Discriminative Stimulus Properties of MDMA: The Role of Serotonin and Dopamine

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Contents

Abstract

Rationale: ±3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") produces unique and complex subjective effects which distinguish it from other recreationally used drugs. An understanding of the neurochemical mechanisms that underlie these effects is important in order to assess the potential for MDMA abuse and to inform researchers exploring of the drug's therapeutic potential. The present thesis investigated the neurochemical mechanisms underlying the subjective effects of MDMA using drug discrimination procedures in laboratory animals. Despite evidence that training dose can markedly impact the results of drug discrimination studies, the impact of training dose on the discriminative stimulus properties of MDMA has been largely overlooked. The broad aims of these experiments were 1) to test the ability of two different doses of MDMA to support drug discrimination learning, and 2) to determine the role of serotonin (5-HT) and dopamine (DA) neurotransmitter systems in producing the discriminative stimulus effects of each MDMA training dose.

Methods: Groups of rats were trained to discriminate MDMA (1.5 or 3.0 mg/kg) from saline or to discriminate MDMA (1.5 or 3.0 mg/kg) from amphetamine (0.5 mg/kg) and saline, using two- or three-lever, food-reinforced drug discrimination procedures. The first experiments determined the impact of training dose on the acquisition of the MDMA discrimination. Reliability of the discrimination was assessed by measuring the impact of changes in acquisition criteria. Once the discrimination had been acquired, generalisation tests were carried out in two-lever experiments with the SSRIs, fluoxetine and clomipramine, the 5-HT_2 agonists, mCPP and DOI, and the 5-HT_1 agonists, 8-OH-DPAT and RU-24969, to investigate the role of 5-HT in the discriminative stimulus effects of 1.5 mg/kg vs 3.0 mg/kg MDMA. Next, the role of DA was investigated in further generalisation test sessions with the DA releasing stimulant, AMPH, the non-selective D_1/D_2 agonist, apomorphine, the D_1 agonist, SKF38393, and the D_2 agonist, quinpirole. Finally, experiments were carried out in which the ability of the 5-HT_{2A} antagonist, ketanserin, the 5-$HT_{1B/1D}$ antagonist, GR-127935, the 5-HT_{1A} antagonist, WAY100635, the D_1 antagonist, SCH23390, and the D_2 antagonist, eticlopride, to attenuate the discriminative stimulus effects of 1.5 mg/kg vs 3.0 mg/kg MDMA was assessed.

Results: A higher training dose of MDMA was associated with a more rapid acquisition of drug discrimination in both the two- and three-lever tasks, and significant differences were observed with respect to the ability of each dose of MDMA to maintain consistently accurate discrimination across both tasks. All of the serotonin agonists that were tested generalised to the discriminative stimulus effects of 1.5 mg/kg MDMA in a two-lever discrimination task. In contrast, only agonists for 5-HT_{1A} or 5-HT_{2A} receptors generalised to the discriminative stimulus effects of 3.0 mg/kg MDMA. Non-selective dopamine agonists generalised to the discriminative stimulus effects of 3.0 mg/kg but not 1.5 mg/kg MDMA, whereas selective D_1 and D_2 agonists failed to generalise to the discriminative stimulus effects of either training dose. None of the DA or 5-HT antagonists tested had a marked impact of the discrimination of 1.5 mg/kg MDMA whereas administration of a D_2 antagonist produced a small but significant attenuation on the discriminative stimulus effects of 3.0 mg/kg MDMA.

Conclusions: The results of the present thesis suggest that the discriminative stimulus effects of MDMA may change both quantitatively and qualitatively as a function of dose. The subjective effects produced by lower doses appear to be mediated primarily via serotonergic mechanisms, whereas higher doses may involve the additional recruitment of dopaminergic mechanisms. These findings have implications for our understanding of MDMA in terms of the drug's potential for dependence and abuse.

List of abbreviations

AMPH	Amphetamine
5-HT	Serotonin
C_{max}	Peak Plasma Concentration
CNS	Central Nervous System
CPP	Conditioned Place Preference
DA	Dopamine
DAT	Dopamine Transporter
DRN	Dorsal Raphe Nucleus
ED_{50}	Half maximal effective dose
FR	Fixed Ratio
GABA	γ-aminobutyric acid
HPLC	High Performance Liquid Chromatography
K_i	Inhibitory constant
LSD	Lysergic acid diethylamide
MA	Methamphetamine
MDMA	±3,4-methylenedioxymethamphetamine
NAc	Nucleus Accumbens
NE	Norepinephrine
PFC	Prefrontal Cortex
PTSD	Post-traumatic Stress Disorder
SERT	Serotonin Transporter
SEM	Standard error of the mean
SDL	State-dependent learning
SSRI	Selective Serotonin Reuptake Inhibitor
VTA	Ventral Tegmental Area

List of Ligands

DOI	5-HT_{2A} agonist
8-OH-DPAT	5-HT_{1A} agonist
CGS10746B	Dopamine Release Inhibitor
Clomipramine	Selective Serotonin Reuptake Inhibitor
Eticlopride	D_2 agonist
Fenfluramine	5-HT releasing agent
Fluoxetine	Selective Serotonin Reuptake Inhibitor
Fluvoxamine	Selective Serotonin Reuptake Inhibitor
GBR12909	Selective Dopamine Reuptake Inhibitor
GR-127935	5-$HT_{1B/1D}$ antagonist
Haloperidol	D_2 antagonist
Ketanserin	5-HT_{2A} antagonist
Mazindol	Selective Dopamine Reuptake Inhibitor
mCPP	5-HT_2 agonist
NAN-190	5-HT_{1A} antagonist
Pirenperone	5-HT_2 antagonist
Quinpirole	D_2 agonist
RU-24969	5-$HT_{1B/1A}$ agonist
SCH23390	D_1 antagonist
SKF38393	D_1 agonist
WAY100635	5-HT_{1A} antagonist

General Introduction

MDMA

History and Legal Status

±3,4-methylenedioxymethamphetamine (MDMA) is the primary psychoactive ingredient in the recreationally used drug most commonly referred to as "ecstasy". The amphetamine analogue was originally patented by the German pharmaceutical company, Merck, in 1912 as a parent compound during the development of haemostatics (Benzenhöfer & Passie, 2010). Some pre-clinical experiments were carried out by the company over the following decades but scientific interest in the psychological effects of MDMA was not piqued until the 1970s, largely due to the contributions of Alexander Shulgin. He described the experience of MDMA as an easily-controlled, altered state of consciousness with emotional and sensual overtones (Shulgin & Nichols, 1978) and the compound was subsequently adopted by therapists who began administering it as an adjunct to traditional counselling sessions (Cohen, 1995). However, optimism regarding the therapeutic potential of MDMA was short-lived. MDMA was made illegal in the UK in 1977 and the United States Drug Enforcement Administration (DEA) placed the drug on the highest schedule of controlled substances in 1985 citing animal studies that suggested neurotoxicity. In New Zealand, MDMA was initially scheduled under part 2 of Class B in the Misuse of Drugs act in 1975 but this status was recently elevated to part 1 in 2005 ("Misuse of Drugs Act," 1975). The current penalty for importing, manufacturing, or supplying MDMA is a maximum of 14 years imprisonment while possession alone can incur three months jail and a fine of up to $500.

Use and Prevalence

Ecstasy is one of the most widely used recreational drugs in New Zealand with a national survey estimating that approximately 6% of the adult population had tried the drug at least once, second only to cannabis in this respect (Ministry of Health, 2010). While the perceived availability of ecstasy in NZ has been gradually declining over the past decade, a reduction in the price and increase in the purity of

MDMA tablets suggests that there is still a robust market for the drug, particularly in major urban centres (Wilkins, Prasad, Wong, & Rychert, 2014).

MDMA use has long been associated with the electronic music scene and with dance culture. The early 1990s saw the rise of the 'rave' phenomenon which involved all night dance marathon parties (McDowell & Kleber, 1994). These events were typically 'underground' in nature, hosted in private, unregulated environments where the open use of ecstasy and other substances was common. In the last decade, electronic music has rapidly become part of the mainstream. Modern day Electronic Dance Music (EDM) festivals such as 'ULTRA' and 'Future Music' attract hundreds of thousands of concert-goers every year. The association between dance music and ecstasy use remains strong (Murphy, Wareing, & Fisk, 2006) and will likely serve to encourage a new generation of MDMA-users. Indeed, several of these large events have been mired in controversy following tragic drug-related incidents. One study analysed wastewater samples over the course of an EDM festival in Australia and detected significant increases in levels of MDMA over the course of the 6-day event (Lai et al., 2013).

Pharmacology

MDMA is consumed as a racemic mixture containing even amounts of its two enantiomers, S-(+)-MDMA and R-(-)-MDMA. Each stereoisomer varies in its potency and behavioural effects, including both reinforcing (Fantegrossi, Ullrich, Rice, Woods, & Winger, 2002) and discriminative stimulus properties (Bondareva et al., 2005; Schechter, 1987). For the purposes of the present thesis, however, all mention of MDMA will refer to racemic MDMA unless otherwise stated.

The pharmacology of MDMA has been studied extensively in animals. MDMA penetrates the blood brain barrier and interacts with several recognition sites in the central nervous system (CNS), but displays the strongest affinity for the serotonin transporter (SERT) followed by the norepinephrine (NET) and dopamine (DAT) transporters (Battaglia, Brooks, Kulsakdinun, & De Souza, 1988). Direct binding of MDMA to α_2 adrenergic, 5-HT_2, H_1 histamine, β adrenergic, dopamine D_2 and D_1 receptors was also reported. Activity at these sites is significantly lower than that of the monoamine transporters and any receptor-mediated behavioural effects are likely to be produced indirectly through release of endogenous neurotransmitters.

The primary effect of acute MDMA exposure is a marked elevation in extracellular 5-HT (Baumann, Clark, Franken, Rutter, & Rothman, 2008; Baumann, Clark, & Rothman, 2008). This elevation is caused by a dual-mechanism whereby the drug stimulates the release of 5-HT while simultaneously preventing reuptake. MDMA first enters 5-HT neurons via the SERT and in doing so reverses the action of the transporter protein so that 5-HT is ejected from the cell (Rudnick & Wall, 1992). Once inside the neuron, MDMA interacts with vesicular monoamine transporter (VMAT) which is responsible for repackaging unbound cytosolic 5-HT into vesicles. When MDMA binds to VMAT, the transporter is reversed preventing the repackaging of 5-HT and increasing 5-HT levels within the cell. This effect synergises with the reversal of the SERT to produce substantial efflux of 5-HT into the synapse.

MDMA produces increases in extracellular DA via a similar mechanism. MDMA binds to the DAT, inhibiting reuptake, and preventing the clearance of DA from the synapse. Microdialysis *in vivo* showed that administration of DA reuptake inhibitors, mazindol and GBR12909, significantly reduced DA levels produced by an acute administration of MDMA (Nash & Brodkin, 1991). Interestingly, administration of the 5-HT reuptake inhibitor, fluoxetine, also attenuated DA release suggesting a possible interaction between the 5-HT and DA systems. MDMA shows a greater affinity for the SERT (K_i = 72 nM) than it does for the DAT (K_i = 278 nM) (D. C. Jones, Lau, & Monks, 2004; Setola et al., 2003) which likely explains why in-vivo microdialysis studies have consistently reported MDMA-produced increases in 5-HT that are markedly higher than the corresponding increases in dopamine (see Table 1.1; adapted from Schenk, 2011). It should be noted that lower doses (1 – 1.5 mg/kg) of MDMA are sufficient to produce marked increases in 5-HT (250-700%) without significantly altering dopamine release (100-200%). Only following doses of MDMA exceeding 2.5 mg/kg are dopamine levels increased to such an extent that observable DA-mediated behavioural effects might be expected.

There is evidence of MDMA-produced neurotoxicity in laboratory animals. Subcutaneous injections of MDMA (10-40 mg/kg) administered twice a day for four days led to reductions in 5-HT, DA and NE levels in the hippocampus, hypothalamus, striatum, and cortex of guinea pigs (Commins et al., 1987). A single subcutaneous injection of 10 mg/kg was sufficient to cause depletion of cortical 5-HT levels – measured by high performance liquid chromatography (HPLC) – which

was detectable 7 days following MDMA exposure. Reductions in synaptosomal 5-HT uptake were observed in the same study (Schmidt, 1987). The same treatment regimen also produced reductions in the activity of the rate limiting enzyme for the synthesis of 5-HT, tryptophan-hydroxylase which persisted for up to 110 days (Stone, Merchant, Hanson, & Gibb, 1987). Functional 5-HT deficits are considered reasonable indicators of neurotoxicity, but whether these changes reflect neuroadaptations or destruction of serotonergic nerve cells or fibres is unclear. Some studies have reported increases in certain indicators of nerve terminal degeneration such as silver staining (Commins et al., 1987; Jensen et al., 1993) and Fluoro-Jade B staining (Schmued, 2003), following MDMA administration. However, the extremely high doses used as well as the presence of increased staining in brain regions containing few 5-HT neurons suggest that this may be a non-specific effect.

Table 1.1

Summary of microdialysis studies comparing extracellular DA and 5-HT release following acute administration of MDMA (Adapted from Schenk, 2011)

	MDMA (mg/kg)	Brain site	5-HT	DA
Baumann et al. (2005)	1.0, 3.0 (IV)	NAc	1.0 - 700% 3.0 - 1445%	1.0 - 150% 3.0 - 285%
Baumann et al. (2008a)	1.0, 1.5, 7.5 (IP)	Caudate NAc	1.0 - 500% 1.5 - 500% 7.5 - 3000%	1.0 - 150% 1.5 - 0% 7.5 - 500%
Baumann et al. (2008b)	1.0, 3.0 (IV)	PFC	1.0 - 400% 3.0 - 800%	1.0 -200% 3.0 - 400%
Bradbury et al. (2014)	1.0, 3.0 (IV) *(Acq, Non-Acq)*	NAc	1.0 - 250%, 500% 3.0 - 500%, 1250%	1.0 - 100%, 125% 3.0 - 175%, 225%
Golembiowska et al. (2015)	5.0, 10.0 (IP)	PFC NAc Striatum	5.0 - 2000% 10.0 - 6000% 5.0 - 1000% 10.0 - 3000% 5.0 - 300% 10.0 - 700%	5.0 - 500% 10.0 - 1000% 5.0 - 300% 10.0 - 500% 5.0 - 400% 10.0 - 500%
Kankaanpaa et al. (1998)	1.0, 3.0, 9.0 (IP)	NAc	1.0 - 300% 3.0 - 300% 9.0 - 400%	1.0 - 150% 3.0 - 200% 9.0 - 400%
Kurling et al. (2008)	5.0 (IP)	NAc	5.0 - 1400%	5.0 - 600%
O'Shea et al. (2005)	2.5, 5.0 (IP) *(20º, 30º)*	NAc Striatum	2.5 - 0%, 200% 5.0 - 200%, 300% 2.5 - 250%, 250% 5.0 - 350%, 350%	2.5 - 250%, 350% 5.0 - 350%, 350% 2.5 - 200%, 200% 5.0 - 300%, 300%
Shankaran et al. (1994)	7.5 (IP)	Striatum	7.5 - 750%	7.5 - 500%

Dosage and Interspecies Scaling

Humans typically consume MDMA orally in the form of pills or tablets, and the concentration of drug within these pills varies widely. While there are reports of a gradual decline in the amount of MDMA actually present in ecstasy tablets, a large and relatively recent study from the Netherlands reported an average MDMA content of 82.5 mg per pill (Brunt, Koeter, Niesink, & van den Brink, 2012). This is slightly higher than estimates from a UK survey which reported that the MDMA content of most pills ranged between 25 and 74 mg (Morefield, Keane, Felgate, White, & Irvine, 2011). However, it was noted that users who consumed pills containing less MDMA were more likely to consume them in greater numbers meaning that the cumulative dose per session was closer to 100-120mg regardless of pill content. Incidentally, this dose range was reported as most likely to produce 'desirable effects' with 'undesirable effects' predominating at doses higher than 120mg (Brunt et al., 2012). Thus, most evidence suggests that recreational users consume MDMA in quantities of between 1.0 – 2.0 mg/kg.

Meaningful extrapolation of preclinical data from animal studies requires that drugs are administered at doses that are comparable to those taken by humans. Within mammals, many physiological aspects, such as metabolic rate, lifespan, and bone density do not scale in direct proportion to body mass (Boxenbaum & D'Souza, 1990) but instead conform to a relatively simple mathematical power law taking into account both mass and surface area. The result is that the clearance of drugs from the circulatory system tends to be faster in smaller mammals than in larger ones. Allometric scaling of this sort has been used to estimate that a 1.28 mg/kg dose of MDMA in a human may be equivalent to as much as 20 mg/kg in a rat (Ricaurte, Yuan, & McCann, 2000). However, others have highlighted that non-linear pharmacokinetics as well as variations in the metabolic pathways between species make this type of scaling irrelevant (Vollenweider, Jones, & Baggott, 2001). Much of the discussion regarding interspecies scaling has focused on MDMA-produced neurotoxicity and, in particular, differences in metabolite concentrations following hepatic first pass (Baumann et al., 2009). An analysis of pharmacokinetic factors including route of administration suggested that an intraperitoneal injection of 2 mg/kg MDMA produced a peak blood plasma concentration (C_{max}) in rats that was

comparable to the C_{max} produced by a 1.3 - 1.7 mg/kg dose administered orally in humans (Baumann et al., 2009; De La Torre et al., 2000).

An alternative scaling method has been proposed which compares threshold doses required to produce a given behavioural or physiological response in each species. Accordingly, similar minimum doses are required to produce a range of responses such as operant reinforcement, secretion of prolactin, neurotransmitter release, and crucially, drug discrimination in both rat and human subjects (see Table III – Baumann & Rothman, 2009). With respect to the present thesis, the literature to date suggests that a dose of 1.5 mg/kg MDMA in rats represents a typical moderate recreational dose consumed by human. Higher doses in rats (3.0 – 4.5 mg/kg), may therefore be equivalent to the consumption of multiple tablets or pills within a session; something that is known to be the case for some users (Green, Mechan, Elliott, O'Shea, & Colado, 2003).

