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BEHAVIOURAL AND NEUROTOXIC EFFECTS OF
3,4 METHYLENEDIOXYAMPHETAMINE

by

Lynn Elizabeth Markert

A thesis submitted to
the Faculty of Graduate Studies and Research
in partial fulfilment of
the requirements for the degree of

Masters of Science

Department of Psychology

Carleton University

Ottawa, Ontario

August 1990

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"Behavioural and Neurotoxic Effects of
3,4 Methylenedioxyamphetamine"

submitted by Lynn E. Markert, B.A.
in partial fulfilment of the requirements for
the degree of Master of Science

Thesis Supervisor

Chair, Department of Psychology

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August 28, 1990

Abstract

3,4 Methylenedioxyamphetamine (MDA), a drug with both stimulant and hallucinogen-like properties, has been used for both medical and recreational purposes. The present study examined the reinforcing effects of MDA in rats and evaluated the resulting neurotoxicity. Self-administration of various doses (0.10, 0.05 and 0.025 mg/injection) was examined on a Fixed Ratio 1 (FR1) and a Progressive Ratio (PR) schedule. MDA supported self-administration at all doses on the FR1 schedule, but overdoses and deaths occurred at the 0.10 mg/injection dose. The breakpoints established on the PR schedule were relatively low. High performance liquid chromatography analyses of the cortex, hippocampus, striatum and nucleus accumbens subsequent to MDA self-administration revealed significant depletion of 5-HT in the hippocampus. The results suggest that MDA is moderately reinforcing and that self-administration of low doses of MDA over several days is selectively neurotoxic.

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"The Truth of Each Man's Inner Being was Put Upon his Shield. And the People Began to Seek their Medicines. As they Sought their own Names, they also Found their Brothers and Sisters."

- Hyemeyohsts Storm

Seven Arrows

TABLE OF CONTENTS

Abstract.....	ii
Acknowledgements.....	iii
Table of Contents	iv
List of Tables	v
List of Figures.....	vi
List of Appendices	vii
Introduction	1
MDA - Physiological Effects	4
Animal Studies/Behavioural.....	5
Animal Studies/Neurotoxicity.....	7
Mechanism of Release/Neurotoxicity.....	8
DRUG SELF-ADMINISTRATION	
Background	9
General Method	10
The Progressive Ratio Schedule.....	11
THESIS EXPERIMENTS	
Introduction.....	13
Experiment #1 MDA Neurotoxicity after Systemic Injection.....	14
Experiment #2 MDA Self-Administration	22
Experiment #3 MDA on the Progressive Ratio Schedule ...	38
Experiment #4 Neurotoxicity at Self-administered doses	46
GENERAL DISCUSSION	
Neurotoxicity	50
Self-Administration	51
Conclusion	55
References	56
Appendix A.....	68
Appendix B.....	70

List of Tables

TABLE	DESCRIPTION	PAGE
1	Monoamine and metabolite concentrations two weeks after 8 MDA injections 20 mg/kg	20
2	Acquisition of MDA self-administration at 0.10 mg/infusion	24
3	Acquisition of MDA self-administration at 0.05 mg/infusion	32
4	Monoamine and metabolite levels two weeks after MDA self-administration	48

List of Figures

FIGURE	DESCRIPTION	PAGE
1	Infusions of MDA at 0.10 mg/injection during acquisition over a 7 day period	28
2	Pattern of infusions for MDA 0.10 mg/inj, cocaine 0.60 mg/inj, and amphetamine 0.06 mg/inj on FR1	30
3	Mean number of infusions and mean drug intake for 4 individual subjects on MDA .025 and 0.05 mg/inj on FR1	35
4	Pattern of responses and infusions for one subject on MDA 0.10 mg/injection on the progressive ratio schedule	41
5	Pattern of responses and infusions for one subject on cocaine 0.60 mg/injection on the progressive ratio schedule	45

List of Appendices

Appendix	Description	Page
A	Sample chromatograph of standard	68
B	Sample chromatograph of rat hippocampus	70

Behavioural and Neurotoxic Effects of 3,4
Methylenedioxyamphetamine (MDA)

Introduction

3,4-Methylenedioxyamphetamine (MDA) is a synthetic amphetamine derivative with the qualities of both amphetamines and hallucinogens (Fehr, 1988). Its unique effects are produced by two active enantiomers: the (+) enantiomer is amphetamine-like while the (-) enantiomer is hallucinogen-like. These dual stimulus effects were determined from subjective reports in humans (Marquardt et al. 1978) and from research with nonhumans, using the drug discrimination paradigm (Glennon & Young, 1984) in which MDA generalized to both amphetamine-like and hallucinogen-like drugs such as amphetamine, cocaine, 1-(2,5-dimethoxy-4-methylphenyl-2-amino-propane (DOM) and lysergic acid diethylamide (LSD) (Glennon & Young, 1984).

MDA was prescribed in the 1950s as an anorectic agent but because of its disturbing mood-altering capacity, it was considered unsuitable for use as an appetite suppressant (Marquardt et al. 1978). Medical use of MDA escalated during the 1960s and early 1970s when psychedelic drugs, such as LSD, were being investigated for altering consciousness. MDA was then promoted for use as an adjunct to psychotherapy (Yensen, et al., 1976). Yensen et al. reported that neurotic patients given MDA (75-200 mg)

benefited from the drug when they experienced visions, mystical experiences, and ego-transcendence. The drug was thus valued for its ability to bring to the surface issues which had been buried in the unconscious and promote "therapeutic insight". According to Marquardt et al. (1978), human subjects described various effects from MDA injections including: a sense of well being, increased self-awareness, increased introspectiveness, mood elevation, increased sense of taste and hearing, a "three-dimensionality" to music and hallucinogen-like properties. The hallucinogen-like properties were not the same as those reported for drugs such as LSD but were alleged to be flashes of visual imagery and changes in visual function. Although it is not clear if MDA is a true hallucinogen, it is still referred to as being a hallucinogenic amphetamine (Ricaurte et al., 1985; Thompson & Moerschbaecher, 1984). During the 1970s, when MDA was being promoted for psychotherapeutic purposes, its illicit use gradually increased, reflecting a trend towards the recreational use of psychedelic drugs (Marquardt et al., 1978; Grinspoon, et al. 1986). In the United States, the Federal Drug Enforcement Agency eventually classified MDA in their Schedule I, meaning that MDA had no accepted medical use and had a high abuse liability. However, recreational use continues and a number of MDA overdose deaths have occurred (Ruangyuttikarn & Moody, 1988).

The recreational use of MDA became an increasing concern when it was revealed that MDA is toxic to the serotonergic system in rats (Ricaurte, 1985). The neurotoxicity of MDA has been the subject of recent controversy in the United States because of concerted efforts to reclassify some hallucinogens for medical use (Barnes, 1988). For example, Grinspoon and Bakalar (1986) have promoted the use of psychedelic/hallucinogenic drugs such as LSD, MDA and methylenedioxymethamphetamine (MDMA) in psychotherapy. Recently Grinspoon was able to have MDMA, a derivative of MDA which is also a neurotoxin, reclassified for medical use (Barnes, 1988). In Canada, MDA is a highly restricted drug and is scheduled in Part IV, Schedule H of the Food and Drug Act (Solomon, 1988). Of the 25 drugs listed in Schedule H, MDA is one of the five most popular in terms of its illegal use (Solomon, 1988). According to the Bureau of Dangerous Drugs, in 1987 there were 13 convictions for possession of MDA in Canada. In addition to these convictions, 65 samples of MDA were gathered as evidence by the police. As recently as November 1989, an active MDA laboratory was seized in Ontario which was fully equipped to manufacture MDA and at the time of seizure contained enough chemicals and paraphenelia to make about 8 kgs of this drug (E. Leader, personal communication, April 12, 1990).

