative to fixed ratio schedules of reinforcement that provides a measure of changes in the reinforcing efficacy of a substance. The traditional operant paradigm is maintained, however, the PR reinforcement schedule requires that an animal respond successively more for each infusion of reinforcer. For example, an animal might be required to make only one response to obtain the first drug infusion, yet two responses are required to obtain the next infusions and then 4, 8, 16 and 32 responses would be required to obtain the third to sixth responses, respectively. Therefore, animals must do more "work", operationally defined as lever responses, to obtain each successive infusion of drug.

Studies examining psychostimulants and food reinforcement have shown that animals responding on a PR schedule of reinforcement will readily complete the response requirements of the schedule when the ratio is relatively low. However, with successive infusions, the response requirements will increase to a point where the animal will eventually fail to respond sufficiently to obtain the next reinforcement. The last ratio completed is labelled the final ratio, and the number of reinforcements obtained is labelled the breaking point (BP) (Hodos, 1961; Bedford et al. 1978; Roberts et al. 1989). The greater the BP the more operant responses the animal had to produce in order to obtain the presentation of the reinforcer. The BP is generally considered to be an indication of the motivation of the animal to obtain an infusion of drug. Increasing the unit dose of a drug commonly results in an increase in BP (Roberts et al. 1989).

Rats responding for intravenous infusions of cocaine on a PR schedule of reinforcement demonstrate a pattern of drug intake similar to that observed in rats self-administering cocaine on a fixed ratio schedule. Rats will obtain an infusion of cocaine at evenly spaced time intervals and the rate at which cocaine infusions are obtained is inversely related to the unit dose of drug. Following each infusion of drug rats will cease responding for a period of time, then they will return to the lever and complete the ratio requirement to obtain the next drug infusion (Roberts and Richardson, 1992). Bedford, Bailey and Wilson (1978) have demonstrated similar findings in rhesus monkeys self-administering cocaine on a PR schedule of reinforcement. Risner and co-workers demonstrated comparable effects in dogs administering amphetamine and cocaine (Risner and Goldberg, 1993; Risner and Silcox 1981). In the latter two studies, the ratio requirements of the reinforcement schedule were increased at the start of each daily testing session and remained the same during that session. The general finding that increases in the unit dose of reinforcing agent resulted in increasing BP was still observed (Risner and Goldberg, 1993; Risner and Silcox 1981).

It is conceivable that the increased BP at higher doses of cocaine may be an artifact of the drug's promotion of behavioural stereotypy. However, the characteristic pause in responding following each infusion of drug argues against this position. If the infusion of cocaine were to promote stereotypy one would expect an increase rather than a cessation in lever responding following each infusion of reinforcer. A typical cumulative response profile of a rat responding for three doses of cocaine under a PR schedule of

reinforcement is presented in Figure 2. The post infusion pause is readily discernable from this record.

Additional studies have demonstrated that other substances with high abuse potentials such as heroin (Roberts and Bennett 1993); 3,4-methylenedioxymphetamine (MDA; Markert and Roberts 1991) and the indirect DA agonists GBR 12909 (Roberts 1993) will also be intravenously self-administered by animals responding on PR schedules of reinforcement.

Figure 2. Example of cumulative response patterns of rat responding for three doses of intravenous cocaine on a progressive ratio schedule of reinforcement. Arrows indicate time of injection. From Roberts et al (1989).

Progressive Ratio Schedule of Reinforcement:

Manipulations of Dopaminergic Systems

A number of studies have examined the effects of manipulations of DA systems on the BPs of rats responding for psychostimulant reinforcement under PR schedules of reinforcement. Pretreatment with the DA system antagonist haloperidol has been shown to result in a dose dependent decrease in the BPs of the animals, when compared with the BPs observed on baseline days prior to the pharmacological challenge (Roberts et al. 1989). Further, Hubner and Moreton (1991) observed decreases in the BPs of rats responding for cocaine following pretreatment with either the D₁ receptor antagonist SCH 23390 or the D₂ receptor antagonist spiperone. These studies support the position that pharmacological agents that compromise DA system function reduce the reinforcing effects of psychostimulants.

In another study, Roberts (1989) examined the effects of DA specific lesions of the nucleus accumbens on the BP's of rats responding for apomorphine. Following the DA lesions rats responded to significantly higher BP's, however no change in the rate of apomorphine SA was observed. This demonstrated that the reinforcing effects of a substance could be altered with affecting the rate of drug self-administration. The finding argues against the critical assumption of studies using FR reinforcement schedules: that a change in the reinforcing properties of a substance will be reflected in changes in the rate of drug self-administration.

Progressive Ratio : Pharmacological Manipulations of Serotonergic Systems

Several studies have used PR schedules of reinforcement to assess the effects of pharmacological manipulations of 5HT systems on cocaine self-administration. It has been shown that rats pretreated with either fluoxetine (Richardson and Roberts 1991) or L-tryptophan (McGregor et al 1993) demonstrate reduced BPs for intravenous cocaine infusions. L-Tryptophan pretreatment had no effect on rat's responding for food reinforcement in the same paradigm (McGregor et al 1993). In contrast, pretreatment of animals with the 5HT specific receptor antagonists MDL 72222 (5HT₃), ketanserin (5HT₂) and methysergide (5HT_{1,2}) did not alter the BPs for cocaine infusions (Lacosta and Roberts 1993).

The effects of clozapine on cocaine self-administration differs from other DA system antagonists and may be related to the effect on 5HT function. Clozapine is classified as an atypical neuroleptic that acts as a potent 5HT₂ receptor antagonist as well as having antagonistic properties at several DA receptor sites (D₁, D₂, and D₄ receptors). As noted earlier, if animals are pretreated with clozapine and allowed to self-administer cocaine on a FR reinforcement schedule, a negative relationship is observed between the dose of clozapine and rate of cocaine self-administration. In contrast, positive dose-response relationship is found when animals are pretreated with a variety of other neuroleptic compounds prior to cocaine self-administration. Increasing doses of clozapine produced a reduction in the rate of intake while all other neuroleptics tested produced an increase in the rate of self-administration (Roberts and Vickers 1984). An explanation for

the effects of clozapine could be determined from studies using FR reinforcement schedules.

However, when we examined the effects of clozapine using a PR schedule of reinforcement, a dramatic increase in rats' BPs for intravenous cocaine infusions was observed (Loh et al, 1992). This would suggest that rats were more motivated to obtain infusion of cocaine following clozapine pretreatment. In contrast, reduction in BP's for cocaine infusions are observed when rats are pretreated with other neuroleptics such as haloperidol (Roberts et al. 1989). It is possible that the antagonistic effects of clozapine at the 5HT₂ receptor contribute to the observed increases in BPs for intravenous infusions of cocaine. This is an important example of how the use of PR schedules of reinforcement can clarify findings from studies using FR schedules.

Progressive Ratio Schedule of Reinforcement: Serotonergic System Lesions

The definitive role of 5HT systems in cocaine self-administration was first demonstrated by Loh and Roberts (1990). In this study we used the PR schedule of reinforcement to investigate the effects of lesions to 5HT systems on cocaine self-administration. The effects of infusions of 5,7-DHT into either the medial forebrain bundle (MFB) or amygdala were investigated. Intracranial infusions 5,7-DHT into the MFB produced global forebrain reductions in both 5HT and the 5HT metabolite 5HIAA. In contrast, infusions of 5,7-DHT into the amygdala resulted in a selective reductions of 5HT in that region alone. A dramatic increase in the BPs for cocaine infusions was

observed in both groups of rats that received infusions of the neurotoxin (Figure 3). An illustration of the increase in BP is represented in Figure 4 which depicts the pre- and post-lesion cumulative response profile of a rat that received infusions of 5,7-DHT into the MFB.

Figure 3. Alterations in breaking points following serotonin-specific lesions to the medial forebrain bundle and amygdala of rats responding for cocaine on a progressive ratio schedule of reinforcement. Data points represent mean (SEM) changes in breaking points for each group expressed as a percentage of baseline testing.

Figure 4. Cumulative response profiles of a rat responding for cocaine following infusions of 5,7-DHT into the medial forebrain bundle. Prior to surgery, the rat obtained 13 infusions of cocaine during the testing session. The rat was required to perform 62 lever responses in order to obtain the last infusion of drug. However, following an infusion of 5,7-DHT into the medial forebrain bundle the same rat obtained 18 infusions of cocaine. In order to obtain the last infusion the rat was required to make 178 lever responses.

This was the first study to demonstrate that manipulations of neurotransmitter systems, other than DA systems, would alter the motivation of rats to obtain IV infusions of cocaine (Loh and Roberts 1990).

It is also important to note that no change in the rate of cocaine intake was observed. This again demonstrates that failure to find a change in rate of drug self-administration does not necessarily indicate that the reinforcing properties of a drug has not been affected.

A goal of this study was to identify the critical site of 5HT depletion responsible for the changes in self-administration. Rats receiving the 5,7-DHT infusions displayed a considerable variability in post lesion BPs (Loh and Roberts 1990). Some animals showed substantial increases in BP, while other animals failed to show a change from baseline. While 5HT specific lesions of amygdala resulted in substantial increases in BP in a number of animals, 5HT lesions of the MFB also produced similar changes in BPs. Correlation between changes in BP and degree of 5HT depletion did not suggest a preferential involvement of a particular neural region.

This study demonstrated that 5HT lesion of the amygdala as well as global forebrain 5HT depletions increase the BPs of rats responding for cocaine (Loh et al, 1990). However, it is unclear if lesions to any forebrain 5HT system would result in increases in BP for cocaine, or if the effect was the result of 5HT depletions of one

specific region. Consequently, the objective of the first of the series studies reported here was to attempt to identify specific neural regions that may have a preferential involvement in the mediation of serotonergic influences on the reinforcing efficacy of cocaine (see Experimental Investigations: Study 1).

The finding that lesions of 5HT systems will alter the reinforcing efficacy of cocaine begs the question of whether 5HT system lesions may also alter the reinforcing efficacy of other reinforcing substances. The finding that manipulation of 5HT systems can affect other reinforcing agents, such as food, would extend function of 5HT systems beyond the mediation of the reinforcing efficacy of powerful pharmacological agents to other reinforcing agents. In order to gain insight into this issue, the first study reported in Experimental Investigations section also examined the effects of serotonergic manipulations on rats responding for food reinforcement using a PR schedule of reinforcement.

Behavioural Effects of Psychostimulants

In addition to having reinforcing properties, psychostimulants produce a variety of changes in behaviour. By definition, the administration of psychostimulants will produce behavioural hyperactivity. However, the relationship between the reinforcing properties of psychostimulants and the behavioural hyperactivity produced by these substances remains unclear. It is common to find that experimental manipulations that alter the reinforcing properties of a psychostimulant will also affect the behavioural activating properties of that substance. However, it has yet to be determined if there is a relationship between behavioural activation produced by a psychostimulant and the reinforcing properties of that drug. The relationship between the reinforcing effects of a substance and the reinforcement process in general remains to be clarified. The following section reviews a number of studies that have examined behavioural stereotypy resulting from the administration of reinforcing drug. Studies 2-4 in the Experimental Investigations section examine common changes in behaviour that result from the administration of reinforcing drugs.

Behavioural Activating Properties of Psychostimulants

Randrup and associates (1963;1967) showed that beyond the behavioural hyperactivity produced by low doses of amphetamine, higher doses would produce behaviour "of grossly abnormal stereotyped character". Further they defined stereotyped behaviour as follows: "A response is stereotyped in character if it is performed repetitively, with little variation and without apparent purpose" (Randrup and Munkvad

1967). The description characterised changes in the behaviour resulting from the administration of relatively high doses of amphetamine and provided a framework for the systematic exploration of the behavioural effects of the drug. Several rating scales have been developed to quantify effects of amphetamine and related compounds. These scales outline a series of behaviours typically found when various doses of psychostimulants are given to animals. An example of a commonly used rating scale is presented in the Table 1.