Abuse and Dependence

Some researchers warn that MDMA is perceived as a "safe" drug by the general public (Green, Cross, & Goodwin, 1995). However, evidence from surveys of both users and non-users suggest that the risks associated with MDMA use are well-known. In one online survey of over 900 subjects, 73% of respondents indicated that they associated ecstasy use with at least 'some risk' and of those, 24% perceived the drug to be 'dangerous' or 'very dangerous' (Gamma, Jerome, Liechti, & Sumnall, 2005). Another study suggested that a large proportion of regular MDMA users attempt to mitigate the risks by employing a range of precautionary strategies such as monitoring fluid intake, attempting to limit consumption, taking rests, and even consuming the serotonin (5-HT) precursor, 5-hydroxytryptophan (Murphy et al., 2006). Despite the apparent widespread use of these strategies and the awareness of potential dangers, there are still individuals who consume a large number of pills during a single session (Parrott, 2001) and some users even met criteria for dependence as defined by the DSM-IV (Cottler, Womack, Compton, & Ben-Abdallah, 2001). In a recent online survey MDMA users were more likely to report at least three DSM-IV dependence symptoms compared to users of other popular club drugs such as cocaine, mephedrone and ketamine. Furthermore, these users were less likely to express a desire to get help or to consume less MDMA

(Uosukainen, Tacke, & Winstock, 2015). Criteria used to define a more general Substance Use Disorder (SUD) in the newly revised DSM-V are also met by some MDMA users including: tolerance to positive effects of the drug (Peroutka, Newman, & Harris, 1988; Yen & Hsu, 2007), escalation in the amount taken per session (Parrott, 2013), and continued use despite the knowledge of negative consequences (Cottler et al., 2001).

Subjective Effects

The development of drug dependence and abuse necessarily begins with an initial period of recreational consumption. As is the case with most drugs, MDMA users often cite the drug's subjective effects as their initial motivation for drug-taking (Cohen, 1995; Solowij, Hall, & Lee, 1992; Verheyden, Henry, & Curran, 2003; B. White et al., 2006). The subjective experience of MDMA use is characterised by a feeling of empathy and closeness with others, leading to its initial classification as an 'empathogen' or 'entactogen' (Nichols, 1986). Acute effects typically peak between 1-2 hours following consumption but some effects can last for several days (Tancer & Johanson, 2001; Vollenweider, Gamma, Liechti, & Huber, 1998). Surveys of current and former MDMA users are consistent with experiments in controlled laboratory settings which report desirable psychological effects including: elation, increased energy, happiness, warmth, calmness, feeling talkative and friendly, closeness, sexual arousal, and others (Brunt et al., 2012; Cohen, 1995; Davison & Parrott, 1997; Nichols, 1986; Tancer & Johanson, 2001; Vollenweider et al., 1998). While some of these effects such as euphoria, and increased energy, resemble those of prototypical stimulants, perceptual changes have also been reported such as increased sensitivity to light and sound, altered perceptions of colour, and enhanced tactile sensitivity (Camí et al., 2000) although such perceptual effects appear to be more prominent in women than in men (Liechti, Gamma, & Vollenweider, 2001). Adverse psychological effects are also commonly reported. These range from anxiety, confusion, nausea, fatigue, bruxism, and headaches (Baylen & Rosenberg, 2006). Some negative effects such as hallucinations, paranoia, depression, and cognitive impairment manifest as part of the peak effects but may remain for several days or even weeks afterwards (Green et al., 2003).

Aside from the obvious hedonic value of MDMA-induced feelings of empathy and well-being, some have proposed that these same properties of MDMA may have considerable therapeutic value in treatment. Political pressure has made clinical testing of MDMA difficult and at least one trial was abandoned before any meaningful results could be obtained (Bouso, Doblin, Farré, Alcázar, & Gómez-Jarabo, 2008). However, more recently, a number of pilot studies have been carried out assessing the safety and efficacy of using MDMA as an adjunct to traditional cognitive behavioural therapy. One study investigated the potential use of MDMA in the treatment of social anxiety in autistic patients (Danforth, Struble, Yazar-Klosinski, & Grob, 2016), and there is now a growing body of evidence supporting the use of MDMA in the treatment of post-traumatic stress disorder (PTSD). Proponents of this idea point out that patients were less likely to drop out of MDMA-augmented therapy programs (Amoroso & Workman, 2016) and that MDMA was associated with improved outcomes on a Clinician-Administered PTSD Scale (CAPS) (Mithoefer, Wagner, Mithoefer, Jerome, & Doblin, 2011) and the self-reported Posttraumatic Diagnostic Scale (PDS) (Oehen, Traber, Widmer, & Schnyder, 2013). Understandably, other researchers have expressed concern over the use of a drug about which much is still unknown regarding its potential for harm and/or abuse (Parrott, 2013).

A small number of studies have attempted to probe the physiological mechanisms that underlie the various subjective effects of MDMA in humans. Co-administration of MDMA with the 5-HT reuptake inhibitor, citalopram, substantially attenuated ‘empathogenic’ effects such as extraversion, and self-confidence, whereas the Dopamine D_2 antagonist, haloperidol, selectively decreased MDMA induced positive mood. The 5-HT_2 antagonist, ketanserin, selectively reduced emotional excitation and perceptual changes (Liechti & Vollenweider, 2001). These findings suggest that different neurochemical mechanisms might be responsible for different components of the MDMA experience. A comprehensive understanding of these different components would prove helpful in the search for therapeutic agents since treatments might be developed which target beneficial effects while mitigating potential unwanted effects. Furthermore, there is also some evidence that the subjective effects of MDMA change as a function of drug-history with feelings of empathy and closeness gradually declining and amphetamine-like stimulant effects

predominating (Peroutka, 1990). Determining the nature of these changes will be crucial for our understanding of MDMA in the context of drug abuse.

The Drug Discrimination Paradigm

Measuring subjective drug effects in human subjects can be difficult from both a practical and an ethical standpoint. Questionnaires rely on the self-reported experience of past and present users, making it difficult to ensure that the data which are obtained are reliably objective. Furthermore, researchers often have incomplete information regarding individuals' drug history and long term studies frequently suffer from high attrition rates. In laboratory settings, the experimental administration of potentially addictive drugs has obvious ethical implications, and the few studies that do so are restricted to very low doses for safety reasons. These limitations can be avoided however, via the use of experimental animals. The drug discrimination paradigm represents a powerful and versatile way to measure subjective drug effects in animal and human subjects.

History of Drug Discrimination Procedures

The ability of certain substances to induce altered states of consciousness has been known for centuries, and the recreational use of many such natural and synthetic compounds persists today. Scientific interest in chemically induced subjective effects can be traced back to the 19th century, when it was first recognised that the psychotic effects produced by some drugs resembled mental disturbances associated with schizophrenia (Beecher, 1959). In those times, before modern neurochemical assays and imaging technologies, these similarities represented early evidence that psychological illnesses might have a physiological basis. Consequently, numerous researchers attempted to characterise the 'psychic effects' of drugs such as mescaline and lysergic acid diethylamide (LSD) in order to better understand schizophrenia and other mental disorders. Understandably, most data from these studies were qualitative in nature (Guttmann, 1936; Klüver, 1928), relying on either an experimenter's own personal experience, or the reported experiences of a brave volunteer. Over several decades, drug-phenomena became the focus of scientific interest in and of themselves, and a more objective approach to drug research was needed.

It was recognised by 19th century clinicians that patients who suffered from somnambulism, or who underwent hypnosis, were later unable to recall events that occurred while they were in the hypnotic state. However, when the altered state was reinstated, the lost memories returned. Similar accounts involving alcohol intoxication emerged. For example, events that occurred while an individual was inebriated and could not be recalled later when they were sober, were clearly recalled when the individual was intoxicated again (Overton, 1984). This led to the hypothesis that an individual's current physiological state, including the presence or absence of drug-effects, determines the availability of memory retrieval at a given time. These concepts were gradually integrated into the fields of learning and memory and labelled state-dependent learning (SDL). The state-dependent learning model is particularly amenable to testing in animals. In a typical SDL experiment, subjects are trained to perform some operant task, but always following administration of a training drug. Once the task is learned, performance of the task is measured in the absence of the drug. The resulting decrease in performance is interpreted as evidence of state-dependence. Studies often employ a range of comparison conditions, such as Saline → Drug group in which animals are trained with saline (drug absent) and then tested under drug conditions, as well as Saline → Saline, and Drug → Drug controls. However, in all cases, subjects only ever receive training with one type of stimulus: drug only or saline only.

The first drug discrimination experiment was designed to address a possible confound produced by SDL type effects. In an investigation into the effect of alcohol on approach / avoidance behaviour, rats learned to avoid a stimulus during alcohol free training sessions. Rats were then divided into two groups which were tested in either the presence (S→D condition) or absence of alcohol (S→S condition). Any resulting decrease in avoidance behaviour in the S→D condition could reflect an intrinsic effect of alcohol. However, the same results could be explained by a type of state-dependent learning elicited by the difference in physiological state during training versus test sessions. Crucially, it was realised that if alcohol did in fact produce a stimulus effect that was distinct from the saline condition, then this could be demonstrated in a discrimination procedure. In other words animals could be trained to approach when drunk but avoid when sober (Conger, 1951). When this experiment was subsequently carried out it represented the first time that a drug was reported to act as a discriminative stimulus in a laboratory setting (Overton, 1991).

An early and important refinement of the discrimination paradigm was the introduction of a symmetrical task. In the avoid/approach procedure described above, stimulus effects of the drug are measured according to the success or failure to perform a trained task. A potential confound is introduced if the training drug inherently produces rate-depressing or disruptive behavioural effects. This confound is mitigated by using a procedure in which stimulus effects are observed in the form of response-selection rather than response-failure. The first such experiments involved a simple T-maze where animals learned to escape a mild electric shock by entering one arm following an injection of drug and the other arm following an injection of saline (Overton, 1961, 1971). The last major refinement was the progression from the T-maze task to a 2-lever operant task. The measurement of food- or water-reinforced lever-pressing allowed for even lower training-doses of drug to be used and greatly reduced the impact of non-specific drug effects. In 1975, a fixed ratio (FR-10) schedule was introduced and an effort was made to standardise the drug discrimination paradigm so that meaningful comparisons could be made between studies from different laboratories (Colpaert, Niemegeers, & Janssen, 1975; Colpaert, Niemegeers, & Janssen, 1976). The basic design of this 2-lever procedure endures and forms the foundation of modern drug discrimination experiments.

The Modern Drug Discrimination Procedure

Modern drug discrimination experiments have become a powerful tool to measure and determine the underlying mechanisms of the subjective effects of drugs. The procedure is equally applicable to human or animals subjects, however animal studies allow for a wider range of doses to be tested as well as greater experimental control (Solinas, Panlilio, Justinova, Yasar, & Goldberg, 2006). In a typical experiment, rats are trained to respond on one lever following the administration of drug and a different lever following the administration of vehicle solution. Training is usually carried out in daily sessions that consist of a number of trials. Each trial within a session requires the completion of a fixed ratio of responses (FR10) on the drug- or vehicle-appropriate lever in order to trigger the delivery of a reinforcer. Under these conditions, only responding during the first trial is controlled exclusively by the stimulus effects of the drug/vehicle injection, since the delivery or non-delivery of a reinforcer may guide subsequent responses. The remaining trials

within a session function only to strengthen the learned association between the training stimulus and its assigned lever. Accordingly, only the distribution of responses during the first trial can be used to indicate whether the discrimination has been acquired.

Training sessions continue until predetermined acquisition criteria are met (discussed in detail in Chapter 3). Briefly, subjects must display a high proportion (often >80%) of responses on the appropriate lever within each session and maintain this level of accuracy for a number of sessions. Figure 1.1 shows hypothetical data depicting the acquisition of a two-lever drug discrimination task. Initially, responses are distributed between drug and vehicle levers approximately evenly. As training continues the number of responses on the drug-lever increases during sessions preceded by a drug injection (closed circles), and decreases during sessions that are preceded by a vehicle injection (open circles). The discrimination is considered to have been acquired once the proportion of correct responses is consistently above 80% (below 20% drug-lever responses during vehicle sessions) which in this example is after approximately 28 sessions.

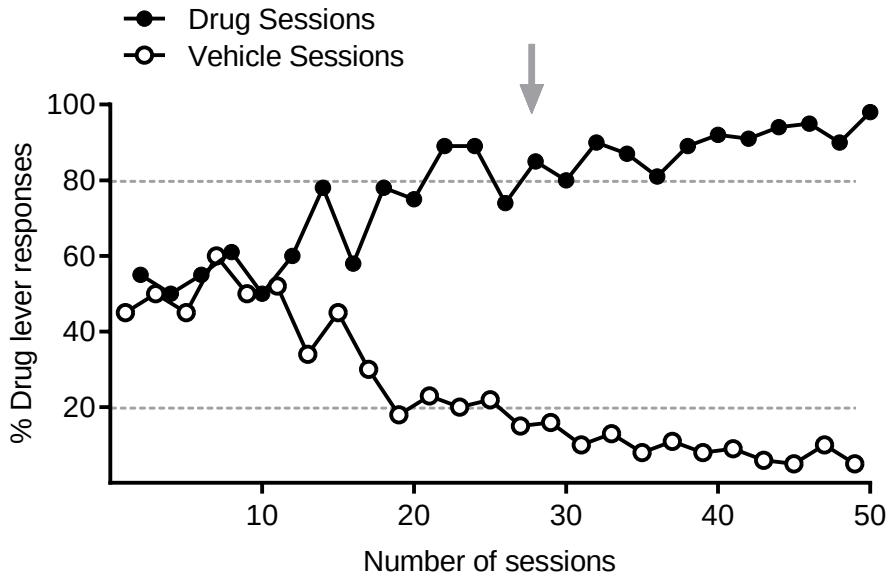

Fig 1.1 Hypothetical data showing the acquisition of drug discrimination. Arrow indicates the point at which drug discrimination has been acquired

Once training is complete, generalisation test sessions can be carried out in order to compare the discriminative stimulus effects of training drug to the discriminative stimulus effects of other compounds. Briefly, a novel compound is administered prior to the session instead of the training drug. If the novel compound elicits responding on the drug-lever then it is assumed to share discriminative stimulus properties with the training drug. When carrying out generalisation experiments, multiple doses are often tested so that dose-effect curves can be generated. Thus, when data from a group of rats is considered, the dose-effect curve takes on a sigmoid shape, as indicated below. Figure 1.2 shows results of a hypothetical generalisation experiment in which sufficiently high doses of a test drug (compound A: > 1 mg/kg) produced effects that fully generalised to the training stimulus. In some cases (compound B in figure 1.2), responding produced by the maximally effective dose appears to be distributed between the two levers. In the case where a test-compound produces only a moderate proportion of drug-appropriate responding – approximately 40-70% – it is said to partially generalise to the training-stimulus.

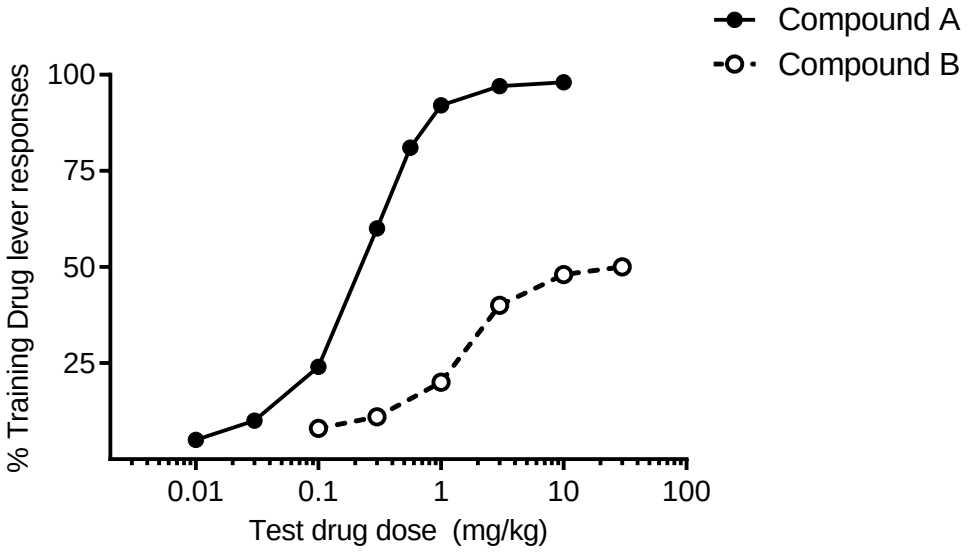

Fig 1.2 Hypothetical data depicting full (compound A) and partial (compound B) generalisation to a previously trained drug stimulus

Partial generalisation is sometimes interpreted to indicate that either the test compound shares at least some discriminative stimulus effects with the training drug, or that it produces a similar but less potent effect. The concept of partial generalisation has led to some controversy regarding the nature of drug discrimination behaviour itself. Some argue that discrimination behaviour reflects a quantal, or 'all or none', response (Colpaert, 1991). That is to say, when asked to discriminate the presence versus absence of the training drug, a subject can only respond with a 'yes' or 'no'. According to this view, intermediate points on a dose-effect curve are simply an artefact produced by averaging multiple data points and that individual subjects in fact switch from 'all-vehicle' responding to 'all-drug' responding once a certain threshold is reached. Others have suggested that drug discrimination responding might be 'graded' so that the distribution of responses between two levers – e.g. 60% of responses on the drug lever vs. 40% on the saline lever – might reflect the degree to which the test compound resembles the training stimulus (Stolerman, 1991). Efforts to reconcile these conflicting ideas usually concede that either approach is valid and that procedural factors such as reinforcement schedule are likely to determine whether quantal or graded responding is observed (McMillan, Li, & Hardwick, 2001).