In 1988 Janiger and others established the Albert Hofmann Foundation to collect and distribute information on the use of LSD and other hallucinogens to enhance the study of the human conscious (Rayl, 1989). Thus, from a scientific, therapeutic and recreational viewpoint, it appears that interest in MDA and other hallucinogens has declined since the 1960s and 1970s (Blackwell, 1988) but has certainly not disappeared.

MDA - Physiological Effects

MDA is an amphetamine analogue which causes release of serotonin in the central nervous system, producing stimulatory or psychotomimetic effects (Stone et al. 1986; Battaglia et al. 1987). However, MDA also effects the peripheral nervous system (Stone et al. 1986) and this is most dramatically demonstrated in the clinical picture of MDA overdose (hyperpyrexia, hypertension, tachycardia, rigidity, seizures, and respiratory distress) (Simpson & Rumack, 1981). In at least one case, none of the standard treatments for drug overdose were successful and the patient succumbed after severe hyperpyrexia, repeated seizures, cardiac and cerebral edema. Retrospective analysis of the symptoms and review of the literature lead to the recommendation that future overdose cases be treated by blocking MDA both centrally and peripherally using

phentolamine (alpha blocker), and methysergide (5-HT antagonist) (Thiessen & Cook, 1973; Simpson & Rumack, 1981). Other research has emphasized the effects of MDA on release of central 5-HT, for example, citalopram, a 5-HT uptake inhibitor, blocked the release of striatal 5-HT in vivo and in vitro (Schmidt et al. 1987), and fluoxetine (5-HT uptake inhibitor) also blocked 5-HT release in hippocampal slices (Nichols et al. 1989). For the purposes of the present series of experiments, the effects of MDA on the central nervous system are emphasized.

Animal Studies - Behavioural

A pattern of behaviours produced by drugs which act on the central serotonergic system has recently been identified. The pattern was named the "5-HT Behavioural Syndrome" (Tricklebank, 1985) and includes forepaw treading with abducted hind limbs, flat body posture, head weaving, Straub tail and wet-dog shakes. The likelihood of occurrence and variability in the pattern of the syndrome are thought to depend on which 5-HT receptor has been activated, as well as on the drug, dose, and species. Specifically, the behaviours are caused by drugs such as p-chloroamphetamine and mescaline which bind to 5-HT receptors and/or cause rapid release of neurotransmitter. MDA binds to 5-HT receptors, (Lyon et al. 1986; Ritz & Kuhar, 1989) causes 5-HT to be released (Johnson et al.

1985), and produces the 5-HT behavioural syndrome. For example, mice injected with 40-90 mg/kg of MDA intraperitoneally (ip) showed stereotypy and "apparent hallucinations" (Marquardt et al., 1978, p.89; Thiessen & Cook, 1973). Hallucinations were believed to occur when mice assumed a "boxing position" and appeared to be fighting an imaginary animal, however, more recent studies indicated that Marquardt et al. were witnessing reciprocal forepaw treading sometimes observed during seizures (Kelly & Markert, unpublished observation) or during the 5-HT behavioural syndrome (Tricklebank, 1985). These mice also demonstrated increased salivation, respiratory rate, motor activity, reactivity, convulsions, stereotypy and ataxia with death occurring in some animals (Thiessen & Cook, 1973; Marquardt et al., 1978).

The doses in the above studies were high and caused obvious physical symptoms and lethality (40-90 mg/kg) (Thiessen & Cook, 1973; Marquardt et al., 1978). However, lower doses of MDA (1.5-0.5 mg/kg) produced more subtle effects and were used to examine operant behaviours. MDA caused a decreased rate of responding in complex operant behaviour in a dose-related manner but there were no adverse consequences in learning or accuracy of performance (Thompson & Moerschbaecher, 1984; Nader et al. 1989). The rate-decreasing effects of MDA were attenuated with the 5-HT antagonists metergylone and ketanserin, but not by the noradrenergic alpha-1 antagonist prazosin (Nader et al.).

Thus, even at low doses, MDA affected behaviour seemingly acting through the serotonergic system.

Animal Studies/Neurotoxicity

Analysis of brain tissue with high performance liquid chromatography (HPLC) revealed that rats injected intraperitoneally (ip) with MDA 1.25 to 40 mg/kg in an acute or chronic manner (Ricaurte et al. 1985; Stone et al. 1986, Schmidt et al. 1987) had dose-dependent depletions. A minimum dose of 5 mg/kg was necessary to cause a statistically significant decrease in 5-HT levels (Ricaurte et al. 1985). Even one injection of MDA, e.g. 10 mg/kg, produced a 32% decrease in 5-HT in the hippocampus (Ricaurte et al. 1985). A consistent finding was the depletion of 5-HT and 5-HIAA in the hippocampus, striatum and frontal cortex (Ricaurte et al. 1985; Stone et al. 1986; Schmidt et al. 1987). These findings, were correlated with silver staining of degenerated terminals in the striatum and hippocampus and reduced numbers of serotonin uptake sites in the hippocampus (Ricaurte et al. 1985), however, immunocytochemistry revealed greater terminal degeneration in the neocortex, striatum and thalamus with partial sparing of the hippocampus (O'Hearn et al. 1988). Battaglia et al. (1987) reported a discrepancy between monoamine depletions and terminal degeneration i.e. although no 5-HT

depletions were found in the hippocampus, terminal degeneration in this area was evident. It is possible that differences in techniques to measure terminal degeneration and depletions resulted in different outcomes, with certain methods considered more sensitive than others (O'Hearn et al, 1988). With the exception of some brief elevation of DA, DOPAC and HVA levels (Stone et al. 1986), the catecholamines remained unaffected in in vivo studies (Ricaurte et al. 1985; Stone et al, 1986; Battaglia et al. 1987; O'Hearn et al. 1988;). In in vitro studies, MDA caused release of DA as well as 5-HT (Johnson et al. 1986; Schmidt et al. 1987; Nichols et al. 1989). However, there is evidence for a regulatory connection between DA and 5-HT systems (Lyness et al. 1980; Lecesse & Lyness, 1984), and it is possible that in vitro studies are not able to reflect this mechanism and release of both neurotransmitters occurred in isolation of any regulatory system.