Score	Identifier	Description
1	Asleep	Lying down, eyes closed
2	Inactive	Lying down eyes open
3	In-place activities	Normal grooming or chewing cage litter
4	Normal, alert, active	Moving about cage, sniffing, rearing
5	Hyperactive	Running movements characterized by rapid changes in position (jerky)
6	Slow patterned	Repetitive exploration of the cage at a normal level of activity
7	Fast patterned	Repetitive exploration of the cage with hyperactivity
8	Restricted	Remaining in the same place in cage with repetitive head and/or foreleg movements (includes licking chewing and gnawing stereotypies)
9	Dyskinetic-reactive	Backing up rearing, jumping seizures, abnormally maintained postures, dyskinetic movements

Table 1. Typical rating scale for behavioural stereotypy. Adapted from Ellinwood and Balster (1974).

With increasing doses of amphetamine, rats tend to exhibit behaviours outlined by higher scores on these rating scales. The rating scale is multivariate in nature, some behaviours elicited by lower doses of the drug will also be elicited at higher doses in conjunction with the emergence of more intense behaviours (Ellinwood and Balister 1974; Fray et al. 1980). The development of such rating systems provided a common measure to examine various aspects of behavioural stereotypy.

Dopamine system antagonists generally attenuate the stereotypy-producing effects of psychostimulants while DA system agonists will potentiate the development of stereotypic behaviours (Randrup and Munkvad 1967; Randrup et al. 1963). For a review see Waddington et al. (1990).

Exploration of Neural Regions That Mediate Stereotypic Behavioural Patterns

Of specific interest to this particular discussion, are studies that have attempted to identify neural sites that are preferentially involved in the mediation of the development of stereotypic response patterns. Creese and Iverson (1973;1974;1975) systematically examined the involvement of the forebrain DA systems in the mediation of the behavioural responses to amphetamine.

In their first study, intraventricular infusions of 6-OHDA in neonatal rats produced massive striatal DA depletions. The animals' response to amphetamine and apomorphine was tested at post natal day 90. Injections of apomorphine produced

substantially higher scores on a stereotypy rating scale when compared with the unlesioned controls. No stereotypical behavioural patterns were observed in the DA depleted animals when challenged with amphetamine, while stereotypical response patterns were observed in the control animals (Creese and Iverson 1973). These data demonstrate that forebrain DA systems play an important role in the mediation of stereotypical response patterns produced by the administration of DA system agonists.

In their second study, Creese and Iverson (1974) examined the effects of 6-OHDA infusions into the olfactory tubercle (tuberculum olfactorium) or caudate nucleus in adult rats. When challenged with injections of amphetamine, rats that received the DA system lesions in the caudate nucleus did not show stereotypical response patterns while such changes in behaviour were still evident after DA specific lesions of the olfactory tubercle. Increases in locomotor activity in response to low doses of amphetamine were apparent in both groups of rats.

In a third study, the effects of 6-OHDA infusions into the substantia nigra were examined (Creese and Iverson 1975). The lesions, which produced a 99% reduction in tyrosine hydroxylase activity in the nigrostriatal pathway and attenuated the stereotypic response to amphetamine. Further, an enhanced response to apomorphine was observed, suggesting a post synaptic DA receptor super-sensitivity. Animals receiving the DA system lesions did not show increased locomotor activity when challenged with amphetamine which was in contrast to the control animals that demonstrated a

considerable increase in locomotor activity. The authors also demonstrated that 6-OHDA lesions of dorsal and ventral NE pathways did not effect the stereotypical response patterns produced by amphetamine.

The role of the NAS in the mediation of amphetamine stereotypy was also examined in a study by Kelly and coworkers (1975). The effects of 6-OHDA infusions into the NAS and caudate nucleus were evaluated. Rats receiving DA specific lesions of the caudate nucleus did not demonstrate stereotypical response patterns when challenged with amphetamine, while rats receiving the DA lesions of the NAS lesions displayed a normal response. When locomotor activity was examined, the opposite was found. Lesions of the NAS resulted in an attenuated locomotor response to amphetamine which was not found in the caudate lesioned animals (Kelly et al. 1975).

Together these studies demonstrated that the nigrostriatal DA pathways are important in the mediation of stereotypical response patterns resulting from the administration of amphetamine. Dopamine systems of the NAS are important in the mediation of a heightened locomotor response resulting from lower doses of the drug (Kelly et al. 1975). Studies examining the reinforcing and stereotypy producing properties of psychostimulants have found that DA systems are in important in the mediation of both effects.

Environmental Influences on Stereotypical Behavioural Patterns

Studies have demonstrated that the stereotypical response patterns produced by amphetamine are dependent upon the environment in which the animal is tested. Ellinwood and colleagues demonstrated that amphetamine promotes behaviours that are largely determined by the behaviour being displayed at the time of drug onset (Ellinwood and Kilbey 1975). Their findings were confirmed in a number of experimental situations (Randrup and Munkvad 1967; Szechtman et al. 1988). In one study, Beck and associates systematically measured a wide variety of behaviours of rats pretreated with amphetamine. The stereotypic response behaviour was found to be influenced by the initial environment in which the rats were placed. However, subsequent changes to this environment had little effect of the stereotypical response patterns initially demonstrated (Beck et al. 1986). Other studies have demonstrated that the administration of psychostimulants leads to improvements on tasks that require repetitive responding (Collins et al. 1979; Lyons and Robins 1975).

These studies indicate that stereotypical response patterns resulting from injections of amphetamine are not simply a result of activation or hyper-activation of motor systems. The response pattern exhibited is sensitive to environmental cues that are present during the onset of the effects of the drug. This suggests that stereotypical response patterns are not "hard-wired" patterns of activity but may have some functional importance in the natural behaviour of animals. While it would appear that almost any behaviour could become stereotyped, the term stereotypy is commonly associated with a

standard pattern of behaviours such as those listed in Table 1 . For the purposes of this discussion the term "perseveration" will be used to identify any series of repetitive behaviours.

Lyons and Robbins Hypothesis

In their classic paper, Lyons & Robbins (1975) described amphetamine induced stereotypy as "an increase in response rates in a decreasing number of behavioural categories". Some behaviours become more frequent and persistent at the expense of others, and at higher doses certain behaviours cease to be exhibited, while the frequency of other behaviours increases. It was explicitly noted that amphetamine does not stimulate behaviour that is not already present in the animal's behavioural repertoire (Lyons and Robbins, 1975; Robbins et al. 1990). This change in behavioural response pattern would lead to a reduction in behavioural variability and a perseveration of specific ongoing behaviours.

Studies examining behavioural stereotypy produced by drugs such as amphetamine have focused on changes in the behavioural repertoire of the animal. The idea that behavioural perseveration might be directly related to reinforcement mechanisms is seldom considered. One hypothesis is that perseveration is a consequence of a high level of reward. A series of experiments examining this possibility is presented as Study 2 in the next section. These studies incorporated a modified version of the RAM paradigm to examine the issue.

It was noted earlier that DA specific lesions of the caudate nucleus attenuate amphetamine stereotypy, while lesions of DA systems in the NAS attenuate the reinforcing and locomotor activating properties of the psychostimulants. The third study reported in the Experimental Investigations section examined the relative contribution of DA systems of the NAS and caudate nucleus to the expression of behaviours that may be related to both stereotypy and reinforcement.

The Modified Radial Arm Maze Paradigm

The traditional RAM paradigm requires that rats explore the arms of a radial maze for food reinforcement. The maze consists of a central hub with eight or 12 equally spaced pathways extending away from the hub. Food is placed at the ends of all, or in only some, of the arms. Since each arm is baited only once, a rat is required to develop a strategy of arm exploration that maximizes the probability of obtaining food reinforcement. If a rat enters a previously entered arm it will not receive additional food reinforcement. This paradigm has proven to be useful in evaluation of manipulations that may effect learning and memory (Olton et al. 1977).

In contrast, the RAM paradigm used in the present studies requires that each arm of the maze to be continually rebaited with food (Devenport and Merriman 1983; Loh and Beck 1989). Food deprived rats are allowed to explore the maze for food reinforcement. Since a rat will receive the same amount of food reinforcement irrespective of its pattern of exploration, animals did not develop a rigid pattern of maze exploration. By evaluating

the order in which a rat explores the arms of the maze it is possible to determine if experimental manipulations effects the pattern of maze exploration. In general, drug naive rats do not demonstrate a specific bias towards any arms of the maze or develop a specific pattern of exploration. However, a perseveration in the pattern of maze exploration will emerge if the rats are pretreated with a number of pharmacological agents.

Behavioural perseveration in the radial arm maze: effects of ethanol and diazepam

It has been demonstrated that both ethanol and diazepam alter the behaviour of animals exploring a RAM for food reinforcement (Devenport and Merriman 1983; Loh and Beck 1989; Beck and Loh 1990). Pretreatment of rats with either ethanol or diazepam produce a dose-dependent reduction in the frequency of non-goal directed behaviours (i.e., grooming, rearing, sniffing, and scratching). As the frequency of these behaviours decreased, the pattern of maze exploration became more rigid (Figure 5). Animals developed biases in the direction of turn made when leaving an arm of the maze. While Lyons and Robbins (1975) have described similar changes in behaviour as stereotyped, the term "perseveration" is used to described the repetitive behaviour of the rats exploring the RAM.

When animals exploring the RAM were pretreated with either ethanol or diazepam, a perseverative pattern of turning that consisted of limiting the direction of turn to a few specific angles was found. This "turning bias" could not be accounted for by a bias towards a specific arm (Loh and Beck 1989). These data suggest that both ethanol

and diazepam produce a perseveration in turning behaviour and a concomitant reduction in variability of maze exploration behaviours. Since these changes in exploratory behaviour did not alter the amount of food reinforcement received, the data indicated that both ethanol and diazepam produce similar alterations in behaviour that are not related to the maximization of food reinforcement. Findings from these studies indicate that the variability of an animal's behaviour observed when exploring a radial arm maze for food reinforcement is reduced when given non-contingent injections of alcohol or diazepam. (Loh and Beck 1989).

Figure 5. Changes in the route of radial maze exploration following injections of diazepam or ethanol. H2 is a descriptive noninferential statistic that provides a measure of the degree of uncertainty of a specific arm entry given a previous arm entry. Calculation of the measure is described in Frick and Miller (1951).

The alterations in behaviour resulting from ethanol or diazepam pretreatment also closely resemble the description of the amphetamine induced changes in behaviour outlined by Lyons and Robbins (1975); "an increase in response rates in a decreasing number of behavioural categories". Increases in turning bias were observed with concomitant decreases in the frequency of other exploratory behaviours (Loh and Beck 1989). It is possible that changes in behaviour as outlined by Lyons and Robbins (1975), and those found in the RAM studies (Loh and Beck 1989) occur with the administration of other reinforcing agents as well. The behavioural effects of other pharmacological agents with high abuse potentials and non-reinforcing agents are examined in Study 2 presented in the next section.

Experimental Investigations

Study 1:

Effects of Lesions to Central Serotonin Systems on Responding On A Progressive Ratio Schedule Reinforced with Cocaine Or Food ¹

Introduction

We previously reported that if 5,7-DHT is infused into either the MFB or the amygdala rats respond to higher BPs on a PR schedule for cocaine reinforcement (Loh and Roberts 1990). While increases in BP's were observed, correlations between changes in BP and the degree of 5HT depletion in a number of neural regions did not indicate a specific neural site that mediated the effect (Loh and Roberts 1990).