Discriminative Stimulus Effects

The three-term contingency proposed by Skinner (1938) forms the central tenet of any operant procedure. Briefly, an operant response consists of three components: (1) Context (i.e. the stimuli that precede the response); (2) The response itself; and (3) Reinforcement/Punishment (i.e. events that occur immediately after the response). The preceding stimulus is often called a discriminative stimulus if it reliably predicts the reinforcement or punishment of a particular response. For example, one might train a subject to press a lever in a chamber containing a light. If lever-presses when the light is switched on lead to the delivery of a reinforcer, but lever-presses when the light is switched off do not, then the subject can learn to discriminate between these two 'light' conditions. Thus, the light acts as a discriminative stimulus. The drug discrimination paradigm operates under the assumption that acute drug effects can act as discriminative stimuli. In fact, tasks requiring the discrimination of drugs from vehicle are sometimes learned more

rapidly than tasks involving the discrimination of classical visual stimuli such as light vs. dark test chambers (Overton, 1971).

A possible mechanism by which drugs may act as discriminative stimuli is via the production of physiological effects outside of the CNS, such as increased heart-rate or muscle flaccidity. Such peripheral effects may be discriminable from the 'normal' state and could therefore direct operant behaviour in the absence of psychoactive effects. To test this idea, rats were trained to discriminate saline from either atropine, an antimuscarinic compound with considerable activity in both the CNS and the periphery, or atropine methyl nitrate, a compound with similar peripheral actions but a 10-fold weaker action in the CNS (Overton, 1971). The discrimination of atropine was acquired far more readily, and the discriminative stimulus effects of atropine were detectable at far lower doses suggesting that peripheral actions were not the basis for discriminative control in this experiment. The barbiturate, pentobarbital, produces pronounced muscle ataxia in addition to centrally mediated effects. Pentobarbital, however, was far more easily discriminated from saline than comparable doses of the muscle relaxant, gallamine, (Overton, 1964) further supporting the idea that peripheral effects are a poor basis for drug discrimination behaviour.

One of the earliest applications of the drug discrimination paradigm was to categorise psychoactive compounds into broad classes according to their centrally mediated effects. Initial generalisation experiments revealed that subjects do not simply differentiate between a 'normal' (drug absent) versus 'abnormal' (drug present) subjective state. Instead it appears that drugs produce distinct and identifiable discriminative stimulus effects which likely relate to their specific pharmacological action. As a result, drugs tend to generalise to other members of the same pharmacological class, but not to drugs of a different class (Overton, 1971). For example, administration of stimulant drugs, amphetamine (AMPH), and methamphetamine (MA), in cocaine-trained rats produced generalised responding on the cocaine lever, whereas administration of the hallucinogens, lysergic acid diethylamide (LSD) and mescaline, did not (Hayes & Greenshaw, 2011). An important observation from such experiments was that animals tended to preferentially select the vehicle (no drug) lever when administered a novel class of compound. In other words the no-drug lever appears to be the default response in all conditions except for those which closely resemble the training drug condition. Thus,

the drug discrimination model may be best understood as the discrimination between the presence versus absence of the specific interoceptive cue of the training drug (Frey & Winter, 1978).

There is remarkable consistency between the categorisation of drugs based on the results of drug discrimination studies in humans versus animals. For example, not only did a range of psychostimulant compounds generalise to amphetamine in humans, rats, and pigeons, but they did so in the same rank order of potency in all three species (Kamien, Bickel, Hughes, Higgins, & Smith, 1993). Humans and rats showed similar abilities to discriminate various opioids based on their activity at mu versus kappa receptors (Dykstra, Preston, & Bigelow, 1997) and the relative potency of benzodiazepines, triazolam, lorazepam, and diazepam, to produce discriminative stimulus effects was almost identical in humans, baboons and rats (Kamien et al., 1993). Importantly, discriminative stimulus effects produced in a laboratory setting correspond closely with self-reported subjective effects when both are measured simultaneously in human subjects (Chait, Uhlenhuth, & Johanson, 1985; Schuster & Johanson, 1988). Taken together, these findings support that idea that drug discrimination studies in animals represent a valid and reliable way to study subjective effects in humans.

Investigating Underlying Mechanisms

Psychoactive drugs produce discriminative stimulus effects by acting directly on the CNS via numerous mechanisms. This activity can be specific to a particular neurotransmitter system or it can involve a combination of several different neurotransmitters, receptor sites, transporter proteins etc. The drug discrimination paradigm can be used to probe the mechanisms by which a training drug produces its discriminative stimulus effects. A common approach is to test the ability of a novel drug to produce discriminative stimulus effects that generalise to those of a training drug which has known pharmacological properties. If the two drugs share discriminative stimulus effects, then it can be inferred that they also share some similar underlying pharmacological mechanisms.

In generalisation experiments, the role of specific neurochemical mechanisms can be studied by selecting test compounds that have highly specific pharmacological action. For example, if administration of a selective DA agonist increases responding

on a lever previously associated with a training drug, it can be inferred that the training drug produces discriminative stimulus effects via dopaminergic mechanisms. An alternative approach involves attempting to disrupt or attenuate the discriminative stimulus effects of the training drug by administering selective antagonists. If for example, blockade of 5-HT receptors reduced the percentage of drug-lever responses produced by the training drug, then the discriminative stimulus effects of the training drug are likely to be mediated by the activation of 5-HT receptors. Since, at present, antagonist drugs are typically far more selective than their agonist counterparts, antagonism studies may be better suited to investigating the role of specific receptor subtypes.

As outlined in earlier sections, MDMA has complex pharmacological profile involving multiple neurotransmitter systems and produces a complex set of subjective effects. The versatility and sensitivity of the drug discrimination paradigm make it ideally suited for use in the present thesis in which the neurochemical mechanisms underlying the subjective effects of MDMA are to be investigated.

Discriminative Stimulus Effects of MDMA

MDMA is structurally similar to the stimulant, AMPH, and to the hallucinogen, mescaline, and recreational users report subjective effects that resemble both drug classes. Unsurprisingly, drug discrimination experiments suggest that MDMA has a complex discriminative stimulus profile incorporating stimulant-like as well as hallucinogen-like properties. In two-choice experiments, the hallucinogen, lysergic acid diethylamide (LSD) fully generalised to the discriminative stimulus effects of MDMA in MDMA-trained rats (Gatch, Rutledge, Carbonaro, & Forster, 2009; Schechter, 1998) whereas MDMA failed to generalise to the discriminative stimulus effects of LSD in LSD-trained rats (Callahan & Appel, 1988). Other hallucinogens such as dimethyltryptamine (Gatch et al., 2009), 2,5-dimethoxy-4-methylamphetamine (Gatch et al., 2009; Goodwin & Baker, 2000) and mescaline (Schechter, 1998), however, did not generalise to the discriminative stimulus effects of MDMA.

MDMA generalised to the discriminative stimulus effects of AMPH in AMPH-trained pigeons (Evans & Johanson, 1986), and rhesus monkeys (Kamien, Johanson, Schuster, & Woolverton, 1986) but not in AMPH-trained rats (Oberlender

& Nichols, 1988), however generalisation was observed in 3 rats in one study (Glennon & Young, 1984). When MDMA was used as the training-drug, AMPH either failed to generalise (Baker & Makhay, 1996) or only partially generalised to the discriminative stimulus effects of MDMA (Glennon & Misenheimer, 1989; Oberlender & Nichols, 1988; Schechter, 1988). This asymmetrical pattern of generalisation is sometimes interpreted to indicate that AMPH and MDMA share some, but not all, of their discriminative stimulus properties. However, an alternative explanation is that differences between the training conditions of these studies may have influenced the results of subsequent generalisation tests. Specifically, rats trained to discriminate AMPH from saline are repeatedly exposed to AMPH injections over several weeks or months, whereas rats trained to discriminate MDMA from saline are instead exposed to MDMA in the same fashion.

Repeated exposure to AMPH led to neuroadaptations which enhanced the behavioural response to subsequent challenge doses of the drug (Pierce & Kalivas, 1997; Robinson & Becker, 1986), and the same is true of MDMA (Kalivas, Duffy, & White, 1998; Schenk & Bradbury, 2015; Spanos & Yamamoto, 1989). A question remains, however, whether these neuroadaptations would affect the discriminative stimulus effects produced by a novel injection of MDMA or AMPH in generalisation tests. It is not unreasonable to suggest that previous exposure to AMPH in AMPH-trained rats lead to adaptations which caused MDMA to more closely resemble AMPH. Conversely, different neuroadaptations following previous exposure to MDMA may not have impacted the discriminative stimulus effects of AMPH.

The potential confound resulting from differential exposure to MDMA versus AMPH during training is somewhat mitigated by the use of three-lever drug discrimination procedures. In such procedures, rats are trained to discriminate saline from MDMA and AMPH, and thus are similarly exposed to each of the two drugs. MDMA (1.5 mg/kg) was readily discriminated from AMPH in several three-lever tasks suggesting that the discriminative stimulus properties of the two drugs are at least distinguishable from each other under these conditions (Broadbear, Tunstall, & Beringer, 2011; Goodwin & Baker, 2000; Harper, Langen, & Schenk, 2014). Nevertheless, whether MDMA produces discriminative stimulus effects that more closely resemble dopaminergic stimulants or serotonergic hallucinogens remains unclear.

The discriminative stimulus effects of MDMA have largely been attributed to serotonergic mechanisms. The serotonin releasing agent, fenfluramine, reliably substituted for MDMA in both two-lever (Schechter, 1986) and three-lever tasks (Goodwin & Baker, 2000; Goodwin, Pynnonen, & Baker, 2003) as did the non-selective 5-HT agonist 3-Trifluoromethylphenylpiperazine (Schechter, 1988). Blockade of 5-HT_2 receptors by the antagonist, pirenperone, attenuated the discriminative stimulus effects produced by a 1.5 mg/kg training dose of MDMA in rats (Glennon, Higgs, Young, & Issa, 1992; Schechter, 1988) while the 5-HT_{1A} antagonist, NAN-190, showed a similar but less potent effect (Glennon et al., 1992).

Damage to 5-HT systems caused by various neurotoxic dosing regimens impaired the ability of rats to discriminate a typical dose of MDMA (1.5 mg/kg). Following the repeated administration of a neurotoxic dose of MDMA (20 mg/kg) twice a day for four days, a significant decrease in the sensitivity to a previously trained MDMA-stimulus was observed by way of a downward shift of the MDMA generalisation dose response curve (Schechter, 1991). Similarly, a neurotoxic regimen of fenfluramine (4.0 mg/kg twice a day for four days) transiently disrupted discrimination of a previously trained MDMA-stimulus (1.5 mg/kg) in the sessions immediately following fenfluramine treatment (Baker & Makhay, 1996). Interestingly, a dose of AMPH which had previously produced no MDMA-lever responding, fully substituted for MDMA in post-treatment test sessions. These findings suggest that in the absence of the usually prominent serotonergic effects, the MDMA stimulus may in fact closely resemble AMPH.

This possibility is of particular concern in light of the large body of evidence suggesting MDMA-produced neurotoxicity of 5-HT neurons. In neuroimaging studies, long term or heavy MDMA users displayed significant reductions in SERT binding (Buchert et al., 2003; McCann et al., 2005). As a result, these individuals may gradually become more likely to perceive MDMA as AMPH-like, thus potentially increasing the likelihood of abuse. Indeed, there is some evidence to support this idea from a human drug discrimination experiment in which subjects were trained to discriminate the 5-HT agonist, meta-chlorophenylpiperazine (mCPP), and AMPH from saline. When given MDMA, some subjects perceived it to be mCPP-like however those subjects with a more extensive drug-history tended to perceive MDMA as more AMPH-like (Johanson, Kilbey, Gatchalian, & Tancer, 2006).

The apparent overlap in subjective effects between MDMA and AMPH following serotonin neurotoxicity likely reflects the involvement of DA since dopaminergic mechanisms have been strongly implicated in the discriminative stimulus effects of AMPH and other stimulants (Brauer, Goudie, & de Wit, 1997; Callahan, Appel, & Cunningham, 1991; Schechter & Cook, 1975). While administration of DA agonists and antagonists did not markedly affect the discrimination of 1.5 mg/kg MDMA (Schechter, 1988), there is evidence that DA may play a more prominent role when higher doses of MDMA are administered. In rats trained to discriminate cocaine from saline, MDMA dose-dependently increased the percentage of cocaine-appropriate responding to the extent that a 3.0 mg/kg dose of MDMA partially generalised to the discriminative stimulus effects of cocaine (Kueh & Baker, 2007). In a three-lever task in which rats were trained to discriminate 1.5 mg/kg MDMA, 0.5 mg/kg AMPH and saline, administration of high doses of MDMA (3.0 – 4.5 mg/kg) produced a significant increase in responding on the AMPH lever (Harper et al., 2014).

It is possible that dopamine-mediated discriminative stimulus effects only become apparent once a threshold dose of MDMA is reached. This type of threshold has been observed with other drugs: In rats trained to discriminate the DA reuptake inhibitor, GBR12909, from saline, generalisation by cocaine and methamphetamine was only observed at doses that elevated DA levels by >200-400% (Desai, Paronis, Martin, Desai, & Bergman, 2010). As described previously, only MDMA doses greater than 2.5 mg/kg were able to increase extracellular DA levels to such an extent (Baumann, Clark, & Rothman, 2008). Unfortunately, few studies to date have employed a training-dose of racemic MDMA in excess of 1.5 mg/kg, or tested higher doses in generalisation experiments.

In summary, the results of drug discrimination studies to date clearly implicate a role of 5-HT in the discriminative stimulus properties of MDMA, especially those produced by a relatively low training dose of 1.5 mg/kg. However, due to inconsistent results from studies comparing the discriminative stimulus effects of MDMA and AMPH, as well as the relatively small number of studies that have tested DA ligands in MDMA-trained animals, the role of DA in the discriminative stimulus effects of MDMA remains unclear. Experiments that employ training doses of MDMA that exceed the typical 1.5 mg/kg dose will be of particular importance,

since it appears that the discriminative stimulus effects of higher doses may involve the recruitment of MDMA-produced increases in dopamine.

The Role of Training Dose

The discriminative stimulus effects of drugs can be influenced by a number of procedural factors including training dose. For example, the acquisition of drug discrimination generally requires fewer training sessions as the training dose is increased. This quantitative change in drug discrimination behaviour likely reflects an increase in the potency of the drug stimulus. An upper limit for this effect is reached when the training dose is sufficiently high to produce behaviourally disruptive effects, such as severe motor impairment which may render the subject unable to perform the lever pressing response. Conversely, very low doses may be below the threshold for producing discriminative stimulus effects and may therefore not be easily discriminable from vehicle. For example, discrimination of pentobarbital from saline was learned more rapidly when the training dose was increased from 10, to 15, to 20 mg/kg, while a low dose (5 mg/kg) could not be adequately discriminated from saline within the timeframe of the experiment (Overton, 1971). A similar pattern has been observed with other drugs: discrimination of a high dose (56 mg/kg) of caffeine from saline was learned by a greater proportion of rats and required fewer sessions than the discrimination of a lower dose (10 mg/kg) (Mumford & Holtzman, 1991); the acquisition of a cocaine (10 mg/kg) discrimination required an average of 35 sessions whereas an additional 75 sessions were required when the cocaine dose was reduced to 2 mg/kg (Kantak, Edwards, & Spealman, 1995).

Decreases in training dose resulted in a leftward shift of the dose-response function for the training drug itself. Figure 1.3 shows hypothetical generalisation functions produced by two different training doses of the same drug. As might be expected, drug-lever responding increases as a function of test dose with maximal responding following administration of the respective training dose. In this example, a clearly visible rightward shift in the dose-response curve can be observed for the 10 mg/kg training dose group. These hypothetical data indicate that some doses which produced generalised responding (i.e. are detectable) in the 1 mg/kg training group are below the threshold to produce significant drug-lever responding in the 10 mg/kg

training group. An easy way to quantify this effect is by calculating the minimum effective dose (ED_{50}) required to produce 50% of the maximum drug-lever responding (represented in figure 1.3 by grey lines). Decreases in ED_{50} values have been observed following decreases in the training doses of cocaine (Callahan, Piercey, & Cunningham, 1992; Schechter, 1997; Terry, Witkin, & Katz, 1994), amphetamine (Stadler, Caul, & Barrett, 2001; Stolerman & D'Mello, 1981), morphine (Young, Masaki, & Geula, 1992), and others.

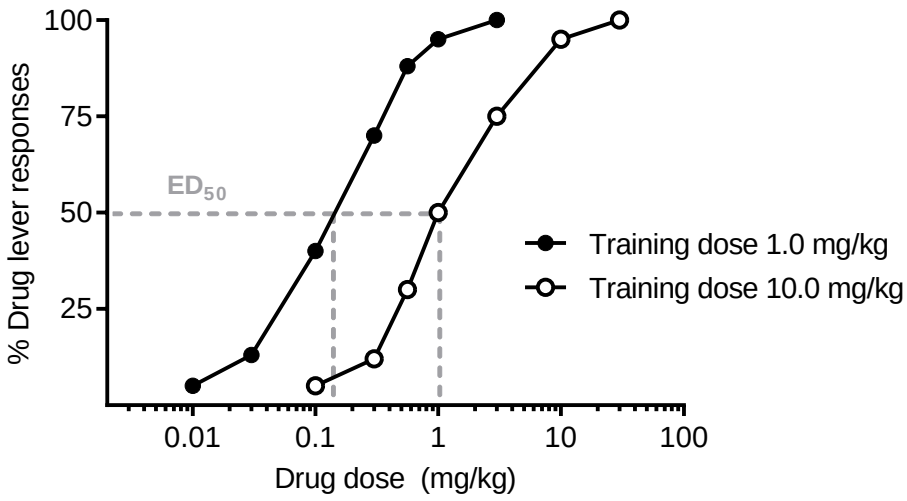

Fig 1.3 Generalisation functions for two different training doses of a hypothetical drug. A test dose (e.g. 0.5 mg/kg) which fully substitutes for the lower training dose stimulus may fail to substitute for a higher training dose

One interpretation of these findings is that extended training with lower doses of drug leads to an increased sensitivity to the discriminative stimulus effects. In other words, subjects that are trained to discriminate low doses of drug become more sensitive to (i.e. better able to detect) the discriminative stimulus effects than subjects trained to discriminate a higher dose (Stolerman, Childs, Ford, & Grant, 2011). Considering that the discrimination of lower training doses often takes longer to acquire, it is unclear whether potential changes in sensitivity are the result of extended practice, or differential exposure to the training drug.