Mechanism of Release/Neurotoxicity

Because 5-HT uptake blockers prevent some of the neurotoxic effects of MDA (Schmidt et al. 1987) it has been proposed that MDA must be taken up into the terminals before it can have neurotoxic effects. It is possible that once inside the terminal, MDA is altered to a toxic metabolite, as has been proposed for other amphetamines (McMillen, 1983; Seiden & Vosmer, 1984; Dougan et al. 1987). There is evidence that metabolites are responsible for the

neurotoxicity of MDA rather than the drug itself since injections of MDA into the cerebral cortex did not destroy 5-HT axons (Molliver, et al. 1986). However, the identity of these metabolites remains unknown and in fact injection of the precursors of the major metabolite of MDA (alpha-methyldopamine; Marquardt et al., 1979) systemically or intraventricularly revealed no evidence of serotonin depletions (McCann & Ricaurte, 1989).

Taken together, the evidence suggests that MDA is toxic primarily to the serotonergic system, however, the exact mechanisms of action and metabolites involved remain unknown.

Drug Self-Administration - Raison d'etre

Background

Many drugs which are considered to have high abuse potential had this distinction verified using the self-administration paradigm (Yokel & Pickens, 1974, Johanson et al. 1976; Collins et al. 1984; Ritz et al. 1987). However, the placement of MDA in this category was based largely on clinical evidence and continuing recreational use of this drug (Blackwell, 1988; Ruangyuttikarn & Moody, 1988). Only one study attempted to measure MDA self-administration (Griffiths et al. 1976) which is surprising, considering that drug self-administration by animals in laboratory

settings is well established as a method of assessing the abuse liability of drugs (Collins et al. 1984). Further, the experimental evidence corresponded with clinical evidence regarding drug use (Griffiths et al. 1978). Verifying the abuse potential of a drug may not seem important until one considers the recent legal battles which resulted in the reclassification of harmful drugs to allow medical use (Barnes, 1988). Perhaps drug self-administration data, were it available, may have provided evidence against the reclassification.

General Method

A typical method to assess the abuse liability of a drug with the self-administration paradigm is as follows: Before being exposed to a drug, animals in the self-administration paradigm are trained in a Skinner box apparatus to press a lever for food reward. These animals are subsequently implanted with an indwelling cannula and trained to press the lever for an injection of cocaine. The cocaine is later substituted with the drug under examination. The capacity of this drug to maintain responding is considered a measure of the "reinforcing" properties of the drug. In most studies, animals are given access to the drug on a fixed ratio (FR) schedule for 3-5 hours per day. An inverse relationship exists between rate of response and drug dose as the animals titrate the dose to

maintain a constant blood level of drug (Yokel & Pickens, 1974; Roberts et al. 1989). Higher doses of a drug are usually more reinforcing than lower doses (Griffiths et al., 1978, Collins et al. 1984, Roberts et al. 1989). Of course, other rate altering effects, such as sedation or hyperactivity, cannot be completely eliminated (Collins et al. 1984; Johanson, et al. 1976). The aforementioned provides a measure of drug reinforcement which demonstrates whether a drug would be self-administered, however, it did not allow comparison of the reinforcing efficacy from one drug to another. To allow comparisons between drugs, a more sophisticated measure such as the progressive ratio (PR) schedule is required (Roberts, 1989; Roberts et al. 1989).

The Progressive Ratio Schedule

The progressive ratio schedule evolved from some of the original obstruction techniques used to measure motivation. For example, an animal was considered motivated to obtain a food reward if it crossed an electrified grid obstruction to get it (Hodos, 1961). When an animal failed to cross the grid, the motivation level was believed to have declined and the animal had reached a "breaking point" in behaviour. The use of repeated electric shocks in these studies lead to variability in results and difficulty in interpretation, prompting Hodos to replace the electric shock obstruction with one of a progressive ratio

schedule. In this paradigm, rats in a Skinner box were rewarded with food initially for pressing a lever twice, then four times, then six times and so on within a session. Hodos found that during a session, rats would work their way up the schedule until a breaking point was reached and responding ceased. The PR schedule has been revised and used to measure the self-administration qualities of various drugs. For example, while the ratios used by Hodos increased throughout a session, Griffiths et al. (1975) used a PR schedule which increased in ratio every 7 days providing that response requirements were met. In subsequent studies, the ratio was increased every day (Griffiths, et al., 1978; 1979). Within-subject comparisons revealed that baboons self-administered drugs on the PR schedule but different drugs yielded different breaking points depending on the dose of drug tested e.g. cocaine yielded higher breaking points than diethylpropion, chlorphentermine and fenfluramine. As well, a dose response curve for cocaine on the PR schedule was obtained (Griffiths et al. 1978; 1979). A similar schedule has been used to examine opiate self-administration in Rhesus monkeys (Hoffmeister, 1979) and amphetamine and cocaine self-administration in beagle dogs (Risner & Silcox, 1981; Risner & Goldberg, 1982; Risner & Cone, 1986). A rank-ordering of some of these drugs paralleled the clinical evidence of their abuse levels (Griffiths et al. 1978; Hoffmeister, 1979). Further revisions to the progressive

ratio schedule reflected a return to Hodos' original concept i.e. a small and gradual increase in ratio throughout a daily session rather than over a period of days (Bedford et al. 1978; Roberts, 1989; Roberts et al. 1989). For example, Roberts et al. found that rats self-administered cocaine when required to respond on a schedule which escalated in the following manner: 2,3,5,7,9,12 ... etc. Within a five hour session, rats would self-administer cocaine but in most cases would reach a breaking point where self-administration would cease before the fifth hour of the session. In terms of Hodos' description of obstructional techniques to measure motivation, perhaps rats on the PR schedule had reached a level of obstruction which they were not motivated to surpass.

In summary, self-administration of drugs on a PR schedule has proven to be a useful tool in assessing the abuse potential of various drugs.

THESIS EXPERIMENTS

Introduction

Based largely on clinical evidence, MDA is considered to have abuse potential (Simpson & Rumack, 1981; Glennon & Young, 1984; Ruanguttikarn & Moody, 1988). However, only one study has examined this experimentally i.e. baboons self-administered MDA on a FR1 schedule (Griffiths, et al.

1976). There have been no reports of MDA self-administration on the PR schedule or on any other schedule in rats. Evidence of MDA-induced neurotoxicity has been restricted to experiments in which rats received noncontingent ip injections. Taking this into consideration, it was apparent that important questions had been left unanswered: At what doses (if any) would rats self-administer MDA on FR and PR schedules? If rats self-administer MDA, to what extent would this be neurotoxic, compared with injected doses? In order to answer these questions, the following series of experiments were carried out.

Experiment # 1

MDA TOXICITY AFTER SYSTEMIC INJECTION

The first study examined the effects of systemic administration of MDA on 5-HT levels in various brain regions. Ricaurte et al. (1985) and Battaglia et al. (1987) reported that 8 injections of MDA (10-40 mg/kg free base every 12 hours X 4 days) produced significant depletions of 5-HT in the striatum, cortex and hippocampus. These effects were obtained in the Sprague-Dawley rat and the present experiment sought to verify these findings in the Wistar strain used in this laboratory. This replication was a necessary step before proceeding to self-administration and related toxicity studies.

Materials

Drugs

Cocaine hydrochloride was purchased from BDH Chemicals Ltd., Toronto, Canada. ($\pm$)3,4 Methylenedioxyamphetamine hydrochloride was obtained from the Bureau of Dangerous Drugs, Health and Welfare Canada (HPB-DID, lot HPB-R5). The MDA used in Experiments 1-3 was from this sample.