The present study re-examined the issue by evaluating the effects of global 5HT depletions by the use of intraventricular infusions of 5,7-DHT. The BPs of rats self-administering cocaine on PR schedule of reinforcement was assessed before and after the 5HT depletions. After behavioural testing, the level of 5HT depletion in a number of neural regions was assessed and correlated with changes in BPs resulting from the 5,7-DHT infusions. A relatively high correlation between changes in BPs and 5HT depletion of a neural region would suggest that 5HT systems in that region may have preferential involvement in the mediation of the reinforcing properties of cocaine.

¹ Results of this study have been published, see reference: Roberts, Loh, Baker and Vickers (1994)

The effects of 5HT depletions on food reinforcement was also examined to determine if the observed changes in BP were specific to cocaine or if the effect could be generalized to other reinforcing agents. The methodology of this experiment matched that of the cocaine self-administration study, with the exception that rats were trained to respond on a PR schedule for food reinforcement instead of intravenous infusions of cocaine. Rats were also tested in sessions in which no food was available (extinction trials).

If the 5HT lesions were found to produce an increase in BPs for food reinforcement, in addition to cocaine infusions, it would suggest that effects of 5HT depletions may be generalized to other reinforcing agents.

Methods

Male Wistar rats were purchased from Charles River Farms (Quebec) at an initial weight of 275-300 g and were immediately quarantined for one week. Rats were maintained on a reverse 12 hour day/night cycle during the study.

Cocaine self-administration study

Rats were food deprived for 24 hours, and trained to press a lever for food reinforcement on an FR 1 schedule. Food was then available ad libitum for the remainder of the study. Each rat was implanted with a chronically indwelling sytastic jugular cannula that exited at the mid-scapular region (Roberts and Goeders 1991). Following cannulation,

each rat was singly housed in 50 cm X 50 cm by 40 cm (h) operant testing apparatus. The jugular cannulae was attached to a counterbalanced swivel apparatus which allowed free movement in the operant chamber.

Rats were allowed access to a lever for a five hour period each day. Initially, each response on the lever activated a syringe driver which delivered 0.1 ml of saline solution containing 0.5 mg of cocaine HCl over a 5 sec. period. This was roughly equivalent to a unit dose of 1.5 mg/kg/injection dose of cocaine. Concurrent with the start of the injection a stimulus light in the testing chamber was activated for 20 seconds. The light signalled a post infusion time-out period when lever responses had no programmed consequence. When the rats had developed a constant rate of responding, which was indicated by the receipt of an infusion of cocaine every 7-10 minutes, a PR schedule of reinforcement was introduced.

The demand characteristics of the PR schedule of reinforcement require an increase in the number of responses to obtain each additional infusion of cocaine. At the start of each daily session the PR schedule was reset to 1. The response requirements for subsequent infusions increased according to the following progression: 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 603. This sequence is derived from the equation: ratio requirement = $5 \times e^{(0.2 \times \text{infusion number})} - 5$ (rounded to the nearest integer).

Typically rats would respond during the first three hours of the session but fail to respond during the last two hours. A rat was assumed to have reached BP if it did not respond sufficiently to obtain an infusion for a one hour period.

If the daily number of infusions did not vary by more than ± 2 BPs for a period of three days, animals were prepared for stereotaxic injections of 5,7-DHT (see below). Following surgery, rats were allowed to recover for one day before being given access to cocaine. Rats were permitted to self-administer cocaine on the PR schedule of reinforcement for seven days following surgery.

Food reinforcement study

Following quarantine, this group of rats was maintained on a food restricted diet (12-15 g of Purina Rat Chow per day) which resulted in a weight gain of 1-2 g/day. Rats were individually housed in opaque plastic tubs except when being tested. The operant testing chambers used for this study were identical to those used to chronically house the rats in the cocaine reinforcement study. Rats were trained to press a lever for food reinforcement using the same PR schedule described above. Completion of each ratio requirement of the schedule, resulted in the delivery of one 45 mg food pellet (Noyes Co., Lancaster, PA). A five second time-out period was signalled by the activation of a stimulus light. The breaking point was defined as the ordinal value of the final ratio achieved prior to either a 10 min period of non-responding or failure to obtain a food pellet within 20 min.

After five days of stable responding, rats were prepared for stereotaxic surgery as described below. Following surgery, animals were allowed free access to food for a 24 hour recovery period and then again placed on the food restricted diet. Animals were tested for a period of 10 days. Rats were also tested for a period of four days during which no food was delivered when the rats completed the ratio requirements of the PR schedule (extinction testing). The stimulus light that indicated the time-out period was activated for a five second period during these testing sessions. Three additional "control" animals were submitted to all testing procedures but did not receive any surgery.

Surgery

Thirty min. prior to surgery animals were injected with imipramine (Sigma Chemicals, St. Louis; 25 mg/kg, i.p.), then anaesthetized with halothane (Fluothane, Ayerst, Montreal). Animals received bilateral infusions (2 μ l) of either 5,7-DHT (Sigma) or the ascorbic acid vehicle solution. A 5.0 mg/ml solution of 5,7-DHT was prepared by dissolving the neurotoxin in a saline solution containing 0.2% ascorbic acid. The 5,7-DHT and vehicle solutions were infused into the lateral ventricles by stereotaxically positioning a 22 gauge cannula at the coordinates :AP -0.8, DV -3.2, ML $\pm$ 1.3 (nose bar -4.2; flat skull orientation) relative to bregma. Once the cannula had been positioned at the appropriate location, a length of plastic tubing that was attached to the cannula was lifted above the level of the head of the animal. This allowed the solution to slowly drain into the ventricles. The amount of solution entering the ventricles was determined by the movement of a small air bubble positioned in the tubing. Once the air bubble had moved a

predetermined distance, indicating a 2 μ l infusion, the tubing was then lowered to stop the infusion process. The cannula was left in place for approximately one minute following each infusion.

Biochemical analysis

Following behavioural testing, rats were decapitated and their brains were removed and quickly dissected on ice. Samples were then frozen in liquid nitrogen for subsequent biochemical analyses. Six specific regions were dissected out: cortex, caudate nucleus, NAS, mesencephalon amygdala, and hippocampus. An additional group of rats (N=3) that did not receive intraventricular infusions was sacrificed to serve as an additional comparison group. This group did not participate in the behavioural testing.

After homogenizing in ice-cold 0.1 M perchloric ascorbic acid (0.05 mM) and EDTA (10 mg%), samples were then centrifuged to remove precipitating protein and the supernatants were used for analysis of 5HT content by high performance liquid chromatography using a modified version of the procedure described in Baker et al. (1987). Biochemical analysis of the samples was conducted by G. Baker, Neurochemistry Research Unit, Department of Psychiatry, University of Alberta.

Results

Cocaine self-administration

Ten rats in the 5,7-DHT group and eight vehicle rats completed the experiment. Figure 6 shows the daily BPs for each group before and after the lesion. Daily BPs of the groups were compared by a repeated measures ANOVA (SPSS-pc, version 3.0). No significant differences were found between the groups of rats on the three baseline days ($F[1,16] = 1.74, p > .05$). Analysis of the post surgical data revealed a significant group by day interaction ($F[6,96] = 2.38, p \leq .05$).

The 5,7-DHT lesioned rats demonstrated an overall average increase in BP of 133% on post-surgical testing days. In contrast, the overall performance of the rats that received the ascorbic acid infusions declined to 95% of control values. Considerable variability in the post-5HT lesion change in BP was observed.

Figure 6. The effects 5,7-DHT lesions on BPs established under a PR schedule of reinforcement for IV cocaine reinforcement. Each point represents the daily mean ($\pm$ SEM) BPs for groups of rats that have received intraventricular infusion of either 5,7-DHT (N = 10) or vehicle (N = 8). Three baseline days are shown followed by data from 7 consecutive post-lesion days beginning 2 days after surgery.

Biochemical results

HPLC analysis of individual brain regions for both 5,7-DHT lesion group are shown in Table 2. A total of five animals from the vehicle control group were assayed along with seven additional non-lesioned animals. Since no differences were observed between these control groups, the data were combined. Compared with the control group, 5HT content was significantly reduced in all brain regions from the 5,7-DHT group. The hippocampus demonstrated the largest depletion (89.5 %), while the remaining regions showed more modest reductions (range 13.8 to 34.3%).

Regional Serotonin Content (ng/g)						
	CTX	Caud	NAS	MES	HIPPO	AMY
Cocaine Reinforcement						
Control (SEM) n=12	199.1 ± 11.8	403.5 ± 22.9	644.3 ± 58.2	506.0 ± 31.3	250.4 ± 21.1	331.6 ± 28.9
5,7-DHT (SEM) n=10	140.3 ± 17.7	268.3 ± 41.8	555.5 ± 81.3	332.4 ± 43.0	26.2 ± 4.6	233.9 ± 40.7
% control	70*	66*	86	65*	10*	71
Food Reinforcement						
Control (SEM) n=12	205.2 ± 10.6	506.0 ± 31.6	593.4 ± 36.6	514.5 ± 25.9	154.4 ± 11.9	539.2 ± 39.0
5,7-DHT (SEM) n=9	38.4 ± 7.0	177.2 ± 33.3	252.4 ± 45.9	316.5 ± 20.2	1.6 ± 1.2	81.9 ± 20.0
% control	18**	35**	42**	61**	1**	15**

Table 2. Effect of intracerebral 5,7-DHT infusions on 5HT concentrations in various brain regions . Numbers represent the concentration of 5HT (± SEM). The percentage reduction in 5HT content relative to the control animals is also shown.

Abbreviations: cortex (CTX); caudate nucleus (CAUD); nucleus accumbens septi (NAS); mesencephalon (MES); hippocampus (HIP) and amygdala (AMY) brain regions. Significant differences between lesion and control groups indicated by *: $p \leq .05$; **: $p \leq .001$.

Comparison of biochemical and behavioural data

To determine the relationship between the behavioural data and the effects of the 5,7-DHT infusions, the mean post surgical percentage change in BP was compared with 5HT content of each of the brain regions. Serotonin content of the mesencephalon was best correlated with post surgical changes in BP ($r = -0.45$, $p \leq .05$). All other correlations were not statistically significant.

Food reinforcement

Repeated measures ANOVA of pre- and post surgery (Days 1-10) BPs receiving either no surgery ($N = 3$) or ascorbic acid infusions ($N = 9$) demonstrated no differences between groups. Data from these two groups were combined for further analysis. For the following discussion of the results, the combined data are referred to as the Control group.

Repeated measures ANOVA comparison of the baseline BPs of the Control and 5,7-DHT lesion groups revealed no statistical differences. Analysis of group differences in BPs on post surgical testing days 1-10 revealed a significant group by day interaction $F[9,171] = 3.65$, $p \leq .001$. Newman-Keuls analysis of the daily BPs converted to percentage of baseline values, revealed significant increases on post-surgical testing days five, six and eight ($p's \leq .05$) when compared with the Control group (Figure 7).

Comparison by ANOVA of group differences in BPs the day that rats had free access to food and 24 hr food deprivation revealed no significant differences in BP.

Repeated measures ANOVA analysis of the four extinction days of testing revealed a significant effect of day of testing $F[3,57] = 20.47, p \leq .001$. Student *t*-tests of the individual days revealed a significant difference between the two groups on the fourth day of extinction testing, $t[19] = 2.84, p \leq .01$, (Figure 8).

Figure 7. The effects of 5,7-DHT lesions on BPs established under a PR schedule for food reinforcement. Each point represents the daily mean ($\pm$ SEM) BPs for groups of rats ($N = 12$) that have received intraventricular infusion of either 5,7-DHT or vehicle. Three baseline days are shown, followed by data from 10 consecutive post-lesion days beginning 2 days after surgery.

Figure 8. The effects of 5,7-DHT lesions on responding during behavioural extinction.

Animals were trained to respond on a PR schedule for food reinforcement. During extinction testing, the pellet dispenser discharged upon completion of ratio of the PR sequence, although no pellet was delivered. Points represent the mean ($\pm$ SEM) for the groups of rats that received either 5,7-DHT or vehicle expressed as a percent of baseline of responding. Asterisk indicates significant differences between groups.