Another interpretation is that different doses of drug produce discriminative stimulus effects that are qualitatively distinct, and involve the recruitment of different or additional neurotransmitter systems. Indeed, qualitative differences between the discriminative stimulus effects of low versus high training doses have been observed with a number of psychoactive drugs. For example, a range of dopamine D_1 receptor agonists including SKF38393, SKF 75670, and CY 208-243, as well as the peripherally acting D_1 agonist, fenoldopam, produced responding that only partial generalised to the discriminative stimulus effects of 10 mg/kg cocaine (Callahan et al., 1991; Witkin, Nichols, Terry, & Katz, 1991), but fully generalised to those of 3 mg/kg cocaine (Terry et al., 1994). This suggests that the discriminative stimulus effects of the 3 mg/kg cocaine dose are mediated primarily by D_1 receptor mechanisms while the discriminative stimulus effects produced by the 10 mg/kg dose may reflect the activation of additional receptors and/or neurotransmitters. In other cases, alterations of training dose resulted in an increased generalisation to one class of compounds but a decrease in generalisation to another. For example, the psychostimulants, cocaine, and amphetamine, produced discriminative stimulus effects that generalised to those of a low dose of caffeine but failed to generalise to those of a high dose (Mumford & Holtzman, 1991). Similarly, the local anaesthetics lidocaine, dimethocaine, procaine, and chloroprocaine substituted fully for a low dose of cocaine but not for a high dose (Wilcox, Paul, Ordway, & Woolverton, 2001).

These findings highlight the importance of testing a range of training doses when investigating discriminative stimulus effects of psychoactive drugs. Considering the complex discriminative stimulus profile of MDMA, and the dose-dependent nature of some of the pharmacological effects of the drug (see previous sections), it is surprising that the role of training dose in the discrimination of

MDMA has so far been overlooked. The majority of drug discrimination studies have employed a training-dose of 1.5 mg/kg of MDMA even though this dose is notably lower than those required to produce a number of other behavioural responses such as conditioned place preference (Marona-Lewicka, Rhee, Sprague, & Nichols, 1996), hyperlocomotion (Brennan & Schenk, 2006), or the reinstatement of drug-seeking (Schenk, Gittings, & Colussi-Mas, 2011). Furthermore, in a three-lever drug discrimination experiment in which rats were trained to discriminate MDMA (1.5 mg/kg) from saline and amphetamine, increasing doses of MDMA (3.0 – 4.5 mg/kg) produced a significant increase in responding on the amphetamine lever (Harper et al., 2014) suggesting that higher doses of MDMA produced qualitatively distinct discriminative stimulus effects.

This is consistent with the pharmacological profile of MDMA since *in-vivo* microdialysis studies have shown that 1.5 mg/kg of MDMA selectively increases extracellular 5-HT but that this selectivity was lost when higher doses of MDMA were administered (Baumann, Clark, Franken, et al., 2008; Baumann, Clark, & Rothman, 2008). In fact, doses in excess of 2.5 mg/kg produced significant increases in extracellular DA (Baumann, Clark, Franken, et al., 2008; Baumann, Clark, & Rothman, 2008; Kankaanpaa, Meririnne, Lillsunde, & Seppala, 1998; O'Shea et al., 2005) which might explain why these doses were required to produce dopamine-mediated behavioural responses in other paradigms. A question remains however, regarding the role of 5-HT versus DA in producing the discriminative stimulus effects of higher doses of MDMA, and therefore testing a range of training doses of MDMA in drug discrimination experiments would provide important and novel information in this respect.

The Current Thesis

Recreational users report that MDMA produces a unique range of subjective effects which resemble both stimulant and hallucinogenic type drugs. The drug discrimination paradigm allows for these subjective effects to be investigated in animals and a number of experiments have examined MDMA-produced discriminative stimulus effects in rats and other animals. Serotonergic mechanisms have been implicated in these effects however few studies have employed selective ligands in order to probe the role of specific 5-HT or other receptor subtypes. Training dose can markedly impact the results of drug discrimination experiments. Despite this however, the majority of studies examining MDMA have only tested a relatively low training dose of 1.5 mg/kg. Higher doses of MDMA increase extracellular DA levels and there is evidence that the discriminative stimulus effects of higher doses may in fact involve the recruitment dopaminergic mechanisms. This raises the possibility that the subjective effects of MDMA change as a function of dose. Exploring this possibility by testing higher training doses of MDMA in drug discrimination experiments will be of particular importance considering reports of gradual escalation in the amount of drug taken per session by some users.

In the present thesis, the discriminative stimulus effects of two different doses of MDMA were compared using drug discrimination procedures in rats. Firstly, the acquisition of drug discrimination was measured to in order to determine the ability of each dose to maintain reliable drug discrimination behaviour. Secondly, the hypothesis that the discriminative stimulus effects of higher doses of MDMA involve the recruitment of dopaminergic mechanisms was tested by administering a range of 5-HT and DA ligands in generalisation and antagonism studies. This thesis therefore provides a broad assessment of the impact of training dose on the role of 5-HT and DA in the discriminative stimulus effects produced by MDMA.

General Methods

This section describes the methods and procedures common to all experiments contained in this thesis. Any deviations from the methods outlined below are described in detail in the relevant chapter.

Subjects

Subjects were male Sprague-Dawley rats weighing 270-300g at the beginning of testing. The rats were bred in the vivarium at Victoria University of Wellington, New Zealand and the colony was maintained on a 12hr reversed light cycle (1900 – 0700 light) in a temperature (21ºC) and humidity (55%) controlled environment. Rats were housed in pairs in standard polycarbonate cages which also contained a short (20cm) PVC 'play' tube. Food was restricted to maintain a bodyweight of approximately 85% of free-feeding weight. Deprivation target weights were periodically adjusted to allow for natural growth. Food was given immediately following experimental sessions while water was available *ad libitum* throughout the study, except during testing.

Apparatus

All experiments were carried out in fourteen commercially available operant chambers measuring 30.5 cm x 24.1 cm x 21.0 cm in the interior (ENV-008: Med Associates Inc.). Each chamber contained three retractable levers (ENV-112CM) and a sugar pellet dispenser (ENV-203-190IR). The dispenser receptacle was located in the centre of the front panel of the chamber with one lever positioned on either side (left and right lever), and the third lever directly above (centre lever). Standard white lights were positioned directly above each of the levers. Experimental events and data collection were controlled by two computers (MED-PC IV ®) and dustless precision sugar pellets were obtained from Bio Serve ® (Frenchtown, NJ).

Procedure

All training and test sessions were carried out during the dark phase of the light/dark cycle (0900 – 1400). Each rat was assigned to a specific operant chamber

for the entirety of the experiment, including all training and test sessions. At the end of each session, rats were returned to their home cage.

Auto-shaping

Experiments began with a series of daily (5 days per week) auto-shaping sessions to establish lever-pressing behaviour. During these sessions, a randomly selected lever was inserted into the chamber and the corresponding light was illuminated for 12 seconds. A single response on the lever (or failure to respond within 12 seconds) resulted in the immediate delivery of a sugar pellet, the deactivation of the light, and the withdrawal of the lever. After a 30 second delay a new trial was initiated and a new lever was inserted. The number of responses required to complete a trial was gradually increased each session until all rats were reliably responding on all available levers at a fixed ratio of 10 responses per reinforcer (FR10). An average of 10 sessions was required for reliable lever-pressing to be established across all experiments.

Drug Discrimination Training

Discrimination training commenced once all subjects had completed auto-shaping sessions. For all subsequent sessions, an intraperitoneal (IP) injection of either vehicle or training drug was administered 15min prior to the first trial. During this delay no lights were activated and no levers were inserted into the chamber. The order in which these daily injections were administered consisted of a 6 session repeating cycle: SMMSSM, where S represents saline sessions and M represents MDMA sessions (two lever experiments) or SAMSMA, where S represents saline sessions, M represents MDMA sessions, and A represents amphetamine sessions (three lever experiments)

For each rat, injections of either vehicle or drug were assigned to a particular lever (e.g. left lever = vehicle, right lever = drug). In two-lever experiments only the left and right levers were used. Vehicle was assigned to the left lever for half of the rats, while drug was assigned to the left lever for the remaining rats. In three-lever experiments, all three levers were used and lever allocations were counterbalanced so that each possible drug × lever combination was evenly distributed between rats.

The first fifteen sessions consisted of an errorless discrimination task in which only the stimulus-appropriate lever was inserted into the chamber (i.e. only the vehicle-appropriate lever following vehicle injections and only the drug-appropriate lever following drug injections) and only the corresponding light was activated. Ten responses on the lever resulted in the delivery of a sugar pellet, the deactivation of the light, and the retraction of the lever. After a 30 second inter-trial interval (ITI), the lever was reinserted signalling the start of a new trial. The session ended after 30 minutes or once 60 trials had been completed. The purpose of these sessions was to establish an initial association between each stimulus and its corresponding lever.

All subsequent sessions consisted of full drug discrimination training. Drug discrimination trials began with the presentation of all available levers (2 or 3 depending on the protocol) and activation of all corresponding lights. Ten consecutive responses (FR10) on the correct lever were required to deliver a sugar pellet and to complete the trial. Responses on an incorrect lever reset the count. In all other aspects, drug discrimination sessions were identical to errorless training sessions described above. Measurements of discrimination performance were based on the allocation of responses during the first trial (FR) of a session.

Generalisation / Antagonist Testing

Generalisation tests were carried out for subjects trained in the two-lever procedure to test the ability of novel compounds to substitute for the training dose of MDMA. The procedure for these sessions was similar to training sessions except that an injection of the test compound was administered prior to the session instead of MDMA or saline. During test sessions, only the first 10 responses on either lever were recorded. Once 10 responses were completed, all lights were deactivated, all levers retracted, and no sugar pellet was delivered.

Antagonist tests were carried out to assess the ability of selective DA and 5-HT ligands to attenuate the discriminative stimulus effects of MDMA. The procedure for these sessions was identical to that of generalisation test sessions except that test compounds were administered prior to an injection of the training dose of MDMA. The training dose of MDMA was administered 15 min prior to the start of the session in the same manner as during training sessions. Pre-treatment times varied between each compound and are described in the relevant chapter.

Drugs

Table 2.1 outlines all of the drugs used in the present thesis. All drugs were dissolved in a 1 ml /kg volume of Saline except for clomipramine, ketanserin, and SKF38393, which were dissolved in a 0.5 ml/kg volume of saline, and for GR-127935 which was dissolved in a 0.5 ml/kg volume of distilled H_2O. All drug weights refer to the salt.

Table 2.1

Summary of experimental compounds

Drug	Doses	Route	Supplier
±3,4-methylenedioxymethamphetamine (MDMA)	0.5 – 3 mg/kg	IP	Environmental Science and Research (Porirua, NZ)
2,5-Dimethoxy-4-iodoamphetamine (DOI)	0.3 – 3 mg/kg	SC	Sigma Aldrich (Australia)
8-OH-DPAT	0.03 – 0.3 mg/kg	SC	Tocris (UK)
Clomipramine	1 – 10 mg/kg	IP	Tocris (UK)
D-amphetamine	0.25 – 0.5 mg/kg	IP	Sigma Aldrich (Australia)
Eticlopride	0.3 mg/kg	IP	Sigma Aldrich (Australia)
Fluoxetine	0.3 – 3.0 mg/kg	IP	Tocris (UK)
GR-127935	3.0 mg/kg	SC	Tocris (UK)
Ketanserin	3.0 mg/kg	SC	Tocris (UK)
meta-chlorophenylpiperazine (mCPP)	0.3 – 2.0 mg/kg	SC	Tocris (UK)
Quinpirole	0.3 – 3 mg/kg	IP	Tocris (UK)
RU-24969	0.3 – 3 mg/kg	SC	Tocris (UK)
SCH23390	0.04 mg/kg	SC	Tocris (UK)
SKF38393	3 – 10 mg/kg	SC	Tocris (UK)
WAY100635	1.0 mg/kg	SC	Tocris (UK)

SC = Subcutaneous Injection
IP = Intraperitoneal Injection

Chapter 3: Acquisition

Parts of this chapter have been adapted from work published in the Journal of Drug and Alcohol Research (Webster, Harper, & Schenk, 2016)

Experiment 1: Two-lever discrimination

The rate at which reliable drug discrimination is acquired provides information regarding the strength of the discriminative stimulus effects produced by the training drug. Specifically, the discrimination of a drug that produces robust stimulus effects should be learned more rapidly and by a greater proportion of subjects than the discrimination of a drug which is not easily distinguished from saline. This idea is apparent when multiple training doses of the same drug are tested since the discrimination of higher training doses, which produce more potent pharmacological effects, is learned more rapidly than the discrimination of lower training doses (Holtzman, 1990; Overton, 1971). In order to ensure that the training dose is sufficient to produce robust discriminative stimulus effects, most drug discrimination studies employ doses that produce other behavioural effects. For example, a dose of 10 mg/kg of cocaine and 1 mg/kg of amphetamine are typically selected. The relatively few studies examining MDMA have generally employed a training dose of 1.5 mg/kg. This is notably lower than doses required to produce other behavioural responses such as conditioned place preference (Marona-Lewicka et al., 1996), hyperlocomotion (Brennan & Schenk, 2006), or the reinstatement of drug-seeking (Schenk et al., 2011). A low dose of 1.25 mg/kg was insufficient to produce reliable discrimination behaviour in rats even after 65 training sessions (Oberlender & Nichols, 1988), raising the possibility that the typical dose of 1.5 mg/kg is just at the threshold for producing discriminative stimulus effects.

Interpretation of data from drug discrimination studies relies on the assumption that subjects are able to consistently and accurately discriminate between the two stimuli, and therefore the choice of acquisition criteria is critical. Rats are typically trained in daily sessions that consist of a number of trials. Each trial within a session requires the completion of a fixed ratio of responses on the drug-appropriate lever – often FR10 – in order for a reinforcer to be delivered. Under this regimen, only responding during the first trial is controlled solely by the interoceptive effects of the drug/vehicle injection since responses in subsequent trials

may be guided by the delivery or non-delivery of a reinforcer. Therefore, the distribution of responses during the first FR10 is often used to indicate whether the discrimination has been acquired. The acquisition criteria usually have two components: 1) Accuracy within the first trial of a session, and 2) The number of daily sessions for which this within-session accuracy must be maintained (Solinas et al., 2006). A within-session accuracy of 80% is commonly reported. Thus, at least 80% of responses emitted before the delivery of a reinforcer must be on the drug appropriate lever. Requirements for the second component vary between studies and are sometimes not even reported, but often require that within-session accuracy must remain above 80% for 10 consecutive sessions (Callahan et al., 1991; Colpaert, Niemegeers, & Janssen, 1980; Kantak et al., 1995; Mantsch & Goeders, 1999; Picker, Doty, Negus, Mattox, & Dykstra, 1990).

It is a matter of concern that most MDMA discrimination studies to date have applied a less stringent acquisition criterion than is typically used for discrimination of other drugs; within-session accuracy must only be maintained for ‘at least 8 out of 10 sessions’ (Oberlender & Nichols, 1988; Schechter, 1986). As with other studies, the order in which MDMA or vehicle is administered typically alternates in a pseudo-random fashion so that vehicle (S) and MDMA (M) sessions are evenly distributed. For example, a ten day sequence may run as follows: SMMSSMSMMS. Thus, in a sequence of 10 sessions, there are a maximum of 6 occasions where the drug administered at the start of a given training session differs from that of the previous session (i.e. there is a change in the discriminative stimulus). It is possible for accuracy to fall below the 80% threshold in two (out of six) of these ‘change’ sessions and to still meet the ‘8 out of 10 sessions’ acquisition criterion. This raises the possibility of spurious results during discrimination training that might be particularly apparent when doses of drug that are just at threshold are tested.

In order to address this issue, the following experiment assessed the discriminability of a low (1.5 mg/kg) versus a higher (3.0 mg/kg) dose of MDMA and determined the impact of changes in criteria on the acquisition of reliable drug discrimination behaviour. It was expected that (1) a higher dose of MDMA would produce a more potent/salient discriminative stimulus and therefore be more readily/rapidly discriminated from vehicle, and (2) employing increasingly stringent acquisition criteria would have greater impact when the lower training-dose of MDMA was used.

Methods

In order to test these hypotheses, two groups of rats (n = 12 per group) were trained to discriminate MDMA (1.5 mg/kg or 3.0 mg/kg) from saline in a typical two-lever discrimination task (see General Methods). A range of different criteria were employed to evaluate responding during drug discrimination sessions. Table 3.1 summarises each of the five different sets of criteria used in the experiment. The first set represents the standard criterion used in the majority of MDMA-discrimination studies to date. This standard criterion was met when at least 80% of responses were on the correct lever for at least 8 out of 10 consecutive sessions. Four additional sets of criteria were also applied. For each of these additional criteria, a within-session accuracy of at least 80% responses on the correct lever was required for an increasing number (4-10) of consecutive sessions (4C-10C).

Table 3.1 Summary of requirements for the various acquisition criteria in 2-lever task

Criteria	Requirements
Standard	*>80% correct for at least 8 out of 10 sessions*
4C	*>80% correct for 4 consecutive sessions*
6C	*>80% correct for 6 consecutive sessions*
8C	*>80% correct for 8 consecutive sessions*
10C	*>80% correct for 10 consecutive sessions*

Only data from full drug discrimination training sessions (i.e. not autoshaping or errorless sessions) are included in the analyses. The number of rats meeting each criterion as a function of test session was determined and the results were used to generate Kaplan Meier survival functions. Data points represent the first session of a sequence that led to the fulfilment of a criterion. The number of sessions required for 50% of rats to meet each criterion was used as a measure of acquisition latency. Response rates were calculated by dividing the total number of responses made during the first trial by the time taken to complete the FR10 requirement. An alpha-level was set at $p < 0.05$ for all statistical tests.