HPLC

(-)-Noradrenaline (NE) hydrochloride, 4-hydroxyphenylethylene glycol (MHPG), 5-hydroxytryptamine (5-HT) creatinine sulphate, dopamine hydrochloride (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxyindole-3-acetic acid (5-HIAA), sodium octyl sulphate (SOS) and 3,4-dihydroxybenzyl-amine (DHBA) hydrochloride were purchased from the Sigma Chemical Co., St. Louis, MO, USA. EDTA and monochloroacetic acid were purchased from J.T. Baker Chemicals, Phillipsburg, New Jersey, USA. Acetonitrile and methanol (HPLC grade) were purchased from the Fisher Chemical Company, New Jersey, USA.

Method

Male Wistar rats weighing 350-380 g (Woodlyn Farms, Guelph, Ontario) were individually housed with food and water ad libitum.

A total of 18 rats were injected subcutaneously (sc)

with MDA 20 mg/kg (free base) at 9:00 a.m. and 9:00 p.m. for four days. Of the 18 rats, 12 had participated in a cocaine self-administration study and 6 were drug naive. Four drug naive rats served as control subjects for the chemical analysis. After the last injection rats were left for two weeks with food and water ad libitum and were then decapitated using a guillotine and the brain quickly removed. A consistent 2 mm slab of tissue was sliced on a brain cutting block over crushed ice, and the frontal cortex, hippocampus, and nucleus accumbens dissected out on ice and stored in 28 x 5 mm micro polypropylene processing capsules at -70 degrees for subsequent analysis with High Performance Liquid Chromatography (HPLC).

High performance liquid chromatography with coulometric detection (Seegal et al. 1986) was used to determine levels of amines and metabolites in these samples which were subsequently sonified in 0.3M monochloroacetic acid containing 0.1 mM disodium EDTA in 10% methanol (HPLC grade). Protein content was measured following the method of Lowry et al. (1951). Following this, the homogenate was centrifuged and the supernatant filtered through a 0.2 μ m regenerated cellulose filter. An aliquot of the filtrate was injected onto a C₁₈ reverse-phase column (Chromatography Sciences Company Inc.) in a high performance liquid chromatography system (Waters) equipped with a coulometric detector (ESA). Output from the detector was plotted and

measured using a Hewlett-Packard HP-3390A plotting integrator. The mobile phase was composed of 0.1M phosphate buffer with 20% acetonitrile and allowed for the separation of the norepinephrine (NE) metabolite, 3-methoxy, 4-hydroxyphenylethylene glycol (MHPG), and the dopamine (DA) metabolite 3, 4-dihydroxyphenylacetic acid (DOPAC). Sodium octyl sulphate (.2mM) was added as an ion-pairing agent and EDTA (.05 mM) was added as an anti-oxidant. Following the method of Mefford (1981), levels of homovanillic acid (HVA), serotonin (5-HT) and its metabolite 5-hydroxyindolacetic acid (5-HIAA) were determined in a separate analysis using 50% acetonitrile added to the phosphate buffer.

Standards were prepared with various concentrations of each compound in the homogenizing solution containing DHBA. 10 microlitres of standard contained 500 picograms of compound for NA, MHPG, DA, DOPAC or 1000 picograms of compound for 5-HIAA, HVA and 5-HT. See Appendix A for a sample chromatograph of a standard and Appendix B for a sample chromatograph of tissue from the hippocampus of a rat brain. The quantities of amines and metabolites in the brain samples were determined from the peak areas of each compound to a standard and comparing them with the standard curve.

Results

A striking difference in mortality rates was noted which seemed dependent on drug history. All 6 of the drug

naive rats which received MDA died after the first injection. Premorbid behaviour consisted of seizure-like activity, unresponsiveness, and rigidity. Five out of twelve cocaine-experienced rats also succumbed after between 1 and 4 of the 8 scheduled injections. Sweating, wet dog shakes, Straub tail, head wagging, forepaw treading and flat body posture were observed after the first two injections of MDA in the cocaine-experienced rats. Stereotypical head wagging continued after the second injection, but the other behaviours subsided.

Biochemical data are based on rats which survived the full dose regimen, i.e. 7 of the cocaine experienced rats, and compared with biochemical data from the 4 drug naive rats. Levels of monoamines and metabolites (expressed as ng/mg/protein) were subjected to two-tailed Student's t-tests (MDA with Control) and the results are shown in Table 1.

Insert Table 1 about here

Analyses of tissue from the hippocampus revealed severe depletions of 5-HIAA, $t(9) = 6.52$, $p < .01$ and a 58% depletion of 5-HT which did not reach significance. 5-HIAA and 5-HT were significantly depleted in the cortex, $t(9) = 3.6$, $p < .01$; $t(9) = 3.8$, $p < .01$ respectively. There was a significant depletion of 5-HIAA in the nucleus accumbens

$t(9)=6.66$, $p<.01$) with a 49% reduction in 5-HT which did not reach significance.

Table 1. Monoamine and metabolite concentrations two weeks after 8 MDA injections 20 mg/kg

HIPPOCAMPUS					
	NE	MHPG	5-HT	5-HIAA	
CONTROL	5.94 ±0.99	2.35 ±0.27	2.14 ±0.20	6.20 ±1.02	
MDA	5.46 ±0.56	2.33 ±0.66	0.90 ±0.44	1.01* ±0.22	
% of CONTROL	92%	99%	42%	16%*	

CORTEX					
	NE	MHPG	5-HT	5-HIAA	
CONTROL	3.46 ±0.51	1.12 ±0.43	1.23 ±0.31	2.85 ±0.80	
MDA	4.60 ± 1.13	0.68 ±1.77	0.24* ±0.10	0.59* ±0.17	
% of CONTROL	132%	61%	20%*	21%*	

NUCLEUS ACCUMBENS					
	DOPAC	DOPAMINE	HVA	5-HT	5-HIAA
CONTROL	44.77 ±7.75	79.41 ±7.00	13.73 ±1.39	8.86 ±2.33	10.05 ±0.60
MDA	49.05 ±4.94	62.23 ±5.67	12.94 ±1.80	4.51* ±0.56	3.42 ±0.66
% of CONTROL	109%	78%	94%	51%*	34%*

Values in ng/mg of protein represent the mean ± SEM. Control N=4. MDA N=7. * p<.01 determined by two-tailed Student's t-test.

Discussion

A consequence was the depletion of 5-HT and 5-HIAA in all mined, although some of these did not reach significance. These biochemical results are similar found in other MDA toxicity studies (Ricaurte et Schmidt et al. 1987; Battaglia et al., 1987). lethality from MDA 20 mg/kg was unexpected. This dose was lethal to all drug naive Wistar rats present study, it was not lethal to the Sprague-Dawley used in other studies (Ricaurte et al. 1985; Schmidt 1987, Battaglia et al. 1987; O'Hearn et al, 1988). Pretesting that rats pre-exposed to cocaine seemed protected from the immediate lethality of MDA injected number of central and/or peripheral mechanisms may be possible, including the capacity of cocaine to act as a uptake inhibitor (Zahniser et al., 1988). However, previous exposure to cocaine did not alter the behavioural response to MDA injections which was similar to that found in studies (Thiessen & Cook, 1973; Marquardt et al. O'Hearn et al. 1988) and resembled the 5-HT behavioural syndrome (Tricklebank, 1985). While cocaine exposures have had some protective effect, it did not prevent depletions of 5-HT and 5-HIAA (49-84%) from occurring in hippocampus, cortex and nucleus accumbens.