Biochemical analyses

Three samples were unavailable for analysis (one NAS sample and a caudate nucleus sample from the Lesion group, and one hippocampus sample from the Control group). Comparison of Control and 5,7-DHT lesion groups by multiple *t*-tests indicated that all brain regions in the lesion group showed statistically significant reductions in 5HT content; the smallest $t = 5.70$, $p \leq .001$ (see Table 2).

Comparison of behavioural and neurochemical results

Correlations between the percentage of baseline change in BPs and the content of 5HT of the various brain regions that were evaluated. In the group of animals that responded for food reinforcement, increases in BPs were statistically significantly correlated with decreases in 5HT content in the regions of the mesencephalon ($r = -0.49$; $p \leq .05$), cortex ($r = -0.46$; $p \leq .05$), and the amygdala ($r = -0.46$; $p \leq .05$).

Discussion

In a previous study (Loh and Roberts 1990), we reported that groups of rats receiving 5,7-DHT infusion into either the medial forebrain bundle or the amygdala responded, on average, to significantly higher BPs when compared with the control group. However, several aspects of our previous study suggested that further characterization of the phenomenon was necessary. Of particular concern was the large variability within the 5,7-DHT groups. Some rats showed dramatic increases in BP values after the lesion, although others continued to respond at prelesion levels. Reductions in 5-HT content within NAS, amygdala and cortex were observed, but the behavioural effect did not appear to correlate with 5-HT depletions in any of these areas. In the present study, we examined whether cocaine self-administration might be similarly affected by intracerebral infusion of 5,7-DHT, and if so, whether the behavioural effect could be linked to the pattern of 5-HT depletions. The results show that 5,7-DHT (ICV) produces increases in BPs on a PR schedule for cocaine reinforcement.

Again, the effect was not observed in all animals. Six of the 10 animals showed a clear increase, elevating their responding by up to seven BPs (corresponding to an increase in total responses ranging from 94 to 894 presses). An attempt was made to account for this variability by examining the pattern of neurochemical depletions produced by the neurotoxin. A small but significant correlation was found between depletions of 5-HT in the midbrain and increases in responding. Depletions of 5-HT in the NAS and amygdala did not correlate significantly with the behavioural data.

The present data are consistent with our previous report (Loh and Roberts 1990).

One interpretation of the data is that the action of cocaine on 5-HT systems may be somewhat aversive, with the result that the motivation to self-administer cocaine increase following the removal of 5-HT systems. This suggestion is consistent with other 5-HT lesion studies that have reported increases in the rate of amphetamine intake following either intraventricular injections of 5,7-DHT (Lyness et al. 1979) or 5,7-DHT lesions of the medial forebrain bundle (Lyness et al. 1980). Conversely, the motivation to self-administer cocaine appears to diminish following stimulation of 5-HT receptors.

Fluoxetine (Richardson and Roberts 1991) and L-tryptophan (McGregor et al. 1993) pretreatments significantly decrease the BP of rats self-administering cocaine on a PR schedule of reinforcement. In addition, a decrease in the rate of both cocaine and amphetamine intake has been reported following either fluoxetine (Carroll et al. 1990; Kleven and Woolverton 1993; Leccese and Lyness 1984; Porrino et al. 1989; Yu et al. 1990), dietary L-tryptophan (Carroll et al. 1990; Smith et al. 1986), systemic L-tryptophan, or quizapine pretreatments (Lyness 1983; Lyness et al. 1980).

However, not all data support the idea of a specific role for 5-HT in cocaine reinforcement. For example, it might be predicted that the blockade of 5HT receptors would enhance the motivation to self-administer psychomotor stimulants by attenuating the hypothesized adverse effect. This prediction is not supported by experimental evidence. Lacosta and Roberts (1993) reported that cocaine self-administration on a PR schedule is unaffected by MDL 72222, ketanserin, or methysergide (5-HT₁, 5-HT₂ and a

nonspecific 5HT agonists respectively). These findings are consistent with those of three other studies that report ritanserin (a 5-HT₂ receptor antagonist) or GR23023F (a 5-HT₁ receptor antagonist) pretreatments fail to alter the rate of cocaine self-administration in rats (Lane et al. 1992; Peltier and Schenk 1991).

To assess whether the increase in BPs was due to a specific alteration in cocaine reinforcement, we examined the effect of 5,7-DHT lesions on responding for food on a PR schedule of reinforcement. The post-surgical changes closely paralleled those observed in rats responding for cocaine reward (Figure 8). This suggests that the lesion effect generalizes to at least one other reinforcer. Previous reports have shown that 5HT depletion (Serbro and Lorens 1975) or pharmacological blockade (Beninger and Phillips 1979; Rosen and Cohen 1973) causes perseveration. These data, together with the demonstration that the 5,7-DHT-lesioned animals show elevated responding during extinction, indicates that the 5HT depletions may have produced a general increase in motivation or inability to withhold responding. It would appear that 5,7-DHT lesions produce a general alteration in behaviour rather than a specific change in cocaine reinforcement mechanisms.

The 5HT depletions produced by the neurotoxin deserve comment. 5,7-DHT caused dramatic reductions in 5HT content in all animals from the food reinforcement study; however, the depletions from the animals in the cocaine self-administration experiment were less striking. Although the cocaine would have cleared from the system

by the time of the 5,7-DHT injections it is likely the chronic cocaine has an effect on the cocaine transporter mechanisms, thus imparting a partial protective effect against the neurotoxin. We have observed this effect previously (unpublished observations) and it requires further study.

In summary, we have replicated earlier work showing 5-HT depletions cause an increase in BP on a PR schedule of reinforcement and we have extended this finding to a second reinforcer. The data show that support for a specific role for 5-HT in the reinforcing effects of cocaine is equivocal and alternate explanations should be considered. Finally, correlational data indicate that the effect is most closely associated with depletion of mesencephalic 5-HT.

Study 2

Evaluation of response perseveration of rats in the radial arm maze following reinforcing and non-reinforcing drugs²

Introduction

A number of studies have examined the behavioural consequences of the administration of amphetamine. Lyons and Robbins (1975) described amphetamine induced stereotypy as "an increase in response rates in a decreasing number of behavioural categories". Some behaviours will become more frequent and persistent at the expense of others, and at higher doses certain behaviours will cease to be exhibited while the frequency of other behaviours will increase. If animals are pretreated with either ethanol or diazepam, similar changes in behaviour are observed (Devenport and Merriman 1983; Loh and Beck 1989) these changes have been labelled behavioural perseveration.

Studies examining the behavioural effects of psychoactive drugs, such as amphetamine, usually examine behavioural stereotypy resulting from high doses of the psychostimulant. Less emphasis has been placed upon the examination of behavioural effects of lower doses of these substances. When examining the behavioural effects of lower doses of a drug one is faced with the problem of quantifying more subtle alterations in behaviour. Further, it is often difficult to examine the effects of various classes of pharmacological agents in the same paradigm. The modified RAM paradigm lends itself to

² Data from this study has been published in Loh et al. (1993).

the examination of a wide variety of pharmacological agents.

It is possible that perseveration of specific ongoing behaviours, with a concomitant reduction in behavioural variability, may be a primary consequence of the administration of a number of drugs with high abuse potential. The following studies examined whether a variety of psychoactive drugs that have been shown to have reinforcing properties (amphetamine, nicotine and heroin), can produce perseveration in the behaviour of rats exploring the RAM for food reinforcement. The effects of two psychoactive drugs that are not considered to have reinforcing properties (haloperidol and scopolamine) were also assessed.

Previous research has shown that a reduction in behavioural variability and perseveration in specific behaviours can be measured within the modified RAM paradigm (Beck and Loh 1990; Devenport and Merriman 1983; Loh and Beck 1989). In these studies, a large number of specific behaviours were quantified. The administration of either drug resulted in an increased perseveration in the angle of turn that an animal made when exiting an arm of the maze. This behaviour was found to be a good reflection of other changes in other behaviours. Therefore, in the present study perseveration in angle of turn was used as a representative measure of a reduction in behavioural variability.

Methods

Animals

Male Wistar rats (Woodlyn Farms, Ontario and Charles River Farms, Quebec), weighing 250 - 300 g at the start of the experiment, served as subjects. Upon arrival from the supplier, rats were housed individually and maintained on a reverse 12 h dark/light schedule (lights on at 9:00 pm). Following at least one week with *ad lib.* access to food and water, food was restricted to 15 grams per day. This reduced the average daily weight gain to 1-2 grams.

Apparatus

Two eight arm radial mazes were used for these studies. The floor of the first maze was constructed from plywood with an arborite surface. The arms (80 X 9 cm) extended from a central octagonal hub measuring 25 cm in diameter. The sides of the arms (25 cm) and the top of the maze were made of 2.5 cm² wire mesh. A second maze had plexiglass sides and a stainless steel bottom. In both mazes a small food cup, that was connected to an electronic pellet dispenser, was positioned at the end of each arm. The first maze required that an experimenter be present in the maze room during testing. The experimenter activated the pellet dispensers and recorded the arm entries. Arm entries in the second maze were detected by weight sensors located in each arm and in the centre of the maze. This information was automatically recorded by a computer. Both mazes were positioned on the floor in the centre of a testing room that was illuminated by a single 15 watt bulb located on one wall. Large objects positioned near or on the walls could

presumably be used by the animal as spatial cues.

Testing procedure

Habituation: Rats were permitted to explore the radial maze for a daily thirty minute period for three consecutive days. During these habituation sessions, rats consumed the food pellets (45 mg Noyes pellet) which were located in the food receptacles at the end of each arm. All food receptacles were frequently replenished during each 30 min. habituation session. This procedure gave the rats the opportunity to learn that returning to a once-visited arm would lead to additional food reinforcement.

Baseline testing

During the three baseline testing sessions, each rat was injected with saline (1.0 ml/kg) and returned to its home cage. After a period of time which corresponded to the drug pretreatment interval (see below), the rats were placed within a large cardboard cylinder in the central hub of the maze. After a moment, the cylinder was lifted and the rat was allowed to search the maze for food. A rat was permitted to explore until it had completed 25 arm entries. Once a rat obtained the food reward from a particular arm and had re-entered the central hub of the maze, the food pellet was replenished. This testing procedure placed no restrictions on the route of exploration the rat selected. A rat was deemed to have made an arm entry when all four paws were situated in the arm.

*Drug testing**Amphetamine and heroin*

Drug testing days immediately followed baseline training. For the next three days, each group of rats was injected i.p. with one of the following doses of amphetamine HCl: vehicle, 0.25, 0.5, 1.0, 2.0 mg/kg, or heroin HBr (N = 8): vehicle, 0.25, 0.5, 1.0, 2.0 mg/kg (N = 8) 30 min prior to testing. Dosages are expressed as the salt. These drugs were dissolved in 0.9% saline and injected in a volume of 1.0 ml/kg.

Nicotine, haloperidol and scopolamine

For the trials using nicotine, haloperidol and scopolamine, rats were tested for one day with the appropriate dose of drug. Rats were injected with nicotine s.c. at doses of: vehicle, 0.1, 0.2, and 0.4 mg/kg, 30 min prior to testing (N's = 23, 9, 23, 13 respectively). Four doses of haloperidol (vehicle, 12.5, 25 and 500 µg/kg; 2 hours prior to testing, i.p.; N's = 23, 6, 6, 17 respectively) and scopolamine (vehicle, 0.1, 0.2 and 0.4 mg/kg; 20 minutes prior to testing, i.p.; N's = 21, 11, 10, 10 respectively) were also tested. Different dosages were tested over a number of replications each containing a saline control group. Data from the rats tested as the control group (vehicle) for a particular drug were pooled for analysis of the effect of that drug. Rats receiving vehicle injections had been exposed to the maze for the same number of days as the groups receiving the drug.