Results

Table 3.2 shows the percentage of rats that met each criterion at the conclusion of the experiment (63 sessions) as well as the number of sessions required for at least 50% of rats to meet each criterion in each condition. Figure 3.1 (top panels) shows the effect of manipulating criterion on the acquisition of drug discrimination for the 1.5 mg/kg (left) and 3.0 mg/kg (right) training-dose conditions. Response rates for each condition during each of the daily saline or MDMA sessions are also shown (bottom panels). Discrimination of a low dose of MDMA (1.5 mg/kg) as determined by the standard criterion was learned rapidly with at least 50% of rats reaching criterion after 11 sessions (see Table 3.2). Manipulating the criteria had a marked impact on the acquisition of the low dose discrimination. As the criterion became more stringent, a smaller percentage of rats acquired the discrimination and more test sessions were required to meet each criterion.

Table 3.2 Summary of latency and proportion of rats meeting each criterion in 2-lever task

	MDMA 1.5 mg/kg		MDMA 3.0 mg/kg	
Criteria	*Percentage of rats that reached criterion*	*First session in which 50% of rats met criterion*	*Percentage of rats that reached criterion*	*First session in which 50% of rats met criterion*
Standard	92%	11	100%	3
4C	100%	12	92%	7
6C	83%	32	92%	8
8C	50%	36	92%	11
10C	42%	-	83%	11

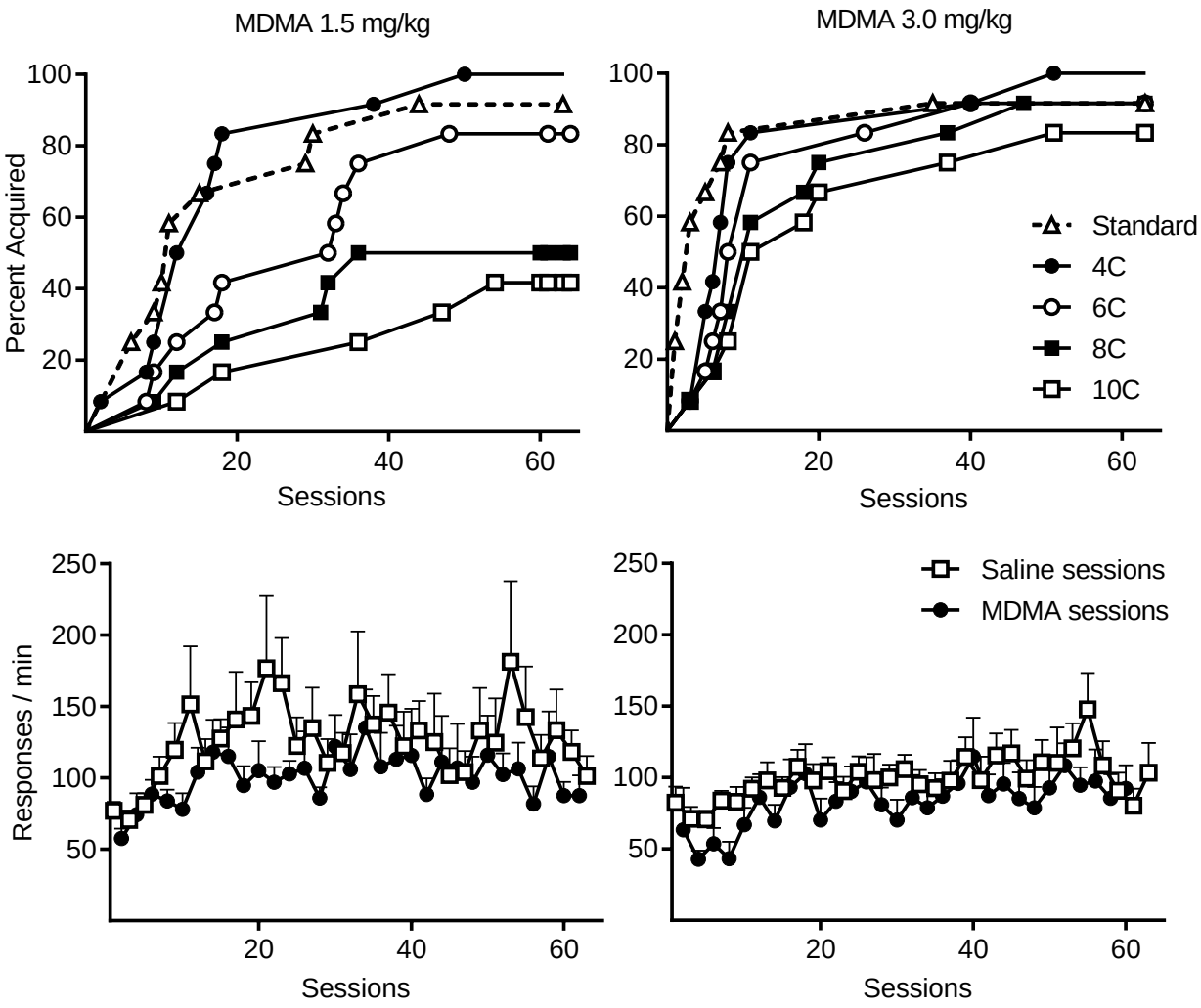

Fig 3.1 Top panels: Effect of manipulating criteria on the acquisition of the discrimination between MDMA 1.5 mg/kg (left) or MDMA 3.0 mg/kg (right) and saline. Symbols represent the cumulative percent of rats that met criterion as a function of test session. Bottom panels: Symbols represent the mean number of responses/min during drug discrimination training sessions and error bars represent standard error of the mean.

Acquisition curves in the low training-dose condition differed significantly as a function of criterion ($\chi 2$ (4) = 24.45, $p < .001$). Follow up tests (Bonferroni corrected) confirmed that the survival function generated by the standard criterion was significantly different from that of the most stringent (10C) criteria ($\chi 2$ (1) = 11.61, $p = .007$). A separate analysis was conducted to determine whether increasing the required number of consecutive correct sessions led to a decrease the percentage of rats that acquired the discrimination. This analysis confirmed that acquisition rates in the low MDMA-dose condition decreased as the required number of consecutive sessions increased ($\chi 2$ (1) = 17.81, $p < .001$).

The impact of criterion on the acquisition of the 3.0 mg/kg MDMA discrimination was less pronounced. The standard criterion indicated that the discrimination was learned very rapidly with at least 50% of rats reaching criterion after 3 sessions (see Table 3.2). Increasing the stringency of the criteria resulted in a moderate increase in the number of required sessions; up to 11 for the most stringent criteria (10C). A log rank test showed that the percentage of subjects that met the acquisition criterion in the 3.0 mg/kg training-dose group did not significantly change as a function of criterion ($\chi 2$ (4) = 9.07, $p = .059$).

The experiment was conducted during a total of 63 sessions which alternated between saline (32 sessions) and drug (31 sessions) as described in general methods. Figure 3.1 (bottom panels) shows the rate of responding during drug or saline sessions for each training-dose condition. Response rates in all three groups remained stable throughout testing with ANOVA revealing no interactions ($F(31,682) = .53$, $p = .98$; $F(31,682) = .36$, $p = .99$).

Discussion

Both doses of MDMA were discriminated from saline however discrimination of the higher dose of MDMA was more rapidly acquired than discrimination of the lower dose. This finding was consistent with other drug discrimination studies in which more than one training dose was used (Holtzman, 1990; Kantak et al., 1995; Overton, 1971; Stadler et al., 2001; Stolerman et al., 2011) and suggests that the higher dose of MDMA produced discriminative stimulus effects that were more easily distinguishable from saline.

This idea was reinforced when the effects of manipulating the criterion for the acquisition of drug discrimination were assessed. The acquisition of the 1.5 mg/kg MDMA discrimination was more easily disrupted by changes in criteria than the acquisition of the 3.0 mg/kg MDMA discrimination. Furthermore, the inability of the majority of rats in the low training dose group to meet the more stringent additional criteria supports the hypothesis that 1.5 mg/kg of MDMA is just at the threshold for producing discriminative stimulus. It must be acknowledged that the present training period was only 63 days in duration. While this was apparently sufficient for the acquisition of the high dose MDMA discrimination, it is possible that the low dose discrimination may have been acquired more reliably following more extensive training.

One explanation for the disrupted acquisition of the 1.5 mg/kg MDMA discrimination is a general disruption of stimulus control. That is, MDMA may produce additional effects that interfere with general aspects of the drug discrimination task such as lever-pressing. One example of this is an increased tendency to continue to respond on a lever that has been previously reinforced even when a task requires a change in response (Harper, 2013). Inflexible, or perseverative, responding of this nature might be particularly disruptive during drug discrimination training in which the training stimulus, and thus the correct lever, alternates between daily sessions. However, this type of disruption would be expected to increase as a function of training dose, and therefore cannot easily account for the apparent lack of disruption of the 3.0 mg/kg MDMA discrimination.

As outlined in general introduction, the primary pharmacological action of MDMA is to increase extracellular 5-HT. Therefore, it might be argued that the more rapid and robust acquisition of the high dose discrimination reflects a greater increase in 5-HT produced by the 3.0 mg/kg dose. However, considering that MDMA (1.5 mg/kg) produced an appreciable increase in 5-HT of approximately 500% (Baumann, Clark, Franken, et al., 2008) it seems unlikely that this would be insufficient to produce robust discriminative stimulus effects. Smaller increases in 5-HT of approximately 350% and 150% were produced by fenfluramine (10 mg/kg) and citalopram (2.5 mg/kg), respectively, and both of these compounds were able to support reliable drug discrimination (>80-85% for at least 10 consecutive sessions) in rats (Auerbach, Minzenberg, & Wilkinson, 1989; Dekeyne & Millan, 2003; F. J. White & Appel, 1981).

Another possible explanation for the difference in acquisition profiles is that the higher dose of MDMA produces additional neurochemical effects that lend to a more easily discriminated stimulus. A likely candidate is the increase in synaptic dopamine that becomes apparent following administration of higher doses of MDMA (Baumann, Clark, & Rothman, 2008). Indeed, evidence from a three-lever task suggested that the discriminative stimulus effects of MDMA became more like the prototypical stimulant, AMPH, when higher doses were tested (Harper et al., 2014), and the discriminative stimulus effects of AMPH have been attributed to dopaminergic mechanisms (Brauer et al., 1997; Callahan et al., 1991; Chait et al., 1985; Ho & Huang, 1975; West, Van Groll, & Appel, 1995). Therefore, in the present two-lever experiment, the additional recruitment of dopaminergic mechanisms by the 3.0 mg/kg dose of MDMA may have produced more salient/potent discriminative stimulus effects than the primarily serotonergic effects of the lower dose.

Experiment 2: Three-lever discrimination

Drug discrimination studies comparing the discriminative stimulus effects of MDMA and AMPH in two-lever experiments have produced an asymmetric pattern of results; MDMA generalised to the discriminative stimulus effects of AMPH in AMPH-trained animals, whereas AMPH did not consistently generalise to MDMA in MDMA-trained animals (Evans & Johanson, 1986; Glennon & Misenheimer, 1989; Glennon & Young, 1984; Kamien et al., 1986; Oberlender & Nichols, 1988; Schechter, 1988). While these findings might reflect similarities in the discriminative stimulus effects produced by the two drugs, they are potentially confounded by neuroadaptive responses to the different training conditions (see General Introduction).

Another way to determine whether two drugs can be discriminated from each other while avoiding these potential confounds is by using a three-lever drug discrimination procedure. In two-lever tasks (i.e. drug × saline) the discrimination can only be learned if the drug produces discriminative stimulus effects that are distinguishable from saline. In a three-lever task (i.e. drug × drug × vehicle), the discrimination can only be learned if each training-drug can be distinguished from each other as well as from saline. This means that the closer the similarity in the

discriminative stimulus effects produced by the two drugs, the more difficult it should be to acquire the three-way discrimination.

Previous studies have shown that a typical 1.5 mg/kg dose of MDMA can be discriminated from AMPH and saline in three-lever tasks (Baker et al., 1995; Goodwin & Baker, 2000; Harper et al., 2014; Smithies & Broadbear, 2011). However, in one study in which rats were trained to discriminate 1.5 mg/kg MDMA from AMPH and saline, increasing doses of MDMA (3.0 – 4.5 mg/kg) produced a significant increase in AMPH-lever responding suggesting that doses of MDMA in excess of 1.5 mg/kg produce discriminative stimulus effects that resemble those of the prototypical stimulant (Harper et al., 2014). This might reflect changes in the pharmacological effects produced by increasing doses of MDMA. In microdialysis studies, 1.5 mg/kg MDMA selectively increased extracellular 5-HT but significant increases in DA were observed following administration of higher (>3.0 mg/kg) doses (Baumann et al., 2005; Baumann, Wang, & Rothman, 2007). AMPH preferentially increases extracellular DA (Baumann et al., 2005) and the discriminative stimulus effects of AMPH have been attributed to dopaminergic mechanisms (Callahan et al., 1991). Thus, the generalisation to the discriminative stimulus effects of AMPH may reflect the recruitment of dopaminergic mechanisms by higher doses of MDMA.

To test this idea, experiment 2 examined whether a higher training dose of MDMA (3.0 mg/kg) could be discriminated from AMPH (0.5 mg/kg) and saline in a three-lever task. If 3.0 mg/kg MDMA produces dopamine-mediated discriminative stimulus effects that closely resemble those of AMPH then the acquisition of the three-way discrimination should be slower and more easily disrupted by changes in acquisition criteria for rats trained with 3.0 mg/kg compared to1.5 mg/kg MDMA.

Methods

Two groups of rats (n = 12 per group) were trained to discriminate saline from AMPH (0.5 mg/kg) and MDMA (1.5 mg/kg or 3.0 mg/kg) in a three-lever procedure. A key finding from experiment one was that the interpretation of drug discrimination learning depended greatly on the acquisition criteria that were employed. Drug discrimination can be understood as the ability to change the allocation of lever-presses following a change in drug stimulus. Thus, in the two

lever task, a sequence of 10 consecutive sessions (SMMSSMSMMS) consists of a minimum of 5 such changes. In a three lever task, subjects must display the ability to change their allocation of responses for all three different stimuli. This means that in a sequence of 10 sessions (SAMSMASAMS), discrimination between all three stimuli can only be observed a maximum of three times: $SAM^1 \mid SMA^2 \mid SAM^3 \mid S$. Accordingly, the additional criteria from experiment one were modified to reflect the greater number of sessions required to allow for a comparable number of discriminations between the available stimuli. Table 3.3 summarises each of the five different sets of criteria used in experiment two. In all other aspects, the design of experiment 2 resembled that of experiment 1. Procedure for the three-lever discrimination task is described in General methods.

Table 3.3 Summary of requirements for the various acquisition criteria in 3-lever task

Criteria	Requirements
Standard	*>80% correct for at least 8 out of 10 sessions*
6C	*>80% correct for 6 consecutive sessions*
9C	*>80% correct for 9 consecutive sessions*
12C	*>80% correct for 12 consecutive sessions*
15C	*>80% correct for 15 consecutive sessions*

Results

Table 3.4 shows the percentage of rats that met each criterion at the conclusion of the experiment (63 sessions) and the number of sessions required for at least 50% of rats to meet each criterion in each group. Figure 3.2 shows the effect of manipulating criteria on the acquisition of three-choice discrimination for the 1.5 mg/kg MDMA training dose group (left) and the 3.0 mg/kg MDMA training dose group (right).

The discrimination of MDMA (1.5 mg/kg) from AMPH (0.5 mg/kg) and saline as determined by the standard acquisition criterion was learned by at least 50% of rats after 21 sessions, and by 100% of rats after 38 sessions. As was the case in experiment 1, manipulating the criteria had a marked impact on the acquisition of the three-choice discrimination when a low training dose of MDMA was used. Even the least stringent of the additional criteria (6C) took longer to be met by 50% of rats than the standard criteria (33 vs 21 sessions). As the criterion became more stringent, fewer rats acquired the discrimination: only 25% and 17% had met the 12C and 15C criteria respectively by the end of the experiment (see Table 3.4).