Experiment # 2

MDA SELF-ADMINISTRATION

Griffiths et al. (1976) compared the self-administration qualities of MDA with other amphetamines and found that baboons self-administered MDA on a fixed-ratio (FR) 160 schedule at doses ranging from 1.0 to 5.0 mg/kg, with the latter dose producing the most number of infusions. However, no studies have examined MDA self-administration in rats. The following experiments were performed to determine whether MDA would support self-administration in rats at various doses, and to evaluate the acquisition pattern and rate of administration.

Method

Male Wistar rats (Woodlyn Farms, Guelph, Ontario) weighing 350-400 g were initially food deprived and trained to press a lever for food reward. Thereafter food and water was available ad libitum. Rats were subsequently implanted with an indwelling jugular cannula (see Roberts and Goeders, 1987) and housed individually in plexiglas chambers with stimulus light and removable lever. Acquisition of MDA self-administration was examined by first training the animals to self-administer cocaine 0.60 mg/injection

(mg/inj) on a FR1 schedule, after which the cocaine solution was replaced by MDA 0.10 mg/inj (expressed as the salt). In rats demonstrating significant intake, MDA self-administration on FR1 was compared to the well-established self-infusion pattern of cocaine. Inclusion criteria included stable MDA intake with no evidence of equipment failure such as leaking or plugged cannulas. Using the same procedure and criteria, other doses were examined in a different group of rats (N=6). Subsequent to training on cocaine, these rats were allowed access to MDA 0.05 μ g/inj for 5 days, then 0.025 mg/inj for a further 5 days. After completion of the self-administration part of the experiment, rats were singly housed in "shoe-box" cages and sacrificed about 2 weeks later.

Results

Insert Table 2 about here

Table 2. ACQUISITION OF MDA SELF-ADMINISTRATION

Number of infusions during daily 5 hour
session at 0.10 mg/injection on FR1

DAY	1	2	3	4	5	6	7
M99	24	15	15	20	15	13	17
M97	(45)*						
M104	(17)	23	16	19	19	9	7
M95	21	16	10	15	7	12	6
M107	(72)	4	3 *				
M124	32	14	23	12	11	13	12
M122	24	26	32	28	28	21	21
M108	(72)	(63)	19	6	10	9	16
M129	(14)	13	14	9	8	6	12
M135	(15)	9	17	9	13	5	3
M137	(45)	17	17	13	8	9	9
M131	13	12	10	7	4	5	5
M136	(24)	13	16	18	12	16	7
M140	13	4	4	7	11	7	35*
M145	24	20	18	14	12	15	18
M146	(19)	25	26	23	18	28*	

Brackets indicate levers were removed during
the session to prevent overdose

Daily intake in mg during acquisition of

MDA 0.10 mg/inj on FR1

DAY	1	2	3	4	5	6	7
M99	2.40	1.50	1.50	2.00	1.50	1.30	1.70
M97	*						
M104	1.70	2.30	1.60	1.90	1.90	0.90	0.70
M95	2.10	1.60	1.00	1.50	0.70	1.20	0.60
M107	*						
M124	3.20	1.40	2.30	1.20	1.20	1.30	1.30
M122	2.40	2.60	3.20	2.80	2.80	2.10	2.10
M108	2.50	2.50	1.90	0.60	1.00	0.90	1.60
M129	1.40	1.30	1.40	0.90	0.80	0.60	1.20
M135	1.50	0.90	1.70	0.90	1.30	0.50	0.30
M137	4.50	1.70	1.70	1.30	0.80	0.90	0.90
M131	1.30	1.20	1.00	0.70	0.40	0.50	0.50
M136	2.40	1.30	1.60	1.80	1.20	1.60	0.70
M140	1.30	0.40	0.40	0.70	1.10	0.70	*
M145	2.40	2.00	1.80	1.40	1.20	1.50	1.80
M146	1.90	2.50	2.60	2.30	1.80	2.80*	

*Rats which died as a result of MDA overdose

The upper part of Table 2 shows the daily infusion rate of rats (N=12) tested on a dose of 0.10 mg/inj MDA. The lower portion of this table shows the daily levels of drug intake for these rats. Two rats (Subjects M97 and M107) died as a result of drug overdose on their first day of exposure and other rats (M108, M124) showed initially high rates of infusions resulting in hyperthermia, piloerection and lethargy. To prevent further overdoses and lethality the experimental procedure for further subjects was changed so that syringes were filled to a daily maximum of 3 mls of MDA/saline solution and levers were removed at signs of overdose i.e. excessive hitting of the lever within the first hour of the five hour session. In cases where the lever was removed and the session shortened, the number of infusions appears in brackets on Table 2. On subsequent days, rats were less likely to overdose and established a reliable pattern of MDA self-administration. However, two overdose deaths did occur after 5-6 days of MDA exposure (See Table 2, Subjects M140 and M146).

Figure #1 shows the mean level of intake over a 7 day period, demonstrating elevated intake over days 1-3 associated with stereotypical headwagging. After this initial high and variable rate the mean intake became more stabilized on days 4-7 i.e. 16 infusions (SD=5) in a five hour period. Although drug intake was relatively stable, some subjects showed daily fluctuations with high levels of

infusions on one day and low levels the next.

Insert Figure 1 about here

Figure #2 shows the daily event records over 7 days for one subject showing a stable intake pattern (M136) and another subject showing cyclic highs and lows (M135).

Insert Figure 2 about here

Figure 1. Mean ($\pm$ SEM) infusions of MDA at 0.10 mg/
injection during acquisition over a 7 day period.

Figure 2. Event records of rats self-administering drugs. Each line represents one day and each upward deflection of the pen represents one infusion of drug. The two upper panels demonstrate the pattern of infusions for MDA 0.10 mg/inj for two subjects (M135 and M136) over 7 days on FR1. The two lower panels show the typical infusion patterns for rats on cocaine .60 mg/inj, (M146) or amphetamine .06 mg/inj (M115) also on FR1.

A different group (N=6) was tested at MDA 0.05 mg/inj and showed the same basic rate of infusion and pattern of intake as the group on 0.10 mg/inj. Daily infusion rates and drug intake for a 7 day period are shown in Table 3. Some subjects showed fluctuations in intake with daily highs and lows (M202), while others showed generally low rates of intake (M216). The mean daily intake was 14 infusions (SD=2). No overdose deaths occurred at any time at this dose. Subsequent to this, four of these rats were tested on MDA 0.025 mg/inj for 4 days. Rats on this dose had a mean daily intake of 19 infusions (SD=2.16), and showed a significant increase in rate of infusions $t(3)=-7.14$, $p<01$ over the rate at 0.05 mg/inj. There was no significant difference in the amount of drug intake ($t(3)=.80$, $p>.05$) between the 0.025 and 0.05 doses. Again, some animals were cyclic in their intake and no overdoses occurred.