Data analyses

Two summary measures were developed to evaluate behavioural biases: (1) the distribution of arm entries into specific arms and (2) distribution of directions of turn when leaving an arm. The sequence of arm entries made in each trial was used to determine these measures.

Arm bias:

A summary score of arm bias was calculated as:

Equation 1:
$$\sqrt{\sum \left[\frac{\text{arm}_n}{25} \right]^2}$$

where arm_n is the number of entries into a particular arm.

A higher arm bias score indicates a higher frequency of entries into a fewer number of arms. The minimum value that can be obtained is 0.356 reflecting a uniform distribution of arm entries, whereas a maximum score of 1.00 would indicate perseverative re-entry into a single arm.

Directional bias:

When leaving an arm, a rat has the option to either re-enter that arm again (0 degree turn) or enter any of the remaining seven arms each of which represents a different direction of turn (0, 45, 90, 135, 180, 225, 270, or 315 degree turns). A summary score

indicating a specific bias in the direction of turn made by a rat when exiting one arm and entering the next was calculated as follows:

Equation 2:
$$\sqrt{\sum \left[\frac{\text{turn}_n}{24} \right]^2}$$

Turn_n is the number of turns made at a particular angle. Scores based on 25 arm entries (24 turns) and can range from 0.354 to 1.0. Increases in preferential turning bias are indicated by higher scores.

To illustrate how the scores reflect biases in the data, Figure 9 shows three histograms which represent possible distributions of arm entries or turns displayed during 25 trials along. The calculated bias scores also shown. Note that when the choices are evenly distributed the bias score is low, whereas an uneven distribution is reflected in higher score. It should be noted that the bias measures are not orthogonal because a strong arm bias could lead to a high directional bias score.

Figure 9. Examples of distribution of arm bias and turning bias scores. Top: typical baseline distribution of arm entries. Middle: Typical baseline distribution of direction of turn. Bottom: Typical distribution of direction of turn following an injection of 1.0 mg/kg amphetamine. Numbers represent calculated directional and arm bias scores.

Results

Directional bias and arm bias scores were analyzed by separate repeated measures analyses of variance. In cases in which rats received amphetamine or heroin, bias scores obtained on the three days when the rats were tested with drug were compared with the three previous baseline days. If rats were pretreated with a drug on only one day (nicotine, haloperidol and scopolamine), the last day of baseline testing was compared with bias scores obtained on the following day when the animal was tested with drug. In the following report of data analysis, "Drug" refers to a independent repeated measure reflecting baseline days vs days of testing when animals received a drug pretreatment. For the amphetamine and heroin groups, the mean of directional and arm bias scores obtained on the three days of testing with drug pretreatment was used in the Newman-Keuls analysis. For *post-hoc* analyses statistically significant values are reported at the 0.05 level.

Amphetamine pretreatment:

Repeated measures analysis of the directional bias measure indicated a significant Drug by Dose interaction $F[4,35] = 6.23$ $p \leq 0.01$, and a significant interaction between Drug and Day of testing $F[2,70] = 4.77$ $p < .05$. Subsequent Newman Keuls analyses indicated a statistically significant directional bias in groups of rats that were pretreated with amphetamine when compared with the control group (Figure 10). Pair-wise t -tests demonstrated that the groups receiving 0.25, 0.50 or 1.0 mg/kg of amphetamine demonstrated significantly higher turning bias scores than during baseline testing.

Analysis of the arm bias measure demonstrated a significant Drug by Day interaction $F[2,70] = 6.79$ $p \leq 0.01$, and Drug by Dose interaction $F[4,35] = 3.67$ $p \leq 0.05$. Subsequent Newman Keuls analysis did not reveal any significant differences between the drug doses. The baseline testing data were compared with drug pretreatment data. Pair-wise t-tests indicated a slight but statistically significant increase in the turning bias of the groups that received the vehicle (mean = 0.371 vs 0.376) and 2.0 mg/kg amphetamine (mean = 0.364 vs 0.377) injections.

Heroin pretreatment:

Repeated measures analysis of the directional bias measure indicated a significant Drug by Dose interaction condition $F[4,35] = 6.64$ $p \leq 0.01$, and a significant Day effect $F[2,70] = 7.52$, $p \leq 0.01$. Subsequent Newman Keuls analyses indicated that groups of rats that received the 0.25, 1.0 and 2.0 mg/kg doses of heroin demonstrated statistically significantly turning bias scores when compared with the group that received the vehicle injection. Pair-wise t-tests indicated an increase in directional bias at the 1.0 and 2.0 mg/kg doses (Figure 11).

No significant effects were found in the repeated measures analysis of the arm bias measures.

Figure 10. Effect of amphetamine on directional bias in the radial arm maze. Points represent means ($\pm$ SEM) for rats pretreated with amphetamine 30 min prior to testing. * Statistically significant difference from vehicle group. † Statistically significant difference from baseline testing.

Figure 11. Effect of heroin on directional bias in the radial arm maze. Points represent means ($\pm$ SEM) for rats pretreated with heroin 30 min prior to testing.

* Statistically significant difference from vehicle group. † Statistically significant difference from baseline testing.

Nicotine pretreatment:

Repeated measures analysis of the directional bias data demonstrated a significant Drug by Dose interaction ($F[3,64] = 7.81, p \leq 0.01$). Newman Keuls analysis indicated that the group that received 2.0 mg/kg demonstrated statistically significantly higher turning bias scores than the group that received saline. Pair-wise t -tests indicated that groups that received the 2.0 and 4.0 mg/kg doses of nicotine showed statistically significantly higher turning bias scores on the day that they received the drug (Figure 12).

A significant effect of Dose was found in the analysis of the arm bias data ($F[3,64] = 3.33, p \leq .05$). Newman Keuls analysis indicated that groups that received the 0.2 and 0.4 mg/kg doses of nicotine demonstrated a lower arm bias (0.380 and 0.375, respectively) than the group that received the vehicle injection (0.409).

Figure 12. Effect of nicotine on directional bias in the radial arm maze. Points represent means ($\pm$ SEM) for rats pretreated with nicotine 30 min prior to testing.

* Statistically significant difference from vehicle group. † Statistically significant difference from baseline testing.

Haloperidol pretreatment:

No significant differences were found in the analysis of the directional or arm bias data (Figure 13).

Scopolamine pretreatment:

Repeated measures analysis of the directional bias measure demonstrated a significant Dose by Drug interaction ($F[3,48] = 2.79, p \leq .05$). Newman-Keuls and pair-wise *t*-tests indicated that, when compared with baseline testing days, or the saline control group, rats receiving all doses of scopolamine showed statistically significantly lower turning bias scores (Figure 14).

Figure 13. Effect of haloperidol on directional bias in the radial arm maze. Points represent means ($\pm$ SEM) for rats pretreated with haloperidol 2 hours prior to testing.

Figure 14. Effect of scopolamine on directional bias in the radial arm maze. Points represent means ($\pm$ SEM) for rats pretreated with scopolamine 20 min prior to testing. * Statistically significant difference from vehicle group. † Statistically significant difference from baseline testing.

Discussion

The present experiments show that the exploratory pattern of animals foraging for food in an eight arm radial maze is altered by a variety of centrally acting drugs. Two dependent variables were developed to quantify the perseverative nature of the exploratory pattern. One measure examined the distribution of arm entries to assess whether individual arms were preferred. The second measure examined whether the pattern of exploration (i.e. direction of turn) was repeated.

The results show that there was little tendency for animals to develop a bias to one particular arm. The minimum arm bias score is 0.356 and the average arm bias scores in the present experiments were remarkably close to this value. This is in accord with the statement by Olton et al (1977). that "animals appear to have a strong predisposition to randomly alternate responses among spatially distinct choices even though there is no differential reinforcement contingent on this behaviour" (p.300).

On the other hand, even during baseline testing animals displayed a slight bias in the way they explored the maze. Each animal was likely to repeat certain angles of turn over the course of the session, reflected in average directional bias scores greater than the theoretical random score.

Amphetamine pretreatment significantly increased directional bias scores; the lower doses of amphetamine appeared to have a greater effect than the highest dose (2.0 mg/kg). Animals showed an increased frequency in a decreased number of possible angles of turn. Thus, for this behavioural measure, the animals showed increased perseveration. These data extend the observation that psychomotor stimulant drugs decrease the variability of behaviour, whether it be locomotor activity (Lyons and Robins 1975; Schiorring 1979; Segal and Mandell 1974) or higher order processing (Ridely et al. 1981;1988).

The present results indicate that the behaviour of animals following heroin pretreatment also becomes less variable and more perseverative. It is well known that low doses of opiates can produce locomotor stimulation (Ayhan and Randrup 1973; Babbini, and Davis 1972; Fog 1970; Norton 1977), and a number of other investigators have previously described opiate-induced stereotyped behaviours (Ayhan and Randrup 1973; Charness et al. 1975; Fog 1970). Just as stimulants produce higher rates of activity in more limited response categories (Lyon and Robbins 1972), the present study shows that opiates, under appropriate circumstances, can also cause a similar perseveration. Similarly, nicotine was also found to increase perseverative patterns in the radial arm maze. This appears to be the first report of nicotine-induced repetitive behaviour.

It should be emphasized that the arms of the maze were continually rebaited; therefore, all animals received identical amounts of food irrespective of their exploratory

pattern. Consequently, the systematic change in the exploratory pattern cannot be accounted for by a change in food reinforcement.

Whereas amphetamine, heroin and nicotine were found to increase perseveration, neither haloperidol nor scopolamine significantly increased the directional bias. In fact, scopolamine reduced turning bias significantly. These data suggest that not all centrally acting drugs produce the same effect on exploratory behaviour. If all drugs tested produced perseveration it might be concluded that the effect was simply a non-specific disruption of normal behaviour.

Ethanol and diazepam have previously been shown to decrease variability of exploratory patterns in the radial arm maze (i.e. increase perseveration; Loh and Beck 1989), and the present data extend the list to include amphetamine, heroin and nicotine. All of these are drugs of abuse. This prompts the speculation that the observed drug-induced increase in perseveration might be related to reinforcement. Reinforcing stimuli have by tradition been defined by their ability to increase the probability of a particular response. Of course, an increase in the response rate of one behavioural category, such as lever pressing, would be at the expense of other behavioural categories, such as exploration. Clearly, reinforcing stimuli usually cause a focusing of the behavioural repertoire, sometimes to the point that one particular response prevails over all others. Such is the case in most operant conditioning paradigms. Conversely, withholding reinforcement (extinction) usually causes behaviour to become more variable and

diversified (Beck and Loh 1990).

The present findings suggest that the behavioural repertoire might be described along a continuum. At one end, an organism perseverates in a single response category (highly reinforced responding), while at the other extreme the behaviour would be more variable. Whether behaviour is perseverative or variable depends on the state of reinforcement. If this formulation is correct, one might predict that the behavioural repertoire might be shifted in the perseverative direction by reinforcing drugs.

We hypothesize that reinforcing drugs produce predictable effects on behaviour despite the absence of a formal response contingency. Psychomotor stimulants enhance those categories of behaviours that happen at the time of drug delivery (Broekkamp and van Rossum, 1974; Carr and White 1987; Ellinwood and Kilbey 1975; Ridley et al. 1981). Predominant behaviours would be expected to increase in frequency, forcing less frequent behaviours out of the repertoire; put another way, there may be "an increased frequency in a decreased number of categories." This description is precisely that used by Lyons and Robbins (1975) to describe stimulant induced stereotypy. It is possible that some of the highly repetitive response patterns seen after high doses of amphetamine or cocaine may be an extreme extension of perseveration which is also evident at low doses. Other reinforcing drugs may produce similar effects, although the degree to which they alter ongoing behaviour would be influenced by such factors as speed of drug onset, clearance rate, and other competing responses.