Table 3.4 Summary of latency and proportion of rats meeting each criterion in 3-lever task

	MDMA 1.5 mg/kg		MDMA 3.0 mg/kg	
Criteria	*Percentage of rats that reached criterion*	*First session in which 50% of rats met criterion*	*Percentage of rats that reached criterion*	*First session in which 50% of rats met criterion*
Standard	100	21	100	15
6C	100	33	90	28
9C	58	51	54	43
12C	25	-	45	-
15C	17	-	45	-

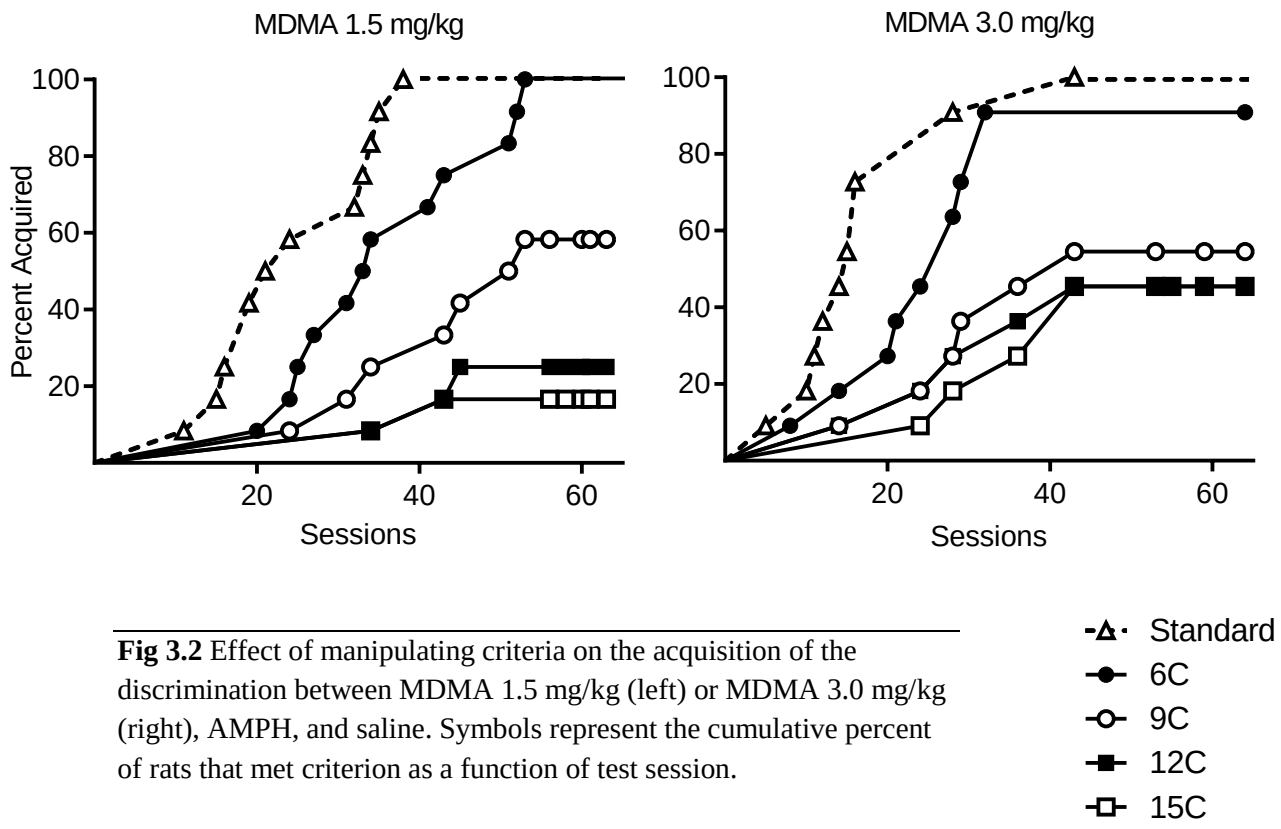

Fig 3.2 Effect of manipulating criteria on the acquisition of the discrimination between MDMA 1.5 mg/kg (left) or MDMA 3.0 mg/kg (right), AMPH, and saline. Symbols represent the cumulative percent of rats that met criterion as a function of test session.

Acquisition curves in the low MDMA training-dose condition differed significantly as a function of criterion ($\chi 2$ (4) = 59.93, $p < .001$). Follow up tests (Bonferroni corrected) confirmed that the survival function generated by the standard criterion was significantly different from that of the 3 most stringent additional criteria: 9C ($p < .001$), 12C ($p < .001$), and 15C ($p < .001$). A separate analysis was conducted to determine whether increasing the required number of consecutive correct sessions led to a decrease the percentage of rats that acquired the discrimination. A log rank test for trend confirmed that acquisition rates decreased as the required number of consecutive sessions increased ($\chi 2$ (1) = 22.83, $p < .001$).

The acquisition of the discrimination of MDMA (3.0 mg/kg) from AMPH (0.5 mg/kg) and saline, as determined by the standard criteria, was learned comparatively quickly as evidenced by a leftward shift in the acquisition curve compared to the MDMA 1.5 mg/kg training dose group. At least 50% of rats had met the standard criteria after 15 sessions, and 100% of rats met these criteria after 43 sessions. Manipulating the criteria had a significant impact on the acquisition of the high dose MDMA ×AMPH × saline discrimination. While nearly all rats met the least stringent criteria (6C) fairly rapidly, the percentage of rats that acquired the discrimination dropped by approximately 50% when the three more stringent criteria were used (see Table 3.4).

Acquisition curves in the high MDMA training dose group differed significantly as a function of criteria ($\chi 2$ (4) = 31.26, $p < .001$). Follow up tests (Bonferroni corrected) again showed that the standard criterion was significantly different from that of the 3 most stringent additional criteria: 9C ($p < .001$), 12C ($p < .001$), and 15C ($p < .001$). When the additional criteria were analysed separately, a log rank test for trend showed that acquisition rates decreased as the required number of consecutive sessions increased ($\chi 2$ (1) = 8.23, $p = .004$). However, follow up tests showed that only the difference between the 6C and 15C criteria reached significance following correction for multiple comparisons (Bonferroni).

Figure 3.3 shows the rates of responding during saline, MDMA, and AMPH sessions for both the 1.5 mg/kg MDMA group (left) and the 3.0 mg/kg MDMA group (right). Response rates did not vary systematically between session types, with ANOVA showing no significant interactions in either group.

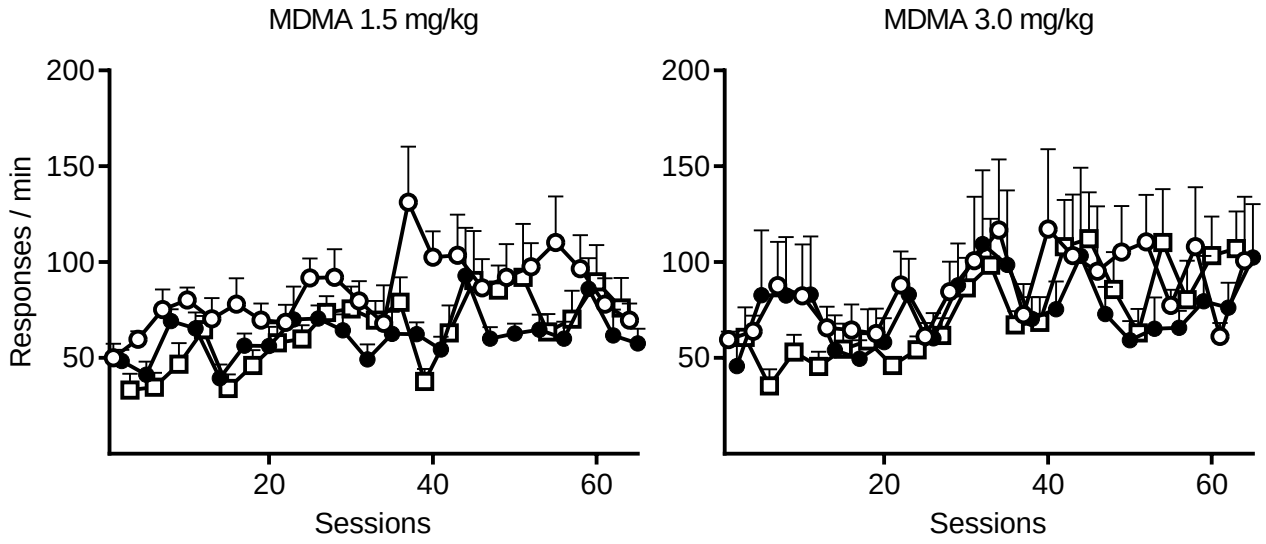

Fig 3.3 Response rates during saline and MDMA sessions remained stable throughout the 63 test sessions in both the 1.5 mg/kg MDMA group (left) and the 3.0 mg/kg MDMA group (right). Symbols and error bars represent the mean number of responses/min and standard error of the mean.

Discussion

Experiment 2 tested whether MDMA could be discriminated from AMPH and saline in a three-choice drug discrimination procedure. It was hypothesised that rats trained to discriminate 3.0 mg/kg MDMA from AMPH and saline would have greater difficulty learning the discrimination than rats trained with a lower 1.5 mg/kg dose. Contrary to this hypothesis however, the standard acquisition criterion was met slightly more rapidly by rats in the high MDMA training dose group and manipulations of criteria had a similar impact on the acquisition of drug discrimination in both training dose groups. These findings might suggest that 1.5 mg/kg and 3.0 mg/kg MDMA are both equally distinguishable from AMPH.

In experiment 1, examining the effect of changes in criteria revealed important differences between the acquisition profiles of the low versus high dose discriminations which may otherwise have gone unnoticed. These differences might suggest an alternative explanation for the results of the three-choice discrimination experiment. In the two-lever task, increasing the required number of consecutive sessions resulted in significant disruption of the acquisition of the low but not high dose discrimination. These findings suggested that rats had difficultly consistently discriminating 1.5 mg/kg MDMA from saline, but had no such difficulty discriminating 3.0 mg/kg MDMA. In the three-lever task, changes in criteria had a significant impact on the acquisition of the discrimination of both 1.5 mg/kg and 3.0 mg/kg MDMA from AMPH and saline. Based on results from the two-lever task, the disruption of the three-lever discrimination in the low MDMA training dose group might be attributed to the inability to reliably discriminate 1.5 mg/kg MDMA from saline. However, since increasing the criterion for acquisition had little impact on the 3.0 mg/kg MDMA vs saline discrimination, disruption of the three-choice discrimination in the high MDMA training dose group may instead reflect difficulty in reliably discriminating 3.0 mg/kg MDMA from AMPH.

During a training session preceded by an injection of MDMA, responses on either the saline or AMPH levers can be considered two distinct types of discrimination errors. If the disruption of acquisition associated with changes in criteria was related to different aspects of the three-way discrimination for each group, then the type of errors made during training may differ as a function of MDMA-training dose. In order to explore this possibility, the distribution of

responses between all three levers during the first trial of each session was examined during the latter stages of training (sessions 40-60). It was hypothesised that if rats were unable to reliably distinguish between the discriminative stimulus effects of 1.5 mg/kg MDMA and saline in the low dose training group, then a significant percentage of incorrect responses might be made on the MDMA lever during saline training sessions and vice versa. Similarly, if 3.0 mg/kg MDMA produced discriminative stimulus effects that were difficult to distinguish from AMPH, then this might be reflected in a higher percentage of responses on the MDMA lever during AMPH training sessions.

Unfortunately, it became apparent that the existing data were unsuitable for this type of analysis. Since rats generally tended to make more responses on the stimulus appropriate lever as training sessions continued, when data from all rats were combined any pattern in erroneous responding became impossible to detect. Future studies could explore different ways to measure a subjects' potential confusion between similar training stimuli. For example, if the distinction between MDMA and AMPH is difficult to make, this may result in an increased latency to make an initial lever press during MDMA or AMPH training sessions.

Chapter 4: The Role of Serotonin

Parts of this chapter have been adapted from work published in Behavioural Pharmacology (Webster, Harper, & Schenk, In press)

Results from two-lever acquisition experiments suggest that 3.0 mg/kg MDMA produces more robust discriminative stimulus effects than the typical 1.5 mg/kg training dose. From acquisition data alone, however, it cannot be determined whether these findings reflect differences between the two stimuli that are purely quantitative in nature – i.e. an increase in potency of the higher dose – or whether they might reflect qualitative differences in subjective effects resulting from the recruitment of additional neurochemical mechanisms. One way to address this question is to examine the pharmacological mechanisms of the discriminative stimulus effects of each training dose of MDMA. This can be done by assessing the ability of selective drugs with different pharmacological effects to generalise to the discriminative stimulus effects of one, or the other, or both doses of MDMA.

MDMA binds to monoamine transporters in the CNS but shows the greatest affinity for the SERT (Battaglia et al., 1988). *In vivo* microdialysis studies have confirmed that 1.5 mg/kg MDMA selectively increased extracellular 5-HT (Baumann, Clark, Franken, et al., 2008; Baumann et al., 2007; Panos & Baker, 2010). It is therefore unsurprising that the discriminative stimulus effects of 1.5 mg/kg MDMA have been attributed to serotonergic mechanisms. For example, 5-HT releasing agents fenfluramine, norfenfluramine, and trifluoromethylphenylpiperazine (TFMPP) generalised to the discriminative stimulus effects of 1.5 mg/kg MDMA (Goodwin & Baker, 2000; Goodwin et al., 2003; Schechter, 1988) whereas the administration 5-HT_2 antagonists , and to a lesser extent 5-HT_{1A} antagonists, attenuated the 1.5 mg/kg MDMA stimulus (Glennon et al., 1992; Schechter, 1988; Smithies & Broadbear, 2011). Furthermore, serotonin neurotoxicity in rats led to a significantly impaired ability to discriminate 1.5 mg/kg MDMA from saline (Baker & Makhay, 1996; Schechter, 1991) suggesting that 5-HT neurotransmission is crucial for the production of the discriminative stimulus effects of the drug at this dose.

While the pharmacological action of 1.5 mg/kg MDMA is to preferentially increase serotonin, this selectivity is lost following administration of higher doses of MDMA. Not only does the magnitude of the 5-HT response increase, but additional

neurotransmitter effects also become apparent. For example, following administration of 3.0 mg/kg MDMA, synaptic DA (Baumann, Clark, & Rothman, 2008), acetylcholine (Nair & Gudelsky, 2006), and norepinephrine (Starr, Page, & Waterhouse, 2008) overflow also increased. The recruitment of any or all of these additional neurotransmitter systems would be expected to impact the discriminative stimulus effects of higher doses of MDMA.

Higher doses of MDMA are seldom tested in drug discrimination experiments however an MDMA training dose of 2.5 mg/kg was used in one study (Mori et al., 2014). The 5-HT_{2A} agonist, DOI, dose-dependently generalised to the discriminative stimulus effects of 2.5 mg/kg MDMA, whereas the 5-HT_{2A} antagonist, ritanserin, but not the 5-HT_{1A} antagonist, WAY100635, somewhat reduced MDMA-appropriate responding. Interestingly, the selective serotonin reuptake inhibitor (SSRI), fluoxetine, did not generalise to the discriminative stimulus effects of 2.5 mg/kg. In fact, partial generalisation was only observed at doses that also produced rate-suppressing effects. Another SSRI, fluvoxamine, did generalise to the discriminative stimulus effects of 2.5 mg/kg MDMA, however this effect was blocked by the σ1 receptor antagonist, NE-100, suggesting that this generalisation may have been due to opioid, rather than serotonergic mechanisms. These findings raise the possibility that serotonergic activity alone may be insufficient to produce subjective effects that resemble those produced by doses of MDMA in excess of 1.5 mg/kg.

This possibility was addressed in the following experiment which compared the ability of serotonergic compounds to generalise to the discriminative stimulus effects of a 1.5 mg/kg versus 3.0 mg/kg training dose of MDMA. Firstly, generalisation tests were carried out with the SSRIs, fluoxetine and clomipramine, in order to determine whether a general increase in extracellular 5-HT could produce MDMA-like discriminative stimulus effects. These two compounds selectively increase extracellular 5-HT via blockade of the SERT (Fuller & Beasley, 1991; Tatsumi, Groshan, Blakely, & Richelson, 1997). If the subjective effects of higher doses of MDMA involve neurochemical mechanisms other than serotonin release, as has been previously suggested (Mori et al., 2014), one might not expect SSRIs to generalise the discriminative stimulus effects of 3.0 mg/kg MDMA.

Next, generalisation tests were carried out a range of 5-HT receptor agonists. MDMA does not bind directly to 5-HT receptors, but rather these receptors are

activated as a result of MDMA-stimulated 5-HT release. There are at least 14 different types of serotonin receptor distributed throughout the brain (Hoyer et al., 1994). These subtypes are categorised into 7 different families based on their specific pharmacological effects. MDMA-stimulated release of 5-HT may activate any number of these receptor, however the 5-HT_1 and 5-HT_2 receptor families have been implicated in MDMA-produced behavioural effects in a number of paradigms including self-administration (Fantegrossi et al., 2002), locomotor activity (Clissold, Choi, & Pratt, 2013; Kehne et al., 1996; Schenk et al., 2016), conditioned reinforcement (Fletcher, Korth, Robinson, & Baker, 2002), pre-pulse inhibition (van den Buuse et al., 2011) and drug discrimination (Glennon et al., 1992; Smithies & Broadbear, 2011). In order to determine the role of these receptor subtypes in producing the subjective effects of each MDMA training dose, test compounds were selected which produced robust discriminative stimulus effects via 5-HT_1 and 5-HT_2 receptor mechanisms.

The 5-HT_2 agonists, mCPP and DOI produce psychoactive effects in humans (Bossong et al., 2009; Shulgin, Shulgin, & Nichols, 1991) as well as discriminative stimulus effects in laboratory animals (Callahan & Cunningham, 1994; Glennon, 1986). Neither compound can be considered highly selective, however DOI displays strongest affinity for 5-HT_{2A} receptors (K_i = 1.65 nM) whereas mCPP shows strongest affinity for 5-HT_{2C} (K_i = 20.0 nM) (Knight et al., 2004). Accordingly, the discriminative stimulus effects of DOI and mCPP have been attributed to 5-HT_{2A} and 5-HT_{2C} receptor mechanisms, respectively (Callahan & Cunningham, 1994; Glennon, 1986).

RU-24969 produces an MDMA-like behavioural syndrome in locomotor experiments in rats (Martinez-Price & Geyer, 2002) suggesting that it may share some important underlying neurochemical mechanisms. RU-24969 is a non-selective agonist which is commonly used to investigate 5-HT_1 receptor mechanisms. It displays strong affinity for the 5-HT_{1B} (K_i = 0.38 nM), as well as the 5-HT_{1A} receptor (Ki = 2.5 nM) however its discriminative stimulus effects appear to be primarily mediated by 5-HT_{1B} mechanisms (Gardner, 1989). The role of 5-HT_{1A} receptor mechanisms was investigated in generalisations tests with 8-OH-DPAT. In contrast to RU-24969, 8-OH-DPAT is highly selective for 5-HT_{1A} receptors (K_i = 1.0 nM) (Peroutka, 1986) and the discriminative stimulus effects of 8-OH-DPAT likely

reflect 5-HT_{1A} receptor activation since these effects were blocked by the selective 5-HT_{1A} antagonist, NAN-190 (Glennon et al., 1988).