Insert Table 3 about here

Table 3. ACQUISITION OF MDA SELF-ADMINISTRATION
 Number of Infusions during daily 5 Hour
 session at .5 mg/injection on FR1

DAY	1	2	3	4	5	6	7
M207 (16)	2	12	30	7	7	8	
M216	13	1	1	4	9	6	6
M220 (16)		17	13	10	16	9	
M218 (19)		13	18	9	8	13	
M204	17	14	29	17	17	31	
M202	23	22	7	13	10	5	

Brackets indicate levers were
 removed at the session
 to prevent overdose

Daily intake mg of MDA during
 acquisition .05 mg/injection

DAY	1	3	4	5	6	7
M207	0.80	0.60	1.50	0.35	0.35	0.40
M216	0.60	0.05	0.20	0.45	0.30	0.30
M220	0.80	0.85	0.65	0.50	0.80	0.45
M218	0.60	0.65	0.90	0.45	0.40	0.65
M204	0.70	1.45	0.85	0.85	1.55	
M202	1.10	0.35	0.65	0.50	0.25	

Figure #3 (upper panel) demonstrates the difference in mean number of infusions between the 0.025 and 0.05 mg/inj doses for individual subjects revealing a compensatory increase in rate of infusion when the dose is decreased. The lower panel of Figure #3 shows the mean drug intake for individual subjects at these doses, revealing that the actual amount of drug intake remained relatively stable.

Insert Figure 3 about here

Figure 3. Upper panel shows the mean number of infusions for individual subjects as they compensate for change in dose between .025 and .05 mg/inj on FR1. Lower panel shows mean drug intake for individual subjects at .025 and .05 mg/injection MDA on FR1.

Discussion

The present data demonstrated that after a 2-3 day acquisition period rats developed a relatively stable level of MDA self-administration at the 0.10 mg/inj dose with daily fluctuations being evident in some subjects. Fluctuations were also evident in the daily intake of some rats on the 0.05 mg/inj and .025 doses. The general pattern of intake (e.g. double hitting) was similar at all doses examined (see Figure 2). As well, when rats were exposed to the 0.05 and 0.025 mg/inj doses, alterations in dose produced a compensatory increase or decrease in self-administration. When compared to cocaine (See Figure 2 and Roberts et al. 1989), MDA infusion is not as consistent and seems somewhat erratic at all doses examined. Specifically, there were relative highs and lows over days - a cyclic pattern which has been demonstrated in MDA, 3,4-methylenedioxymethamphetamine (MDMA) (Griffiths et al. 1976; Lamb & Griffiths, 1987) and d-amphetamine self-administration (Griffiths et al. 1976; Markert & Roberts, unpublished observation; see Figure 2) which may relate to an initial high rate of intake (called a "loading dose") followed by a decrease in drug infusion until the loading dose wears off (Wilson et al. 1971). However, on average, self-administration behaviour was maintained. Unfortunately, in the case of MDA, the highest dose tested sometimes caused rats to become ill or overdose.

Of interest were the two types of overdose phenomena revealed here. Overdose deaths occurring at the initial exposure to MDA were similar to that from d-amphetamine self-administration studies (Yokel & Pickens, 1974) and is believed to result from a delay in metabolism and consequently a delay in awareness of the drug's effects. A high loading dose is taken at the beginning of a session, followed by a decrease in responding (Wilson et al. 1971; Leccese & Lyness, 1984). However, a second overdose phenomenon occurred on MDA about one week after initial exposure. This phenomenon has been observed in animals with unlimited access to a drug but is not common during limited access (Johanson et al. 1976). It is possible that overdoses at this time resulted from a cumulative effect of MDA causing neurotoxic effects. Increased self-administration of amphetamine and/or overdose deaths have been reported after intracerebroventricular destruction of 5-HT -containing neurons with 5,7-dihydroxytryptamine (5,7-DHT) (Lyness et al., 1980) or after injections of methysergide or cyproheptadine to rats with 5,7-DHT lesions of the medial forebrain bundle (Lecesse & Lyness, 1984). Such lesions may lead to stereotypy, so it is also possible that the bursts of lever responding were a form of stereotyped behaviour (Lyness et al., 1980; Lecesse & Lyness, 1984).

In summary, Experiment #2 demonstrated that despite variations in intake over days, MDA was self-administered on a FR1 schedule in a robust fashion and rats compensated for changes in dose with changes in rate of self-administration.

Experiment #3

MDA ON THE PROGRESSIVE RATIO SCHEDULE

In Experiment #2, MDA was shown to act as a reinforcing stimulus on a FR1 schedule, providing a qualitative measure of this drug. However, this schedule did not measure motivational factors. In order to obtain a comparative measure against cocaine or other drugs, MDA self-administration on a PR schedule was assessed.

Method

Rats (N=9) were implanted with intravenous catheters and trained to self-administer cocaine as described in Experiment #2 except that after demonstrating a regular response pattern for cocaine on a FR1 schedule, rats were switched to a PR schedule on cocaine reinforcement. This consisted of a 5 hour daily session during which rats had to respond on a schedule which increased throughout the session i.e. 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 603, 737, 901, 999. The last ratio completed was defined as the "breaking

point". After at least one week of baseline training on cocaine PR, rats (N=6) were switched to MDA (0.10 mg/injection) on the same PR schedule for 7 days and breaking points recorded. Rats were returned to cocaine FR for 1-2 days and the pattern examined in order to confirm the integrity of cannulas and other equipment.

Results

Experiment # 2 demonstrated that rats would self-administer MDA (0.10 mg/injection) on FR1, however, the present experiment suggested that rats will not respond to higher ratios to receive an injection of the drug (See Figure 4). On the PR schedule, rats failed to earn more than 9 infusions in 5 hours and maintained a low level of MDA self-administration over a period of 7 days. The highest ratio completed was never more than 25. Because of the self-limiting nature of the PR schedule, no overdose deaths occurred. Preliminary data at other doses (0.05 and 0.025) again demonstrated that MDA did not support high breaking-points on the PR schedule.

Insert Figure 4 about here

Figure 4. Pattern of responses and infusions (indicated by arrows) for one subject on MDA 0.10 mg/injection on the progressive ratio schedule during one daily five hour session.

Discussion

Insert Figure 5 about here

While the highest breakpoint on MDA was 25, rats on cocaine PR have reached breakpoints of 999 (Roberts et al. unpublished observation). Figure 5 shows an example of cocaine self-administration on the PR schedule. At least two interpretations are possible for the relatively low level of response to MDA on the PR schedule. One interpretation is that MDA is modestly reinforcing compared to cocaine, and rats are not as motivated to receive this drug. This is reflected in the difference in rates of self-administration between MDA FR1 e.g. a mean of 16 infusions on FR compared to a mean of 6 infusions on PR. While rats may take a high number of infusions on FR1, when forced to pay a high behavioural price the number of infusions decreases. Such a phenomenon has been reported for apomorphine self-administration which did not support the PR schedule, despite robust responding on FR1 (Roberts, 1989). Thus, MDA was more like apomorphine than cocaine, and could be considered a moderately reinforcing drug. Another explanation may be that MDA is a powerful stimulus but the PR schedule failed to capture the motivation. This might occur if MDA disrupted operant behaviours (Thompson & Moerschbaecher, 1984; Nader et al., 1989), making the self-administration self-limiting. Alternatively, the PR

schedule may not be the appropriate one to measure motivation for this drug. Another schedule has been suggested to assess heroin self-administration, and this may be more appropriate (Bennett & Roberts, 1990) (see General Discussion).