In summary drugs of abuse, including ethanol, diazepam, amphetamine, heroin and nicotine, increase perseveration, suggesting that perseveration may be related to drug reinforcement. Further, the present data demonstrate that exploratory behaviour in the radial arm maze can be used to detect and quantify drug-induced perseveration.

Study 3

Effects of 6-OHDA Lesions of Either the NAS or Caudate on Rats Exploring a Radial Arm Maze for Food Reinforcement: Effects of Psychostimulants on Behavioural Variability

Introduction

Findings from the first RAM study suggested that perseverative behavioural patterns demonstrated by animals receiving high doses of amphetamine may also be observed, in a more subtle fashion, in rats receiving low doses of the drug. It is possible that the perseveration observed is a less pronounced form of behavioural stereotypy. If this were the case, then it would be expected that experimental manipulations which compromise the expression of amphetamine stereotypy would also compromise behavioural perseveration in RAM.

A number of studies have demonstrated that DA systems play an important role in the mediation of stereotyped patterns of behaviour produced by amphetamine (Creese and Iverson, 1973;1974;1975). Mesolimbic dopamine systems have also been shown to be important in the mediation of the reinforcing effects of amphetamine. However, previous research has suggested that the integrity of DA systems in the caudate nucleus may predominately mediate amphetamine induced stereotypy, while lesions of the DA systems innervating the NAS attenuate the reinforcing effects of psychostimulants (Creese and Iverson 1974, Kelly et al. 1975; Lyness et al. 1979, Roberts et al. 1980). It was of interest

to determine if amphetamine-induced perseveration observed in the RAM is specifically mediated by DA systems in the NAS or caudate regions of the brain.

The objective of the present study was to determine if amphetamine induced perseveration in the RAM is mediated by DA systems in the NAS or caudate nucleus or if both systems are important in the mediation of the response. The effects of 6-OHDA lesions to each of these regions was examined. It was hypothesized that if DA systems of either NAS or the caudate nucleus played are important in the mediation of the amphetamine induced perseveration effect then a DA specific lesion should attenuate the effect.

Methods

Male Wistar rats underwent the same habituation and baseline testing procedures as was described in Study 2. Following three days baseline testing, rats received bilateral infusions of 6-OHDA or a vehicle solution (sham lesion) into either the NAS or caudate regions of the brain (see below). Rats were allowed to recover for a period of one week before recommencement of behavioural testing. Rats were again food deprived and then tested for one day in the RAM using the same procedure as was followed during baseline testing days (day 4). Then, for the next three days, rats were given an injection of amphetamine (1.0 mg/kg), 30 minutes prior to testing (days 5-7).

In total 56 rats were tested. The control groups consisted of two groups of 15 rats that received infusions of the vehicle solution (caudate control, NAS control). Sixteen rats received DA system lesions of caudate nuclei and ten rats received DA system lesions of the NAS (nine completed all behavioural testing sessions).

Surgery

Thirty min. prior to surgery animals were injected i.p. with imipramine (Sigma Chemicals, St. Louis; 25 mg/kg, i.p.), then anaesthetized with halothane (Fluothane, Ayerst, Montreal). Animals received bilateral infusions (2 μ l) of either 6-OHDA or the ascorbic acid vehicle solution. A 6.0 mg/ml stock solution of 6-OHDA was prepared by dissolving the neurotoxin in a saline solution containing 0.2% ascorbic acid. The 6-OHDA and vehicle solutions were infused into the lateral ventricles by stereotactically positioning a 22 gauge cannula at the coordinates of either the caudate nucleus :AP +9.7, DV +4.4, ML $\pm$ 2.7 or the NAS: AP +9.8, DV +2.7, ML $\pm$ 1.2 or the NAS (nose bar -4.2; flat skull orientation).

To control the amount of solution being infused into the brain, one end of a length of polyethylene tubing was attached to the infusion cannula while the other end was attached to a 10 μ l Hamilton syringe. The syringe was mounted on a syringe pump (Razel Scientific Instruments, Roanoke, VA) that permitted the solution to be slowly infused into the brain.

Results

Effect of lesions of the nucleus accumbens septi

Arm Bias

Comparison of arm bias scores of rats did not reveal any statistically significant differences with the exception of an analysis of the scores on the three days during which rats received amphetamine pretreatments that indicated a significant between groups effect ($F[1,22]=6.43$; $p \leq .02$; Figure 15). Examination of mean arm bias scores indicated that rats receiving the NAS - 6-OHDA infusions demonstrated statistically significantly greater arm bias scores when compared with the control group. However, the magnitude of the effect was so small that it is unlikely that the increase in arm bias represented any meaningful effect.

Turning bias

One rat in the DA lesion group was removed due to illness. Analysis of the turning bias scores recorded during baseline testing and on the first day of testing following surgery did not reveal any statistically significant between groups or within group effects (Figure 16).

Comparison of turning bias scores for rats receiving either sham or 6-OHDA infusions into the NAS on the three days when rats received the 1.0 mg/kg amphetamine pretreatment did not reveal a significant between groups effect ($F[1,22]=.05$; $p > .05$) or effect of Day of Testing ($F[2,44] < .01$; $p > .05$). However, a visual inspection of the mean

turning bias scores on individual testing days indicated an increase in turning bias scores on the first of the three days of amphetamine testing (See Figure 16, Day 5). A *post-hoc* between groups comparison of turning bias score demonstrated that the group receiving the DA specific lesion of the NAS demonstrated statistically significantly higher turning bias scores than the group receiving the sham lesion ($F[1,22]=4.59$, $p \leq .05$).

The mean turning bias score for the three days during which rats received the amphetamine was combined into one measure and compared with both the mean baseline turning bias score and turning bias scores from the testing day following surgery. A statistically significant effect of amphetamine pretreatment was demonstrated when the baseline scores were compared with the mean turning bias scores during drug testing ($F[1,22]=22.05$; $p \leq .001$). Further comparison of amphetamine pretreatment days with post surgical turning bias scores demonstrated a significant Group (NAS lesion vs non-lesion) by Day (no drug vs drug) interaction ($F[1,22]=4.25$, $p \geq .051$). The interaction indicated a statistically significant increase in turning bias scores in the group of rats that had received infusions of the DA specific neurotoxin in the NAS when compared with the control group (Figure 16).

Effect of lesions of caudate nucleus

Arm Bias

A comparison of the mean of the three days of testing prior to surgery and arm bias score on the first testing day following surgery indicated a significant Group by Day interaction ($F[1,29]=16.62, p < .001$). This resulted from a small but statistically significant increase in the arm bias of the group of rats that received the DA specific lesion to the caudate nucleus.

Analysis of the mean baseline arm bias scores with the scores on the testing day following surgery indicated a significant interaction ($F[1,29]=16.62, p < .001$). Further, a significant effect of Drug was demonstrated between the post surgical day of testing and the mean arm bias score on the three days when amphetamine was administered prior to testing ($F[1,29] = 9.52; p \geq .004$). However, the functional significance of these changes in arm bias are questionable. The overall mean of arm bias values approximated a uniform distribution of arm entries (c.f. Figure 10) and the largest change in arm bias of any group was less than 0.05 units (Figure 15). While statistically significant effects in changes in arm bias were demonstrated, it is doubtful if the changes had any functional significance.

Turning bias

No significant between groups differences in turning bias was found on any of the three days of baseline testing ($F[1,29]=0.08, p > .05$). No effect of Day of testing ($F[2,28]=.32, p > 0.05$) or Day by Group interaction ($F[2,29]=0.53, p > .05$) was found.

A comparison of mean turning bias scores on baseline testing and the scores on the day following surgery demonstrated a significant effect of surgery in both groups ($F[1,29]=11.41$ $p \leq .002$). Both 6-OHDA infusions and infusions of the vehicle alone produced a statistically significant increase in turning bias when compared with the mean baseline turning scores (Figure 17). While the increase in perseveration was statistically significant, it is likely that the effect is simply an artifact of an exceptionally stable turning bias scores in group of rats receiving the 6-OHDA infusions. Note that the test of a Group by Day interaction was not statistically significant ($F[1,29]=.05$, $p > .05$).

No significant between-lesion groups effects were demonstrated in the turning bias score on the three days that rats received amphetamine pretreatments prior to testing ($F[1,29]=0.20$, $p > .05$) nor was an effect of Day of Testing indicated ($F[2,58]=1.65$, $p > .05$). However, a statistically significant increase in turning bias was found in the mean turning bias score obtained on the three days when rats were pretreated with amphetamine were compared with either mean baseline turning bias scores ($F[1,29]= 31.85$, $p > .001$) or turning bias scores following surgery ($F[1,29]= 15.88$, $p > .001$; Figure 17).

Amphetamine produced an overall increase in turning bias in both groups of rats. However this increase in bias of turn was significantly greater in the group of rats that received infusions of 6-OHDA in the NAS.

Figure 15. Mean ($\pm$ SEM) arm bias scores during baseline, post-lesion and testing days when rats were pretreated with amphetamine.

Figure 16. Mean ($\pm$ SEM) turning bias scores during baseline, post-lesion and testing days when rats were pretreated with amphetamine.

Figure 17. Mean ($\pm$ SEM) turning bias scores for rats exploring an eight arm radial maze prior to infusion of either 6-OHDA or vehicle into either the nucleus accumbens or caudate nucleus and effects of post-lesion amphetamine pretreatment.

Discussion

A number of studies have been reviewed that demonstrate that amphetamine has reinforcing properties and that at high doses will produce stereotypical response patterns. Dopamine systems of the NAS have been shown to be important in the mediation of the reinforcing effects of amphetamine while DA systems in the caudate appears to be important in the mediation of amphetamine induced stereotypic response patterns.

The first RAM study showed that amphetamine, among other substances with high abuse potential, produce an increase in the perseveration in the angle of turn a rat makes when leaving an arm of the RAM. The perseveration in turn had no bearing upon the food reinforcement the rat received when exploring the maze. The objective of the present study was to determine if the amphetamine-induced turning perseveration was mediated by either DA systems of the NAS or caudate nucleus. If a preferential role for either of these systems could be demonstrated, it would provide evidence that turning perseveration may be specifically related to either the reinforcing or stereotypy-producing properties of amphetamine.

An increase in turning bias was observed in all groups when pretreated with 1.0 mg/kg of amphetamine. However, on the first day of amphetamine testing the group of rats receiving the DA specific lesion in the NAS demonstrated a statistically significantly higher turning bias score when compared with the appropriate control group. This may be a transient effect, as turning bias scores approached the scores of the control group on the

second day of testing. Turning bias scores increased again, although not statistically significantly, on the third day of testing. In comparison, turning bias scores exhibited a slight but gradual decline in all other groups over amphetamine testing days (Figure 17). These data would suggest that, in some manner, DA systems of the NAS are important in the mediation of amphetamine-induced turning perseveration in the RAM.

Examination of changes in turning bias resulting from infusions of 6-OHDA or vehicle solution into the caudate nucleus demonstrated that the infusion of either neurotoxin, or the vehicle alone, produced an increase in turning perseveration on the test day prior to amphetamine testing (Day 4). Turning bias was slightly enhanced in the 6-OHDA lesion group over and above the increase observed in the control group on the first day that animals received amphetamine (see Figure 16). However, no statistically significant differences were found between the control and lesion groups suggesting that it is unlikely that the DA specific lesions to the caudate nucleus enhanced turning perseveration.

If turning perseveration is preferentially mediated by DA systems in the NAS one would expect that lesions of this system would attenuate the increase in turning bias produced by amphetamine pretreatments. In contrast, the opposite was found as turning bias was enhanced in the DA lesion group. It is possible that the dose of amphetamine used in this study (1.0 mg/kg) may have compromised the likelihood of finding a significant reduction in turning bias. Study 2 examined the effects of four doses of

amphetamine on turning bias (0.25, 0.50, 1.0 and 2.0 mg/kg). Pretreatment of rats with the three lowest doses of amphetamine produced nearly identical increases in turning perseveration (c.f. Figure 10). If the DA depletions resulted in an attenuation of the effects of amphetamine by 50 to 75 percent, it is possible that the effect would not be reflected as a decrease in turning bias scores. It is possible that if the study were replicated with a lower dose of amphetamine (0.25 mg/kg) a reduction in turning bias could be revealed.