Methods

Subjects were the two groups of 12 rats from the two-lever experiments in the previous chapter. One group was trained to discriminate 1.5 mg/kg MDMA from saline and the other group was trained to discriminate 3.0 mg/kg MDMA from saline. The first test sessions established an MDMA dose-response curve for each group. MDMA (0.5 – 3.0 mg/kg) was administered (IP) 15mins prior to the session (see General Methods for full procedure). Once all MDMA generalisation sessions were complete, test sessions were conducted with the selective serotonin reuptake inhibitors (SSRIs), fluoxetine (0.3 – 3.0 mg/kg) and clomipramine (1 – 10 mg/kg); the 5-HT_{2C} receptor agonist and releasing stimulant, meta-chlorophenylpiperazine (mCPP, 0.3 – 2.0 mg/kg); the 5-HT_{2A} receptor agonist, 2,5-dimethoxy-4-iodoamphetamine (DOI, 0.3 – 3.0 mg/kg), the 5-HT_{1A} receptor agonist 8-OH-DPAT (0.03 – 0.3 mg/kg), and the 5-$HT_{1A/1B}$ receptor agonist, RU-24969 (0.3 – 3.0 mg/kg). All test compounds were administered 15 min prior to the start of the session. Dose ranges were chosen that have been shown to produce robust discriminative stimulus or other behavioural effects in previous studies (Callahan & Cunningham, 1994; Dekeyne & Millan, 2003; Gardner, 1989; Glennon, 1986; Glennon & Young, 2000; Maurel, Schreiber, & De Vry, 1997).

Results

MDMA dose response

Figure 4.1 displays the percentage of responses on the MDMA lever (left panel) and the corresponding response rates (right panel) during the MDMA generalisation tests for both training dose groups. Significant behavioural disruption (<5 responses) was observed in two rats in the low training dose group following administration of 3.0 mg/kg MDMA and these data were excluded from the figure and analysis. Saline injections produced marginal responding (<10%) on the MDMA lever in both groups. A rightward shift in the dose-response function was observed for rats trained with 3.0 mg/kg MDMA. The dose estimated to produce 50%

MDMA-appropriate responses (ED_{50} ± 95% CI) was calculated by linear regression of the ascending portion of the log dose response curve. Comparisons of fit confirmed that the ED_{50} significantly increased from 0.51 (0.27-0.96) mg/kg in the 1.5 mg/kg MDMA group to 1.1 (0.68-1.67) mg/kg in the 3.0 mg/kg MDMA group [$F(1,69) = 4.93$, $p = .030$].

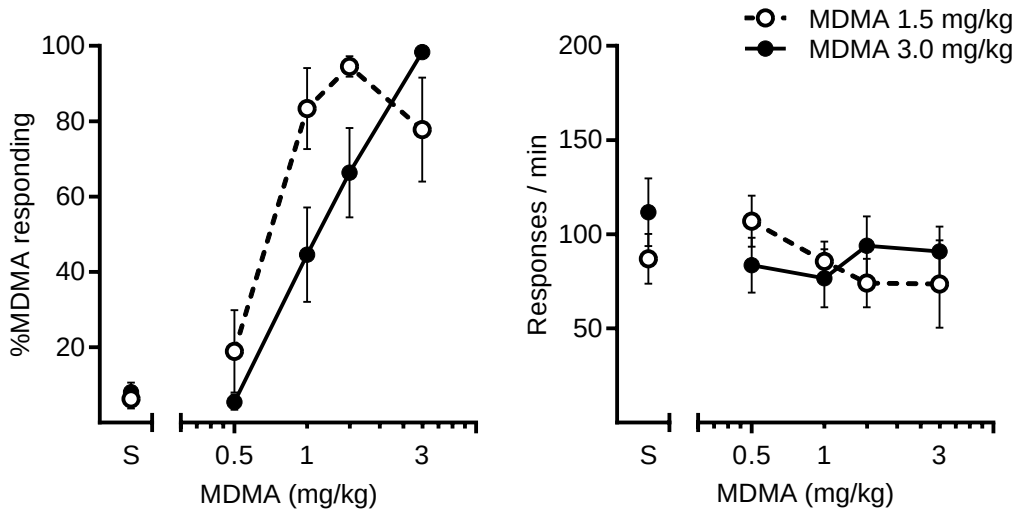

Fig 4.1 Mean percentage (±SEM) of MDMA appropriate responses (left panel) and corresponding response rates (±SEM) (right panel) following administration of various doses of MDMA. Open versus closed symbols represent data from low versus high training dose groups, respectively

ANOVA on the percentage of MDMA-appropriate responses as a function of dose revealed a main effect of dose in the low training dose group [$F(4, 32) = 23.17$, $p < .001$]. Pairwise post-hoc tests (Bonferroni) confirmed that MDMA-appropriate responding following 0.5 mg/kg MDMA was significantly lower than responding produced by all other doses ($p < .05$). The percentage of MDMA-appropriate responding following 1.0 mg/kg and 3.0 mg/kg MDMA was not significantly different from the percentage of MDMA-appropriate produced by the training dose

of 1.5 mg/kg. In the high training dose group, ANOVA on the percentage of MDMA-appropriate responses as a function of dose revealed a main effect of dose [$F(4, 40) = 23.19$, $p < .001$]. Post-hoc tests confirmed that the percentage of MDMA-appropriate responding following the training dose of 3.0 mg/kg MDMA was significantly higher than the percentage of MDMA-appropriate responding following all other doses. No significant change in response rates was observed in either group following administration of any dose of MDMA.

SSRIs

Figure 4.2 displays the results of generalisation tests with the SSRIs, fluoxetine (left panels) and clomipramine (right panels). Top panels display the percent of responses on the MDMA lever following administration of various doses of each compound, and bottom panels display the corresponding rates of responding. Fluoxetine produced a dose-dependent increase in MDMA lever responding in the low dose training group, but only produced marginal MDMA lever responding in the high training dose group. Two-way ANOVA (repeated measures) confirmed that there was a significant group × treatment interaction [$F(2,40) = 5.47$, $p = .008$]. Post hoc tests (Bonferroni) showed that the percentage of MDMA responses following administration of the highest dose of fluoxetine (3 mg/kg) was significantly higher in the low training dose group.

A similar pattern was observed following administration of clomipramine. The highest dose tested (10 mg/kg) partially generalised (76%) to the 1.5 mg/kg MDMA stimulus but none of the doses tested produced appreciable MDMA lever responding in the 3.0 mg/kg MDMA group. Two- way ANOVA failed to reveal a significant interaction but main effects of group [$F(1,18) = 5.46$, $p = .031$] and treatment [$F(2,36) = 11.65$, $p < .001$] were observed. Post hoc tests (Bonferroni) confirmed that the percentage of MDMA-responses following administration of the highest dose of clomipramine (10 mg/kg) was significantly higher in the 1.5 mg/kg training dose group. Neither compound had a significant impact on response rates

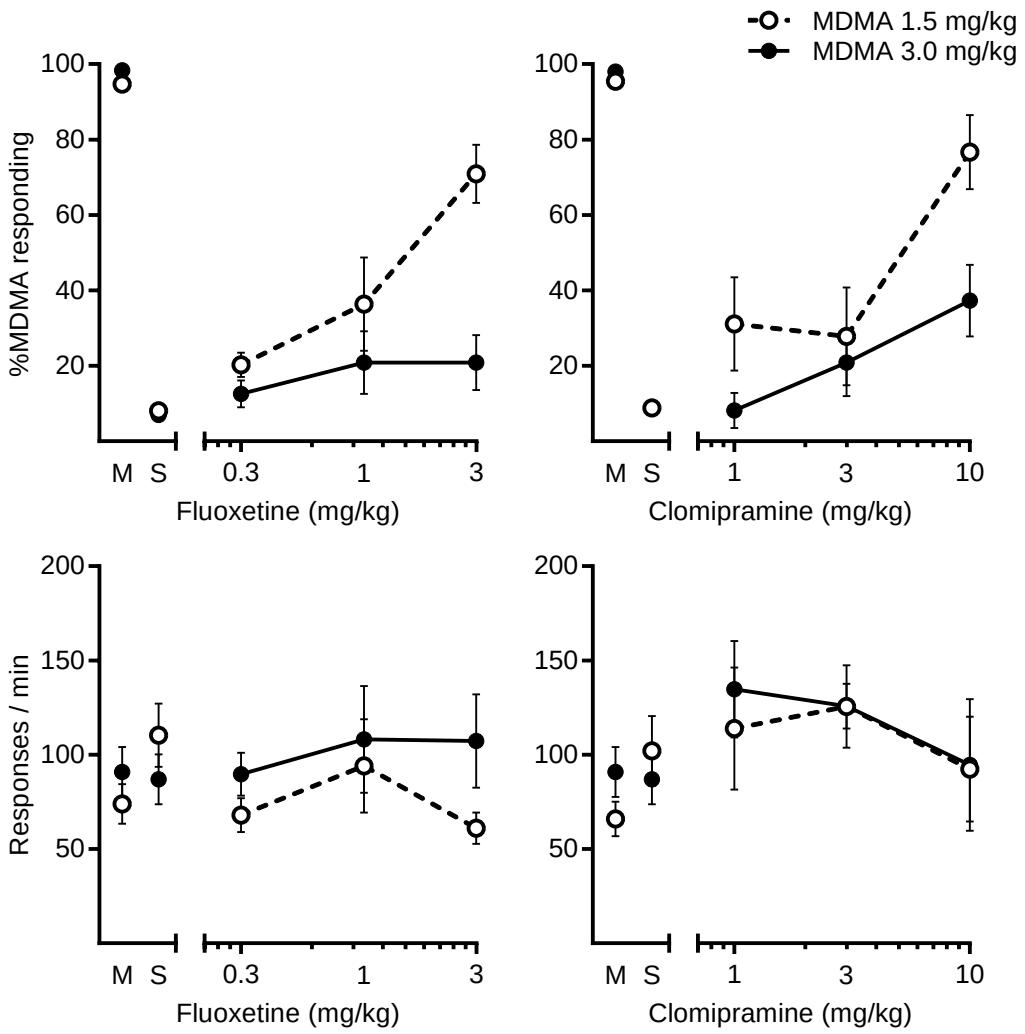

Fig 4.2 Top panels display the mean (±SEM) percentage of responses on the MDMA lever following administration of fluoxetine (left) or clomipramine (right). Bottom panels display the mean (±SEM) number of responses per minute during test sessions. Baseline responding following the training dose of MDMA (M) and saline (S) are also shown.

5-HT$_2$ receptor agonists

Figure 4.3 displays the generalisation functions and response rates produced by mCPP (left panels) and DOI (right panels). Both the 1.0 mg/kg dose and the 2.0 mg/kg dose of mCPP substituted for the 1.5 mg/kg MDMA stimulus. In contrast, none of the doses tested generalised to the discriminative stimulus effects of 3.0 mg/kg MDMA; the maximum percentage of MDMA responses, produced by the highest dose tested, was 43%. Two-way ANOVA revealed a significant group × treatment interaction [$F(2,40) = 4.84$, $p = .013$] with post-hoc comparisons (Bonferroni) confirming that the percentages of MDMA-responding following 1.0 and 2.0 mg/kg mCPP were significantly higher in the low training dose group ($p < .05$). A significant dose-dependent decrease in response rates produced by mCPP was observed in both groups [$F(2,40) = 12.48$, $p < .001$] which prevented the testing of higher doses.

The two highest doses of DOI fully generalised to the discriminative stimulus effects of 1.5 mg/kg MDMA. Fewer MDMA-lever responses were observed in the 3.0 mg/kg MDMA group with only the highest dose of DOI producing a percentage of MDMA-lever responding (72%) that approached the generalisation threshold of 80%. A two-way ANOVA confirmed a main effect of group [$F(1,20) = 7.34$, $p = .014$]. Response rates did not significantly decrease following administration of DOI.

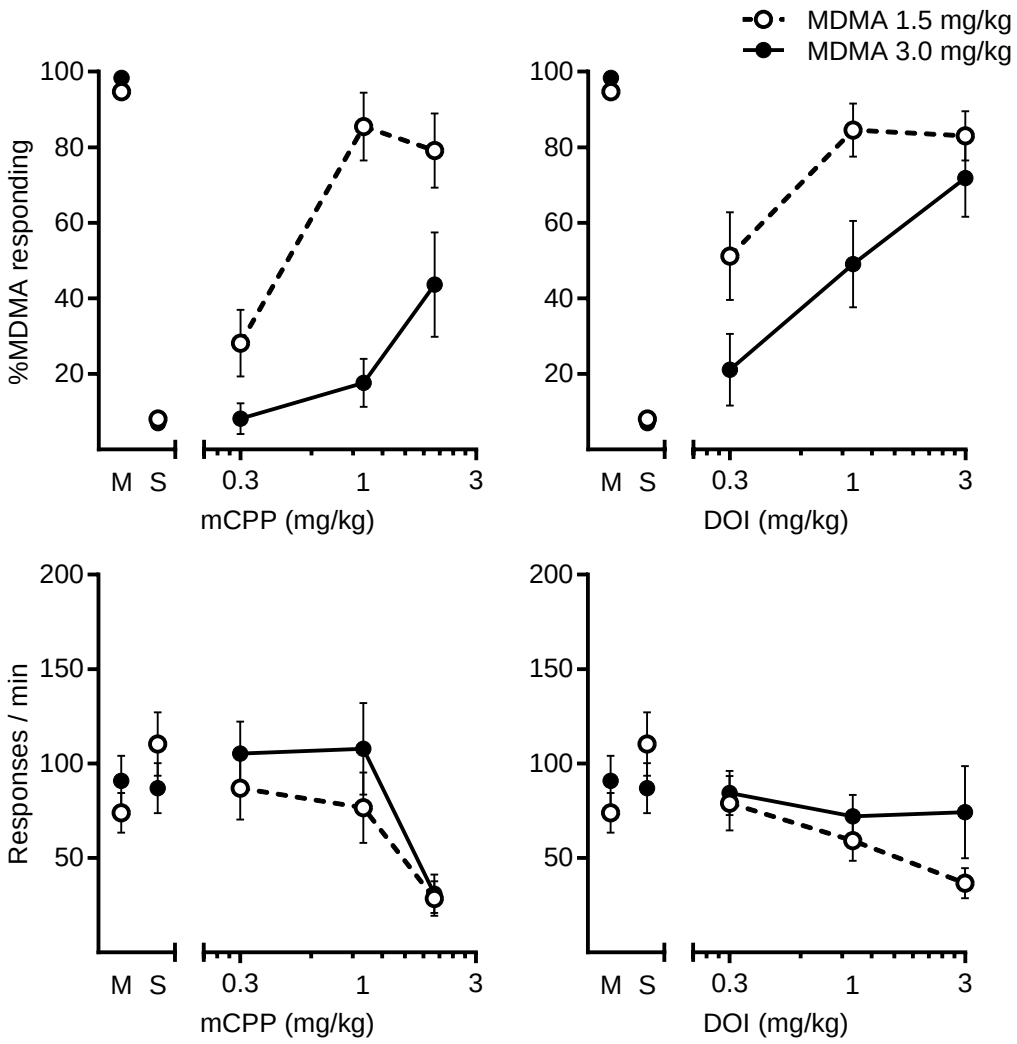

Fig 4.3 Top panels display the mean (±SEM) percentage of responses on the MDMA lever following administration of mCPP (left) or DOI (right). Bottom panels display the mean (±SEM) number of responses per minute during test sessions. Baseline responding following the training dose of MDMA (M) and saline (S) are also shown.

5-HT$_1$ receptor agonists

Figure 4.4 displays the generalisation functions and response rates following administration of the 5-HT$_{1A}$ agonist, 8-OH-DPAT (left panels), and the 5-HT$_{1B/1A}$ agonist, RU-24969 (right panels). Both 8-OH-DPAT and RU-24969 were highly disruptive as evidenced by very low response rates following administration of either drug. However, only three rats failed to complete the FR10 requirement during testing of the highest dose of 8-OH-DPAT and so only data from these rats were excluded.

8-OH-DPAT dose-dependently increased MDMA responding in both training dose conditions [$F(2,30) = 14.84$, $p < .001$] but there was no significant interaction or main effect of group. A similar pattern of results was observed following administration of RU-24969. A significant dose-dependent increase in MDMA lever responding was observed [$F(2,32) = 11.75$, $p < .001$] but there was no significant interaction or main effect of group. Response rates following administration of 8-OH-DPAT decreased in a dose-dependent fashion [$F(2,36) = 20.45$, $p < .001$] but were significantly lower in the 1.5 mg/kg MDMA group overall [$F(1,18) = 5.00$, $p = .038$]. Response rates following administration of RU-24969 also decreased in a dose-dependent fashion [$F(2,32) = 13.18$, $p < .001$] however this effect was comparable for both training dose conditions..