Figure 5. Pattern of responses and infusions (indicated by arrows) for one subject on cocaine .60 mg/injection on the progressive ratio schedule during one daily 5 hour session.

COCAINE SELF-ADMINISTRATION

In summary, assuming that this PR schedule measured reinforcement adequately, the above data suggest that in comparison to cocaine, MDA has a low to moderate reinforcing capacity as measured by the PR schedule used here. The possibility exists that this is a more highly reinforcing drug but that was not evident from this paradigm.

Experiment # 4

NEUROTOXICITY AT SELF-ADMINISTERED DOSES

Depletions of serotonin in the hippocampus, striatum and cortex have been reported following ip injections of MDA (Ricaurte et al. 1985; Stone et al. 1986; Schmidt et al. 1987). Neurotoxicity after self-administered doses had not been considered, therefore, Experiment #4 examined the hippocampus, striatum, cortex, and nucleus accumbens from rats which had self-administered MDA on the FR1 schedule. These tissues were assessed for evidence of monoamine and metabolite depletions using high performance liquid chromatography.

Method

Rats from Experiment #2 which had self-administered MDA 0.10, 0.05 and 0.025 mg/inj. (N=17) were housed singly in shoebox cages for two weeks with food and water ad libitum. These rats, along with drug naive rats to be used for comparison, (N=9) were decapitated using a guillotine and

the brain quickly removed. A consistent 2 mm slab of tissue was taken on a brain cutting block over crushed ice and the frontal cortex, hippocampus, striatum and nucleus accumbens dissected out on ice and stored in 27 x 5 mm micro polypropylene capsules at -70 degrees. High performance liquid chromatography was performed as described in Experiment #1.

Results

Levels of monoamines and metabolites (expressed as ng/mg/protein) were subjected to t-tests (MDA with Control) and the results are shown in Table 4. There was a significant depletion of 5-HT in the hippocampus, $t(23) = 2.81$, $p < .01$. This depletion occurred subsequent to the pattern of drug administration demonstrated in the lower portion of Tables 2 and 3 which show the daily intake in mg of MDA over 7 days. No other depletions reached statistical significance. Average daily drug intake ranged from 0.38 to 2.45 mg and total overall drug intake ranged from 6 to 18 mg. Total intake and 5-HT level were negatively correlated (-0.52 , $p < .05$) suggesting that as cumulative drug intake increased, serotonin levels decreased.

Insert Table 4 about here

Table 4. Monoamine and metabolite levels two weeks after MDA self-administration

HIPPOCAMPUS					
	NE	MHPG	5-HT	5-HIAA	
CONTROL	5.72 ±0.80	1.53 ±0.50	0.95 ±0.26	5.26 ±0.30	
MDA	6.50 ±0.49	0.99 ±0.27	0.36* ±0.06	6.09 ±0.31	
% of CONTROL	113%	65%	38%*	115%	
CORTEX					
	NE	MHPG	5-HT	5-HIAA	
CONTROL	5.08 ±0.50	0.61 ±0.13	0.89 ±0.20	3.32 ±0.44	
MDA	4.51 ±0.28	0.55 ±0.05	0.99 ±0.19	4.47 ±0.50	
% of CONTROL	89%	90%	112%	135%	
NUCLEUS ACCUMBENS					
	DOPAC	DOPAMINE	HVA	5-HT	5-HIAA
CONTROL	42.98 ±2.45	89.22 ±8.08	8.43 ±0.95	10.26 ±1.13	16.69 ±1.57
MDA	37.38 ±2.94	91.52 ±5.52	7.75 ±0.72	11.89 ±0.76	20.66 ±3.75
% of CONTROL	87%	103%	92%	116%	124%
STRIATUM					
	DOPAC	DOPAMINE	HVA	5-HT	5-HIAA
CONTROL	42.53 ±2.60	133.20 ±9.13	12.45 ±1.85	8.21 ±1.92	14.63 ±2.29
MDA	53.16 ±3.21	142.41 ±6.83	17.00 ±1.74	6.60 ±0.68	16.86 ±1.28
% of CONTROL	125%	107%	137%	80%	115%

Values in ng/mg of protein represent the mean ± SEM. Control N=9. MDA N=17. * p<.01 determined by two-tailed Student's t-test.

Discussion

Ricaurte et al. (1985) and others (Stone et al. 1986; Schmidt et al. 1987) reported that ip injections of MDA were toxic to the serotonergic system. However, the question of whether MDA would be neurotoxic at self-administered doses was not addressed. The present study revealed that MDA is neurotoxic at self-administered doses.

Previous evidence suggested that the hippocampus was the first region to experience a decrease in serotonin levels after exposure to MDA e.g. one injection of MDA 0.5 mg resulted in serotonin depletions in this region (Ricaurte et al. 1985). Battaglia et al. (1987) found immunocytochemical evidence that MDA ablated a population of fine axons throughout CA1 while many of the thicker axons in all regions were spared. In order to deplete serotonin in other regions, and to cause a concomitant decrease in 5-HIAA, MDA must be administered chronically at high doses, e.g. 20 mg/kg (see Experiment #1, and Battaglia et al. 1987; O'Hearn et al. 1988; Ricaurte et al. 1985). Silver staining after this treatment also revealed degeneration of nerve terminals in the hippocampus and striatum which appeared to be serotonergic (Ricaurte et al. 1985). In the present study, as indicated in Tables 2 and 3, MDA self-administration occurred in small doses over several days. With this pattern of drug intake, MDA toxicity was specific to the serotonergic system, consistent with results from the

injection paradigms. Further, the specificity of neurotoxicity at self-administered doses was confined to the hippocampus. As well, these depletions were present two weeks after cessation of MDA self-administration, indicating that perhaps some form of degeneration had begun.

GENERAL DISCUSSION

Neurotoxicity

MDA was shown to have profound effects on the serotonergic system both in terms of behaviour and neurotoxicity. There was evidence that the Wistar strain was susceptible to the effects of MDA after injections produced the 5-HT Behavioural Syndrome (Tricklebank, 1985), however, it was unexpected that doses such as the ones used in the literature (20 mg/kg) would be lethal to this strain. Nevertheless, the injection paradigm revealed significant 5-HT and 5-HIAA depletions in the cortex, hippocampus and nucleus accumbens. Subsequent to daily self-administered doses, MDA was again neurotoxic, and this toxicity was most evident in the serotonergic system of the hippocampus. Evidence of toxicity at self-administered doses is of concern because self-administration more closely resembles human drug-taking behaviour than does systemic injection (Collins et al. 1984). The possibility exists that human drug abusers also are vulnerable to selective neurotoxicity.