Findings from this study provide some indication that DA systems are important in the mediation of amphetamine induced turning perseveration in the radial arm maze. However, the increase in turning perseveration in the NAS-lesion group was opposite to what was anticipated. The present results must be followed-up to verify the effect and establish its importance.

Study 4

Effects of intraventricular infusions of 5,7-DHT on Rats Exploring a Radial Arm Maze for Food Reinforcement

Introduction

Serotonergic systems are important in the mediation of the reinforcing effects of psychostimulants (see Study 1; Loh and Roberts 1990). Other research has demonstrated that depletion of forebrain 5HT and the administration of 5HT antagonists produce a reduction in behavioural variability or a resistance to the extinction of a behaviour (Study 1 Food reinforcement extinction data; Woger et al., 1991). These studies would suggest that the amphetamine induced perseveration in the RAM may also be affected by 5HT depletions.

The objective of this study was to examine the effects of forebrain 5HT depletions on the exploratory behaviour of rats in the RAM. The effects of cocaine and amphetamine injections were also evaluated.

Since no specific neural region had been identified to mediate the perseveration inducing effects or the reinforcement enhancing effects of 5HT lesions, the present study incorporated intraventricular infusions of 5,7-DHT to produce global forebrain 5HT depletions.

Methods

Male Wistar rats underwent habituation to the RAM and baseline testing as described in Studies 2-3. Following baseline testing each rat received an intraventricular infusion of 5,7-DHT (N = 8) or the vehicle solution alone (N = 6) following the surgical protocol outlined in Study 1. Following surgery rats were allowed to recover for four to five days and then were tested for 6 days. The rats were tested for three more days during which time they received an injection of saline (1.0 ml/kg) five minutes prior to testing. Rats were then tested on an descending series of doses of cocaine (10.0, 5.0 and 2.5 mg/kg; - 5 min) and on an ascending series of doses of amphetamine (0.5, 1.0, 1.5 mg/kg; - 30 min). Prior to each dose of drug that was tested, rats were tested for a period of 2-3 days where they received injections of saline (re-baseline days).

Rats were also tested for two periods of three consecutive days during which they received an injection of either 5.0 mg/kg or 2.5 mg/kg of cocaine five minutes prior to the start of the testing. Prior to the days during which rats received cocaine, rats were tested for a period of three days during which they received a saline injection (1.0 ml/kg) to control for any effect of the injection itself. Experimental manipulations are summarized in Table 3.

Stage of testing	Duration
Baseline	3 days
Intraventricular 5,7-DHT or vehicle infusions	1 day
Recovery	4-5 days
Re-baseline and evaluation of the effects of surgery	6 days
Baseline with saline injection at - 5 min	3 days
Cocaine 10 mg/kg at - 5 min	4 days
Baseline with saline injection at - 5 min	3 days
Cocaine 5 mg/kg at - 5 min	3 days
Baseline with saline injection at - 5 min	3 days
Cocaine 2.5 mg/kg at - 5 min	3 days
Baseline with saline injection at - 30 min	3 days
Amphetamine 0.5 mg/kg at - 30 min	1 day
Baseline with saline injection at - 30 min	2 days
Amphetamine 1.0 mg/kg at - 30 min	1 day
Baseline with saline injection at - 30 min	2 days
Amphetamine 1.5 mg/kg at - 30 min	1 day

Table 3. Summary of experimental manipulations incorporated in Study 4.

A modification in the design of the radial maze was completed prior to testing the animals in the study. A new eight arm radial maze was constructed out of plexiglass, this maze retained the same dimensions as previous mazes. However, the manner in which arm entries were recorded was altered. A small hinged door was placed in front of the food receptacle at the end of each arm. Rats exploring the maze were trained to push the door aside to obtain food pellets. The door was attached to a small sensor that recorded the door opening. Further a sensor was placed underneath the central platform in the maze. An arm entry was recorded when the rat pushed the door aside to obtain the food pellets, however the rat had to return to the central hub of the maze, thereby activating the sensor beneath the floor before another arm entry was recorded. Since a rat must enter the center of the maze to enter another arm, all arm entries were recorded.

Results

Overall, the effects of serotonergic lesions and psychostimulant pretreatment were ineffective in altering the arm bias or turning bias scores of rats exploring the maze. The data were analyzed by first conducting a repeated measures analysis of variance with Day of Testing as a within groups factor and Lesion (5,7-DHT vs vehicle solution) as a between groups factor. This analysis was conducted for both arm bias and turning bias scores for all parts of the study that lasted for more than one day (Figures 19 and 20). Only three statistically significant effects were found through these analyses. A significant Lesion by Day of testing interaction was found in turning bias scores on the days that rats received saline injections (days 17-19) following the days that rats received 10 mg/kg

cocaine pretreatment ($F[2,24]=3.73$, $p \leq .05$; days 13-16). The interaction was a result of an increase in turning bias scores on the second of three days of saline testing (see Figure 20). A statistically significant Lesion by Day interaction was found in arm bias scores on the six days of testing immediately following surgery ($F[5,60]=2.47$, $p \leq .05$; days 4-9). A significant effect of Day of Testing was also found on the days that rats were pretreated with the 2.5 mg/kg dose of cocaine ($F[2,24]=3.33$, $p \leq .053$; Figure 19). All other tests were not statistically significant. This was a result of a relative reduction in turning bias on the second of the three days of testing. However, it is unlikely that the effect represents any meaningful change in behaviour.

Figure 18. Mean daily turning bias and arm bias scores of rats prior to and following lesion of forebrain serotonergic systems. Effects of cocaine pretreatments are also illustrated. Arm bias scores are represented as the two lower sets of data points. Effect of saline and cocaine pretreatment are indicated by horizontal lines located between the turning and arm bias scores. See Table 3 for a review of experimental manipulations.

Figure 19. Effect of amphetamine pretreatments on mean daily turning bias and arm bias scores of rats that had previously received lesions of forebrain serotonergic systems. See Table 3 for a review of the experimental manipulations. Effect of an ascending series of doses of amphetamine (0.5, 1.0, 1.5 mg/kg; - 30 min) are illustrated. Values above X axis indicate the dose of amphetamine received on the day of testing. Rats received injections of saline on all other days of testing.

Discussion

Overall, the results of the study were disappointing as no definite conclusions from the results can be determined. Of particular interest was the finding that the amphetamine pretreatments did not significantly alter turning bias in this study while in Studies 3 and 4 amphetamine pretreatments produced substantial increases in turning bias scores. There are a number of possible reasons why no effect was found. Prior experience with cocaine in the maze may have also altered the rats' response pattern in a fashion that was not reflected by changes in the turning bias measure. The design of the radial maze was altered prior to this study. However, other studies (Beck and Loh, 1990; Studies 2 and 3) have used mazes that have varied in design and robust effects of drug pretreatment were observed.

Finally, a relatively small number of animals were used in the study this would have compromised the power of the statistical analyses. Examination of mean turning bias scores in Figure 19 would suggest that overall the turning bias scores were elevated in the group of rats that received the infusions of 5,7-DHT and this was augmented by pretreatment of either of the two highest doses of amphetamine. Findings from this study must be replicated to confirm present findings or establish if the results are an artifact of additional factors that were not taken into consideration.

General Discussion

The studies presented here provide clarification of a number of questions that are important in the development of our understanding of mechanisms of action of reinforcing substances. Study 1 provided additional evidence that 5HT systems appear to have an important function in the mediation of the reinforcing effects of cocaine. Further, lesions of 5HT systems would also appear to affect the reinforcing properties of food. Investigations incorporating the modified RAM paradigm demonstrate the utility of the paradigm in the investigation of behavioural effects and neural mechanisms that mediate behavioural perseveration. Findings from these investigations also suggest that many reinforcing substances share the common property of promoting behavioural perseveration. The level of behavioural perseveration can be considered to be represented a continuum contingent upon the magnitude of the reinforcing agent received.

The first study demonstrated that depletions of 5HT by means of intracerebral infusions of 5,7-DHT, resulted in increasing breaking-points on a PR schedule for cocaine reinforcement. This confirmed the findings of a previous study that showed that 5,7-DHT infusions into the amygdala or medial forebrain bundle would increase breaking points for cocaine on the PR schedule (Loh and Roberts 1990). The ability to replicate the previous study further supports the contention that compromise of 5HT system function increases the reinforcing efficacy of cocaine.

The use of intraventricular infusions of 5,7-DHT produced statistically significant reductions in forebrain 5HT. A number of discrete brain regions were dissected out and assessed for 5HT content. However, correlations between reductions in 5HT and changes in BP did not conclusively identify a preferential site that may mediate the 5HT depletion effect. Serotonin depletions in the mesencephalon were best correlated with post- surgical changes in BP ($r = -.45$), however, only 20% of the variation is accounted for. Further investigations may involve a more detailed examination of this region to determine if specific structures in this region play a key role in the mediation of the effect.

The second study examined the effects of 5HT depletions on food reinforcement; serotonin depletions also promoted increases in BPs when reinforcement was available and during extinction testing. This finding would suggest that 5HT systems may play a more general role in the mediation of reinforcement, rather than being specific to cocaine. Woger and associates (1991) explored the effects of 5,7-DHT infusions into the medial and dorsal raphe nuclei of rats responding for a sucrose solution. Their findings provided evidence that the 5HT lesions increased the reinforcement value of the sucrose solution when compared with the control group (Woger 1991). This study supports the hypothesis that 5HT systems play a general role in the mediation of reinforcement.

Findings from the food reinforcement study during extinction testing suggested that the 5HT lesions produced a reduction in the rate of extinction or a perseveration in responding. This can be clearly seen in Figure 8 on the fourth day of extinction testing

when rats continued to respond to higher BPs than the control group. This suggests that 5HT systems may play a role in the mediation of the rate of extinction.

No specific neural site could be identified that may preferentially mediate the 5HT depletion effect. Serotonin depletion in the mesencephalon, amygdala and cortex, were all moderately correlated (r 's = -0.46 to -0.49) with changes in BP. The mesencephalon was again indicated to have a particular involvement in the mediation of the effect.

In both the cocaine and food reinforcement studies, no specific neural site of action could be definitively identified that may mediate the 5HT depletion effect. A number of explanations for this can be entertained. First, the preferential site(s) of action were not specifically examined. Second, there may not be a specific site of action, but rather the effect may be mediated by a number of widely distributed sites. Finally, 5HT depletions indirectly modulate the reinforcing effects of psychostimulants by regulating other systems. Further investigations are necessary to clarify the issue.

Findings from Study 1 presented here and previous work (Loh and Roberts, 1990) provide substantial evidence that forebrain 5HT systems are important in the mediation of the reinforcing efficacy of cocaine. Further, findings from Study 2 suggest that the effects of 5HT system depletions are not specific to cocaine, the 5HT neurotransmitter system may be important in the modulation of the reinforcing effects of a variety of substances.

Study 2 demonstrated that a number of substances with a high abuse potential (amphetamine, heroin and nicotine) all produced a perseveration in the turning bias rats made when exploring a RAM for food reinforcement. In contrast, the psychoactive substances tested that did not have a high abuse potential (scopolamine and haloperidol) did not promote perseveration. In fact pretreatment with scopolamine actually produced a reduction in turning bias. It should be emphasized that rats would obtain the same number of food pellets regardless of the pattern of exploration used. This would suggest that the reinforcing substances tested all have the common property of promoting preservation in behaviour. Previous research has shown that both ethanol and diazepam also promote turning perseveration in the RAM (Devenport and Merriman 1983; Loh and Beck 1989). Together these findings suggest that many, if not all, substances with high abuse potentials produce a reduction in behavioural variability.

Study 3 attempted to determine if DA systems of the NAS or caudate nucleus were important in the mediation of amphetamine induced perseveration in RAM. Previous studies have shown that the integrity of DA systems in the NAS are essential for the demonstration of the reinforcing effects of psychostimulants (Lyness et al. 1979; Pettit et al. 1984; Roberts 1989; Roberts, et. al. 1977; 1980). Therefore, if the amphetamine induced turning perseveration in RAM is related to reinforcing properties of the drug one would expect an attenuation of the turning perseveration if DA function in the NAS were compromised. Alternatively other studies have demonstrated the amphetamine stereotypy is mediated by DA systems in the caudate nucleus (Creese and Iversen 1974; 1975; Kelly

et al. 1975); and it would be expected that compromise of these systems would reduce turning perseveration if the effect was related amphetamine stereotypy. The findings from these studies were surprising as 6-OHDA lesions of the NAS produced an increase, rather than the predicted decrease, in turning perseveration. This may suggest that DA systems of the NAS may mediate RAM perseveration in a manner not previously considered.

It is difficult to determine why animals receiving the 6-OHDA lesions of NAS demonstrated an increase in turning perseveration over and above that demonstrated by either the rats receiving DA specific lesions in the caudate nucleus or the control groups. However, the findings do suggest that the NAS may play a role in the mediation of amphetamine induced perseveration in the RAM.

The results of the 5HT lesion study in the RAM were somewhat surprising. A consistent change in turning bias was not observed when animals received either cocaine or amphetamine pretreatment. In studies 2 and 3 an increase turning bias was observed when they pretreated with the amphetamine. Further, Study two and other research (Loh and Beck 1989) have demonstrated that a number of other substances with high abuse potentials promote behavioural perseveration in the RAM. The results might suggest that cocaine does not promote behavioural perseveration in the RAM. However, the finding that amphetamine did not promote perseveration in this study while the drug promoted a robust effect in previous studies suggests that other factors may have compromised the results.

A number of reasons could account for the present findings. Inspection of the mean turning bias scores in Figure 19 indicates an increase in turning bias for all groups of rats when receiving the highest and intermediate dose of amphetamine. The effect would appear to be augmented further in the group that received the 5HT depletions. The findings may be compromised by the relatively small number of animals used in each group (N 's = 6, 8). More detailed studies examining these possible confounds must be conducted to clarify the issue.

Overall, findings from the present RAM studies demonstrated that the modified RAM paradigm is a viable tool for the exploration of behavioural effects of many reinforcing substances. Further, the measures of arm bias and turning perseveration have been shown to be useful measures for the quantification of behaviours in the maze. The turning perseveration measure has been shown to be sensitive to both changes in the dose of the drug being tested and to the effects of neurotoxic lesions. It is of interest to note that the turning bias measure is not only sensitive to increases in perseveration but will also allow for the measurement of reductions in turning preservation below baseline levels. The latter effects were demonstrated by the reduction in turning perseveration resulting from scopolamine pretreatment (Figure 14).

Beyond the finding that a number of addictive substances reduce behavioural variability in rats exploring the RAM for food reinforcement, a number of theoretical conceptions can be entertained. Of key importance is that the increase in behavioural

perseveration did not have any consequence to the amount of reward the animal received. This would suggest that the perseveration in behaviour observed was not contingent upon the behaviours related to the receipt of drug reinforcement; but rather perseveration is a general consequence of the administration of the drug.

The predictions from Herrnstein's (1970) Matching Law are of interest in interpretation of the findings from the RAM studies. The Matching Law predicts that the probability or rate of a response is increased when the magnitude of a reinforcer it is associated with increases. In other words, increasing magnitude of a reinforcer will increase the associated response rate. The conceptualization is based upon the observation of changes in an animal's behaviour in relation to the acquisition of naturally occurring reinforcers such as food. The hypothesis is an extension of the predictions by Thorndike and the empirical verification of the Law of Effect by Skinner. Herrnstein took exception to the 'simple notion of "stamping-in"' (Herrnstein 1970, p 243) which he believed was too simplistic. "Animals do not just repeat the first successful act; they are likely to improve upon it until they find something like optimal performance" (Herrnstein 1970, p 243). Rather the matching law predicts a more gradual change in behaviour that allows for the modification of the behavioural pattern until reinforcement is maximized. Further, other reinforcers that have greater reinforcing properties can shift a behavioural pattern towards the acquisition of the greater reinforcer. The probability of a behavioural pattern being exhibited is a function of the relative reinforcing value of that reinforcer in the context of other reinforcers. Consistent with this prediction are the results from the RAM

studies demonstrating an increase in turning perseveration when animals were treated with increasing doses of heroin (Figure 11), nicotine (Figure 12), ethanol or diazepam (Figure 5; Loh and Beck 1989). In the cases of both food reinforcement and the administration of psychoactive substances with a high abuse potential, the receipt of a reinforcer results in a narrowing of a behavioural pattern rather than the promotion of a specific behaviour. Therefore, the present data suggest that the predictions made by Herrnstein's Law can be extended to include the effects of drugs with reinforcing properties.

If Herrnsteins' predictions apply to reinforcing drugs, the model can be viewed as a parallel to the Lyon and Robins hypothesis. As noted earlier, Lyons & Robbins (1975) described amphetamine induced stereotypy as "an increase in response rates in a decreasing number of behavioural categories". Lyons and Robbins predicted that the frequency of specific behaviours will be increased as a result of injections of amphetamine. Further Lyons and Robbins "regarded stereotypy as an inevitable concomitant of extreme psychomotor stimulation, and did not consider that it has any particular function" (Robbins et al. 1970, p 26). Herrnstein's matching law predicts that the reduction in the variability of behaviour is contingent upon the association of the behaviours with relative reinforcing magnitude of the contingent reinforcers. Both hypotheses argue that the administration of a reinforcing agent, be it either contingent or non-contingent on specific behaviours, will result in a reduction of behavioural variation resulting from a perseveration in specific behaviours.

It has been shown that stereotypical behaviours resulting from amphetamine infusions are contingent upon the environment where the effects of the drug take effect (Beck et al. 1986). This supports Herrnstein's conception that an association between the environment and a perseveration in behaviour does exist. If reinforcement alone promotes a narrowing of an animals' behavioural repertoire it would be predicted that at high doses, one specific behavioural response would predominate regardless of whether or not the reinforcement was specifically associated with a particular behaviour. This is precisely what is observed in stereotypy studies when animals are under high doses of amphetamine (c.f. Table 1). Since the reinforcing agent is arbitrarily administered no connection can be made between a specific behaviour and the receipt of reinforcement yet a reduction in behavioural variability still occurs. The narrowing of the behavioural repertoire may be simply a result of reinforcement not the association between reinforcement and a specific behaviour. While it is common to observe increases in specific behaviours in relation to the acquisition of reinforcement, it is possible that the narrowing of the behavioural repertoire to these behaviours is not a direct result of the promotion of the specific behaviours. An alternative explanation is that reinforcers directly promote specific behaviours that lead to the acquisition of reinforcement. If reinforcement promotes specific behaviours, the question arises of what determines which specific behaviour is promoted. Promotion of a specific behaviour could lead to maladaptive behavioural patterns. A narrowing of the behavioural repertoire of an animal would permit the development of behaviours that result in the acquisition of reinforcement through a series of successive approximations.

It is predicted that all reinforcing agents will promote a narrowing of an animal's behavioural repertoire resulting from an increased frequency of specific behaviours (behavioural perseveration) regardless of whether the reinforcement is associated with a particular behaviour or not. This is precisely what was observed in the RAM studies presented here. At high levels of reinforcement the animal's behavioural repertoire maybe reduced to such an extent that only one specific behaviour is exhibited in high frequency (behavioural stereotypy). Behavioural perseveration may be thought of as a continuum from a slight bias towards a behaviour to extreme cases where rigid behavioural stereotypy is observed. Degree of perseveration is related to the magnitude of the reinforcement.

Before the neural bases of the drug reinforcement can be understood, a solid theoretical framework which accounts for the behavioural phenomenology of reinforcement is required. At present, it is difficult to argue that such a framework exists.

A large literature has been established that has examined drug reinforcement from an operant perspective. Drug self-administration studies which use an operant paradigm have proven to be useful in the delineation of neural systems important for the expression of the reinforcing effects of drugs. These studies are, however, based upon a theoretical framework that examines changes in an operant response for non-drug reinforcing agents such as food. Operant studies typically examine the rate at which animals perform an operant response in order to obtain the reinforcement. In contrast, self-administration

studies commonly examine the rate at which the infusions of the reinforcing agent are received. While both operant and self-administration studies use similar experimental paradigms, the behaviours that are quantified are considerably different. Consequently, the self-administration literature can not benefit from the theoretical framework developed in the operant literature. Attempts to interpret findings from self-administration studies from an operant perspective result in conflicting interpretations of the meaning of a change in the rate of drug administration.

In an attempt to clarify the problem of the interpretation of changes in rate, the PR schedule of reinforcement has been introduced to establish how much "work" an animal will perform in order to obtain an infusion of the reinforcing agent and how experimental manipulations can affect the motivation of animals to obtain the reinforcement. While results of self-administration studies have a considerable face validity, the lack of a theoretical basis for the paradigm compromises our ability to incorporate these findings from these studies to a larger theoretical framework.

Ultimately, the findings from self-administration studies only examine the behaviour of animal's prior to the onset of reinforcement. The effects of drug reinforcement on on-going behaviours are not addressed. The RAM studies presented here demonstrate that a wide variety of reinforcing substances all produce similar changes in behaviour, namely, behavioural perseveration. This finding is of key importance as it demonstrates that drug reinforcement produces consistent changes in behaviour after the

onset of the effects of the drug. With this information, it is now possible to begin to make predictions of how drug reinforcement affects animal behaviour. Our ability to make predictions on how drug reinforcement changes the behaviour of animals will allow the development of a theoretical framework from which further predictions can be tested.

In summary, the studies presented here have examined the behavioural effects of reinforcing substances and the neural substrates that mediate the behavioural effects using both a traditional and novel paradigm. Findings from the traditional self-administration paradigm demonstrated that the reinforcing efficacy of both cocaine and food can be altered by forebrain 5HT depletions. This would suggest that 5HT systems may play a more general role in the mediation of the reinforcing efficacy of substances than previously thought. A novel paradigm was also introduced that examined the behavioural effects of a variety of substances in the RAM. The first study demonstrated that only substances with a high abuse potential will promote a perseveration in turning behaviour. This perseveration in behaviour closely resembled the description of amphetamine stereotypy presented by Lyons and Robbins (1975); with the exception that the changes in behaviour were less pronounced. The terms behavioural stereotypy and perseveration both imply an increase in a specific subset of behaviours at the expense of others. Further, behaviours that are intensified do not appear to have any adaptive advantage in the experimental environment. The findings would suggest that behavioural perseveration may be a general consequence of reinforcement. In the case of high doses of amphetamine, perseveration in

behaviour becomes so extreme that it is easily observed as behavioural stereotypy. At lower doses of amphetamine and other reinforcing agents, the perseveration in behaviour is more difficult to observe, however, the RAM would appear to be a useful tool in the examination of the process. Finally, studies examining the effects of neurotoxic lesions to either DA or 5HT systems in the RAM paradigm suggest the paradigm may be a useful tool in the examination of the effects of surgical manipulations on behavioural perseveration. While the results of these lesion studies were not conclusive, further investigations using the RAM paradigm are likely to clarify the role of DA and 5HT systems in the mediation of behavioural perseveration. Overall, these studies have incorporated two distinct paradigms to identify common elements in the reinforcement process.

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