Self-Administration

MDA supported self-administration on the FR1 schedule and rats titrated their doses indicating that they were maintaining a "preferred" blood level (Yokel & Pickens, 1974) of MDA. If one were to observe only this schedule, it would indeed seem to indicate that MDA was reinforcing, however, examination of results from the PR schedule showed that this was not a clear-cut issue. MDA self-administration on the PR schedule did not yield high breakpoints, suggesting that although the drug is reinforcing, it was not worth working for in terms of surpassing an obstruction to get a reward (Hodos, 1961; Roberts, et al., 1989). However, in order to give a fair assessment of motivational factors, future studies should evaluate different schedules such as one used to measure heroin self-administration (Bennett & Roberts, 1990). This schedule examined how the presence or absence of drug in the system might influence drug self-administration i.e. the motivation to begin self-administration when no drug is present in the system vs the motivation to continue intake as blood levels increase. Bennett & Roberts found that rats would respond to reach high breaking points to receive the first heroin injection of the day, but subsequently were less motivated i.e. injections of heroin decreased motivation to seek more drug. Conversely, they found that self-injection of cocaine increased motivation to seek more drug resulting in high breaking points. Perhaps the

motivation to receive an MDA injection is strongest at the beginning of the session or sometime during the session e.g. after a period when pressing the lever ceased.

The possibility also exists that MDA decreased the capacity to respond on a more complex schedule. Studies in which monkeys or pigeons were required to respond on multiple schedules of fixed interval/fixed ratio and four response chains revealed that MDA injections (1-3 mg/kg) caused a disruption in rate of response (Thompson & Moerschbaeher, 1984; Nader et al., 1989). Rats on the PR schedule received between 2 and 5 mg, perhaps causing a decrease in rate of response making responding self-limiting. Considering the important role of the hippocampus in learning and memory processes (Gray, 1987), it is possible that toxicity in this region may adversely affect performance on schedules such as the one used in progressive ratio studies. The relationship between neurotoxicity to the hippocampal system and operant behaviour warrants further investigation.

Therefore, with regards to how "rewarding" MDA is, experimental evidence seems to reflect the drug statistics in that while MDA is an abused substance, it is not one which is self-administered to a large extent (Bureau of Dangerous Drugs, 1987). On neither schedule did MDA reach the levels shown for cocaine (Griffiths, et al. 1978;

Roberts et al., 1989), for which the experimental evidence of extremely high levels of self-administration reflect population trends. This is not surprising given that drugs which bind to 5-HT receptors like MDA and LSD are not as readily self-administered as are drugs which bind to DA receptors such as cocaine and d-amphetamine (Ritz & Kuhar, 1989). Another consideration is the slow onset of the effects of MDA (Jacobs et al. 1987) and likely slow metabolism. This would mean that because of slow clearance of this drug, high rates of drug intake are not necessary to maintain significant blood levels. The relationship between drug clearance and drug self-administration is further supported with the self-administration pattern of cocaine (see Figure 2), a drug which metabolizes rapidly, requiring it to be replaced quickly to maintain a preferred blood level (Cone et al. 1989).

Tolerance to MDA is difficult to assess in that MDA metabolites and their effects are largely unknown and lack of tolerance could be due to central or peripheral mechanisms. Future research on MDA must include detailed studies of its metabolites to help clarify the issue of tolerance and toxicity i.e. while it is clear that the serotonergic system is being adversely effected, it is not known exactly how this takes place (Molliver et al. 1986; McCann & Ricaurte, 1989).

Other important findings were the observation of the 5-HT behavioural syndrome and of the lethal effects at injected doses of 20 mg/kg in drug naive Wistar rats. How the differences in lethality between strains and the neurotoxicity of this indole-like drug relate to human MDA intake remains to be seen, however, there is evidence that symptoms of a fatal overdose in humans resemble those of experimental animals (Simpson & Rumack, 1981).

Finally, a conclusion regarding a larger issue is the capacity of the self-administration paradigm to provide information on the characteristics of various drugs and drug classifications. For example, the hippocampus was the first brain region to experience significant serotonin depletions after MDA self-administration. This suggests a variable sensitivity of brain regions to drug toxicity, which may affect subsequent self-administration of this or other drugs. The danger of neurotoxicity from illicit drug use is being further complicated with the increase in the manufacture and use of designer drugs or drugs which are manufactured by the "street chemists" (Doweiko, 1990), especially considering the dangers inherent in incorrect methods of synthesis. For example, Shulgin and Jacob, (cited in Davis and Borne, 1984) have warned of the dangers of the incorrect synthesis of MDA, resulting in equally or more toxic drugs.

Conclusion

To conclude, this series of experiments provided evidence that MDA is toxic at self-administered doses. They also provided vital information on how to approach a relatively unknown substance and test it for lethality, toxicity and abuse potential at self-administered doses, providing groundwork for development of a model on which to base studies of unknown compounds which are becoming increasingly available. As well, considering that MDA began as a legal drug, prescribed by physicians for many years (Yensen, 1976; Glennon & Young, 1984), the importance of such models should be emphasized in all drug research.

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Appendix A

Chromatograph showing standards for monoamines and metabolites.
10 microlitres of standard contained 500 picograms of
compound for NE, MHPG, DA, DOPAC or 1000 picograms
of compound for 5-HIAA, 5-HT or HVA.

Appendix A

PULL # 1380
ID 1

MAY/10/90 18:56:26

ESTD

RT	AREA	TYPE	CAL#	AMOUNT
3.84	1460500	PB	2R	140.700
5.20	1.1857E+08	SPB	4R	5190.900
6.06	6.7410E+07	DSPB	5R	4174.400
7.41	1.1769E+08	SPB	6R	5527.300
9.01	1202800	D BB		0.000
10.01	80957	D BB		0.000
10.91	4.7329E+07	SPB	7	7719.200
12.46	122560	D BB		0.000
12.83	369480	D BB		0.000
13.37	1260100	D BB		0.000
14.68	5.8708E+07	SPB		0.000
16.24	683400	BB		0.000
17.17	1753600	BB		0.000
20.03	1.1504E+08	SPB		0.000
24.57	2846500	BB		0.000

8 MVR

9 SHIP

TOTAL AREA= 5.3453E+08
MUL FACTOR= 1.5000E+01

Appendix B

Chromatograph of tissue from the hippocampus of a rat brain from a drug-naive rat. This analysis was performed specifically to measure the amount of 5-HT present. The standard for this tissue is shown in Appendix A.

Appendix B

RUN # 1389
ID 1

MAY/10/90 15:22:54

ESTD

RT	AREA	TYPE	CAL#	AMOUNT
3.62	9389900	D BB	1R	400.370
4.54	1021700	BB	3R	74.770
5.45	1.8241E+08	SPB	4R	7985.600
7.73	547810	D BB	6R	25.720
9.41	1.9041E+07	BB		0.000
10.37	1218100	D BB		0.000
11.87	4644800	BB	7	757.550
13.51	794090	BB		0.000
14.57	2893500	BB		0.000
15.44	1763000	BB	8R	353.580
17.12	297520	BB		0.000
18.28	125900	D BB		0.000
18.82	344830	D BB		0.000
19.59	1662200	BB	9R	0.000
21.89	671170	BB		68.791
22.48	689510	D BB		0.000
24.62	155780	D BB		0.000
24.71	168890	D BB		0.000
25.31	132010	I BP		0.000

TOTAL AREA= 2.2709E+08
NUL FACTOR= 1.5000E+01

END

1|5|0|4|9 1

FIN