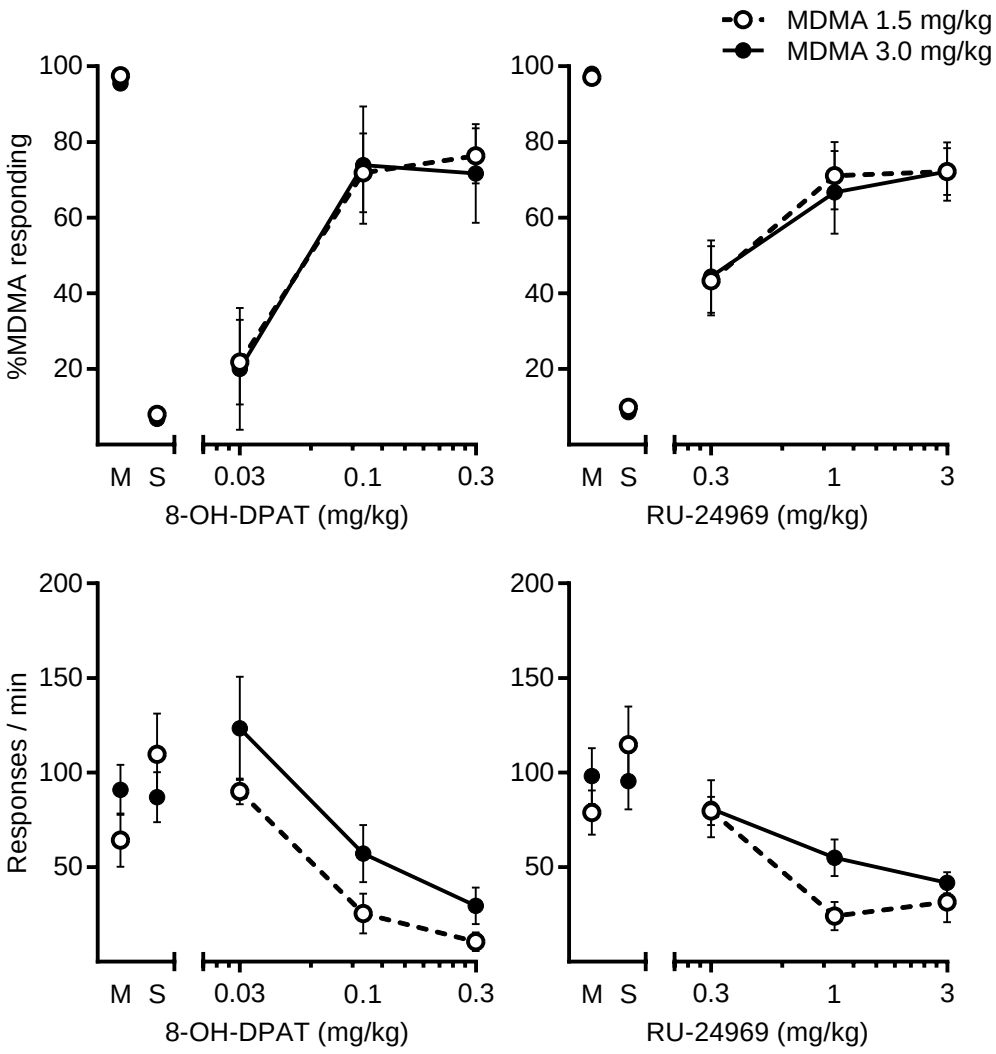

Fig 4.4 Top panels display the mean (±SEM) percentage of responses on the MDMA lever following administration of 8-OH-DPAT (left) or RU-24969 (right). Bottom panels display the mean (±SEM) number of responses per minute during test sessions. Baseline responding following the training dose of MDMA (M) and saline (S) are also shown.

Discussion

The present experiments investigated the role of serotonin in the discriminative stimulus effects of a low versus high training dose of MDMA. These data were consistent with numerous previous studies which have implicated 5-HT as a critical determinant of 1.5 mg/kg MDMA discriminative stimulus. However, some differences were observed in terms of the ability of various ligands to generalise to the discriminative effects of 3.0 mg/kg MDMA, suggesting that the role of 5-HT may be a function of training dose.

Both of the SSRIs, fluoxetine, and clomipramine, generalised to the discriminative stimulus effects of 1.5 mg/kg but not 3.0 mg/kg MDMA. Fluoxetine also failed to generalise to discriminative stimulus effects 2.5 mg/kg in a previous study (Mori et al., 2014). These findings suggest that a general increase in extracellular 5-HT is sufficient to produce discriminative stimulus effects that resemble a low dose of MDMA, but a question remains as to why the SSRIs failed to generalise to the 3.0 mg/kg MDMA stimulus.

SSRIs increase synaptic 5-HT, but the magnitude of the increase is limited by 5-HT-mediated autoreceptor activation (Hervás & Artigas, 1998; Romero, Hervás, & Artigas, 1996). MDMA-produced 5-HT release is not, however, dependent on impulse-mediated release and synaptic levels are therefore not impacted to the same extent by negative autoreceptor feedback mechanisms. SSRIs increased 5-HT overflow by about 150 % (Hervás & Artigas, 1998), which is comparable to the effects of the low dose of MDMA on synaptic 5-HT overflow (Baumann, Clark, & Rothman, 2008). A higher dose of MDMA, however, produced a substantially greater increase in 5-HT (Baumann, Clark, Franken, et al., 2008) and so it is possible that the moderate increases in 5-HT produced by SSRIs were not sufficient to generalise to the discriminative stimulus effects of 3.0 mg/kg MDMA in the present study.

All of the 5-HT receptor agonists tested in the present study generalised to the discriminative stimulus effects of 1.5 mg/kg MDMA. These findings are consistent with the selective increase in extracellular 5-HT produced by this dose of MDMA in microdialysis studies (Baumann et al., 2005; Baumann, Clark, Franken, et al., 2008; Bradbury et al., 2014) and strongly suggest that the subjective effects of the low training dose of MDMA are mediated by 5-HT receptor activation. The 5-HT_{1A}

agonist, 8-OH-DPAT and the mixed 5-$HT_{1B/1A}$ agonist, RU-24969, also generalised to discriminative stimulus effects of 3.0 mg/kg MDMA suggesting that 5-HT_{1A} and 5-HT_{1B} receptor mechanisms may also contribute to the subjective effects of the higher training dose.

A differential pattern of generalisation was observed, however, when 5-HT_2 agonists were tested. The 5-HT_{2A} agonist, DOI, was approximately three-fold less potent in generalisation tests in the 3.0 mg/kg MDMA training dose group as indicated by a rightward shift in the DOI dose-response curve. The 5-HT_{2C} agonist, mCPP, failed to generalise to the discriminative stimulus effects of 3.0 mg/kg MDMA. A similar rightward shift in the mCPP dose-response curve might also have been observed but the testing of higher doses was not possible due to the appearance of behaviourally disruptive effects. In any case, these findings suggest that the activation of 5-$HT_{2A/2C}$ receptors was less able to produce MDMA-like subjective effects in the high training dose group.

One possible explanation for these findings is the development of tolerance to 5-HT_2 receptor mediated subjective effects in the high relative to the low MDMA training dose group. There is some evidence suggesting a down-regulation of 5-HT_{2A} receptors following exposure to MDMA. Experimenter administered MDMA (10 mg/kg s.c) reduced cortical 5-HT_{2A} receptor density in rats and SPECT imaging techniques in human subjects revealed a significant decrease in 5-HT_{2A} receptor densities in recent, compared to ex-MDMA users (Reneman et al., 2002). The effect of MDMA exposure on 5-HT_{2C} receptors is less clear. On the one hand, repeated (15 mg/kg per day for 3 days) or 'binge' (4 × 10 mg/kg, every 2 h) exposure to MDMA failed to alter the behavioural response to the 5-HT_{2C} agonist, mCPP (Bull, Hutson, & Fone, 2003; Jones, Brennan, Colussi-Mas, & Schenk, 2010). On the other hand, an MDMA 'binge' (3 × 5 mg/kg, every 2 h) led to a decrease in 5-HT_{2C} receptor density in the hippocampus of adolescent rats (García-Cabrerizo & García-Fuster, 2015).

In the present study, rats were repeatedly exposed to MDMA during drug discrimination training sessions however it is not clear whether intermittent injections of a relatively low training dose of MDMA would be sufficient to produce neuroadaptations comparable to those mentioned above. It is also unclear whether any changes in 5-HT_2 receptor function would be significantly different following repeated exposure to 1.5 mg/kg compared to 3.0 mg/kg MDMA. It would be interesting to compare 5-HT_2 receptor densities, and 5-HT_2 mediated behavioural

responses, between rats exposed to a dosing regimen that more closely resembles drug discrimination training procedures (e.g. 1.5 – 3.0 mg/kg, 3 times per week for 10 weeks) and drug-naïve controls. If repeated intermittent exposure to 3.0 mg/kg but not 1.5 mg/kg MDMA produced a downregulation of 5-HT_2 receptors then this might explain the differential generalisation of 5-HT_2 receptor agonists in the present study.

An alternative explanation for the present findings is that the discriminative stimulus effects of 3.0 mg/kg MDMA are qualitatively distinct from those of the 1.5 mg/kg dose and in fact involve the recruitment of additional neurochemical mechanisms. One candidate is the increase in synaptic DA that becomes apparent following administration of higher doses of MDMA (Baumann, Clark, & Rothman, 2008; Kankaanpaa et al., 1998). If DA rather than 5-HT release was more important in producing the discriminative stimulus effects of 3.0 mg/kg MDMA, then the 5-HT_{2C} agonist mCPP would not be expected to generalise to the 3.0 mg/kg MDMA stimulus since activation of 5-HT_{2C} receptors decreased DA levels in microdialysis experiments (De Deurwaerdère, Navailles, Berg, Clarke, & Spampinato, 2004). Furthermore, the ability of DOI to generalise to the discriminative stimulus effects of 3.0 mg/kg MDMA could be explained in terms of post-synaptic effects on dopaminergic systems.

In the CNS, 5-HT_{2A} receptors are expressed on glutamatergic neurons in the PFC which project to DA neurons in the ventral tegmentum (VTA). Activation of these receptors via local infusion of DOI (300 µM) produced a substantial increase in extracellular DA (~ 220%) as measured by microdialysis (Bortolozzi, Díaz-Mataix, Scorza, Celada, & Artigas, 2005). Systemic administration of a low dose of DOI (0.5 mg/kg s.c) produced a moderate DA increase (~134%) (Bortolozzi et al., 2005), however larger increases were produced by a higher dose of 2.5 mg/kg (~ 200-300%) (Pehek, McFarlane, Maguschak, Price, & Pluto, 2001; Pehek, Nocjar, Roth, Byrd, & Mabrouk, 2006). An increase in DA of about the same size (~ 200-350%) was produced by systemic (IP) administration of MDMA (2.5 – 3.0 mg/kg) at doses comparable to the high training dose used in the present study (Kankaanpaa et al., 1998; O'Shea et al., 2005). Thus, while low doses of DOI may have been sufficient to produce serotonergic effects that generalised to the subjective effects of 1.5 mg/kg MDMA in the present study, higher doses of DOI may have been required in order to produce a dopamine response sufficient to generalise to the discriminative stimulus

effects of 3.0 mg/kg MDMA. To explore this idea, it would be interesting to test whether the generalisation of DOI to the discriminative stimulus effects of 3.0 mg/kg is attenuated by DA antagonists in future experiments.

Chapter 5: The Role of Dopamine

Parts of this chapter have been adapted from published in Behavioural Pharmacology (Webster et al., In press)

Serotonergic mechanisms clearly contribute to the subjective effects of MDMA. However, the differential generalization of SSRIs and other 5-HT agonists suggests that the discriminative stimulus effects of 1.5 mg/kg and 3.0 mg/kg MDMA can be dissociated and that the subjective effects of the higher dose might reflect activation of additional neurochemical systems. In experiments described in the previous chapter, 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{2A} but not 5-HT_{2C} receptor agonists generalised to the discriminative stimulus effects of 3.0 mg/kg MDMA. In addition to producing a range of 5-HT mediated effects, activation of 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{2A} receptors also facilitate DA release (Gronier, 2008; Ichikawa & Meltzer, 1995; Parsons, Koob, & Weiss, 1999; Rasmusson, Goldstein, Deutch, Bunney, & Roth, 1994) whereas activation of 5-HT_{2C} receptors decreased DA release (De Deurwaerdère et al., 2004). This raises the possibility that dopaminergic mechanisms may have contributed to the ability of some 5-HT ligands to generalise to the discriminative stimulus effects of MDMA. One way to investigate this possibility is to test the ability of DA agonists to generalise to the discriminative stimulus effects of each training dose of MDMA. Alternatively, DA antagonists can be administered to determine whether they attenuate the discriminative stimulus effects of MDMA.

A small number of studies have determined the effect of DA antagonists on the discriminative stimulus effects of 1.5 mg/kg MDMA. The D_1 receptor antagonist, SCH23390 (Broadbent, Appel, Michael, & Ricker, 1992; Harper et al., 2014), the D_2 receptor antagonist, haloperidol (Glennon et al., 1992; Schechter, 1988), and the DA release inhibitor CGS10746B (Schechter, 1988) all failed to attenuate the discrimination of 1.5 mg/kg MDMA suggesting that dopaminergic mechanisms were not a critical component. This is consistent with data from microdialysis studies in which 1.5 mg/kg MDMA selectively increased 5-HT (Baumann et al., 2005; Baumann, Clark, Franken, et al., 2008; Kankaanpaa et al., 1998) and supports the idea that the discriminative stimulus effects of 1.5 mg/kg MDMA are primarily due to serotonergic rather than dopaminergic mechanisms. In other paradigms, higher doses of MDMA (>2.5 mg/kg) were required to produce DA-mediated behaviours such as conditioned place preference (Marona-Lewicka et al., 1996),

hyperlocomotion (Brennan & Schenk, 2006), and the reinstatement of drug-seeking (Schenk et al., 2011). DA mediated discriminative stimulus effects might, therefore only be expected when higher training doses of MDMA are tested.

No drug discrimination studies to date have tested the effects of DA agonists or antagonists in animals trained to discriminate doses of MDMA in excess of 1.5 mg/kg, however higher doses have been administered during generalisation experiments in animals trained to discriminate other drugs. For example, 3.0 mg/kg MDMA partially generalised to the discriminative stimulus effects of cocaine (Khorana, Pullagurla, Young, & Glennon, 2004). Since the discrimination of cocaine is mediated by dopaminergic mechanisms (Callahan et al., 1991; Colpaert, Niemegeers, & Janssen, 1978; Kantak et al., 1995; McKenna & Ho, 1980), this partial generalisation suggests that dopaminergic mechanisms might also contribute to the discriminative stimulus effects of the higher dose of MDMA. Additionally, in a three-lever drug discrimination task in which rats were trained to discriminate 1.5 mg/kg MDMA from AMPH and saline, increasing doses of MDMA (3.0 – 4.5 mg/kg) produced a significant increase in AMPH-lever responding (Harper et al., 2014). As with cocaine, the AMPH-produced discriminative stimulus is mediated by dopaminergic mechanisms. Dopamine (Callahan et al., 1991) but not 5-HT (Arnt, 1992; Moser, 1992; Moser, Moran, Frank, & Kehne, 1995; Silverman & Ho, 1980) antagonists completely abolished the discriminative stimulus properties of AMPH. The shift in generalisation from the MDMA lever to the AMPH lever as the dose of MDMA was increased is therefore consistent with the idea that the discriminative stimulus properties of higher doses of MDMA involve the recruitment of dopaminergic mechanisms.

The following experiments were designed to investigate this possibility by determining the ability of a range of dopamine agonists to generalise to the discriminative stimulus effects of either a 1.5 mg/kg or 3.0 mg/kg training dose of MDMA. MDMA inhibits the reuptake of DA by binding to dopamine transporters (Nash & Brodkin, 1991), and the resulting increase in extracellular DA may contribute to the discriminative stimulus effects. AMPH is structurally related to MDMA and produces substantial increases in extracellular DA via reversal of the DAT, albeit with far greater potency (Baumann et al., 2007). The greater efficacy of AMPH in terms of DAT versus SERT inhibition makes AMPH an ideal candidate for

use in generalisation tests in order to assess the role of DA release in MDMA-produced discriminative stimulus effects.

Dopamine produces behavioural effects by binding to either D_1-like or D_2-like receptors, however simultaneous activation of both receptor subtypes appears to be important for the production of cocaine- and AMPH-like discriminative stimulus effects (Callahan et al., 1991). Thus, in order to investigate the role of direct D_1/D_2 receptor activation, generalisation tests were carried out with the non-selective D_1/D_2 agonist, apomorphine. The role of each individual DA receptor family was further investigated by testing the more selective D_1 and D_2 agonists, SKF38393 and quinpirole. SKF38393 shows a >100-fold selectivity for D_1-like over D_2-like receptors (Neumeyer, Kula, Bergman, & Baldessarini, 2003) whereas quinpirole shows an approximately 20-fold selectivity for D_2-like over D_1-like receptors (Mottola et al., 2002).

Methods

Subjects were the two groups of 12 rats from the two-lever experiments in previous chapters. One group was trained to discriminate 1.5 mg/kg MDMA from saline and the other group was trained to discriminate 3.0 mg/kg MDMA from saline. Generalisation test sessions were conducted with the prototypical psychostimulant AMPH (0.25 – 0.5 mg/kg), the non-selective D_1/D_2 agonist, apomorphine (0.125 – 0.25 mg/kg), the D_1 receptor agonist, SKF38393 (3 – 10 mg/kg), and the selective D_2 receptor agonist, quinpirole (0.03 – 0.3 mg/kg) (see General Methods for full procedure). All compounds were administered 15 min prior to the start of the session. Dose ranges were chosen that have been shown to produce robust discriminative stimulus effects in other studies (Callahan et al., 1991; Colpaert, Niemegeers, Kuyps, & Janssen, 1975; Cunningham, Callahan, & Appel, 1985).

Results

Non-selective DA compounds

Figure 5.1 shows the generalisation functions and response rates produced by AMPH (left panels), and the non-selective D_1/D_2 agonist, apomorphine (right panels). Administration of AMPH produced a dose-dependent increase in MDMA-lever responding in the high training dose group with the highest dose (0.5 mg/kg) fully generalising (93%) to the discriminative stimulus effects of 3.0 mg/kg MDMA. In contrast, only generalisation (56%) was observed in the low training dose group. ANOVA confirmed a significant group × treatment interaction [$F(1,20) = 5.81$, $p = .026$]. Post-hoc comparisons (Bonferroni) confirmed that the percentage of MDMA-responding following administration of 0.5 mg/kg AMPH was significantly higher in the high compared to low training dose group ($p < .05$). No significant change in response rates was observed following administration of either of the tested doses. Preliminary testing of a higher dose of AMPH (1.0 mg/kg) produced significant behavioural disruption in these subjects and therefore higher doses were not tested.

The highest dose of apomorphine (0.25 mg/kg) partially generalised (76%) to the discriminative stimulus effects of 3.0 mg/kg MDMA whereas neither dose produced substantial increases in MDMA-lever responding in the low training dose group. The group × treatment interaction approached statistical significance [$F(1,14) = 4.52$, $p = .052$]. Response rates following administration of apomorphine were significantly reduced compared to baseline [$F(2,28) = 6.95$, $p = .004$] and severe behavioural disruption prevented the testing of higher doses.

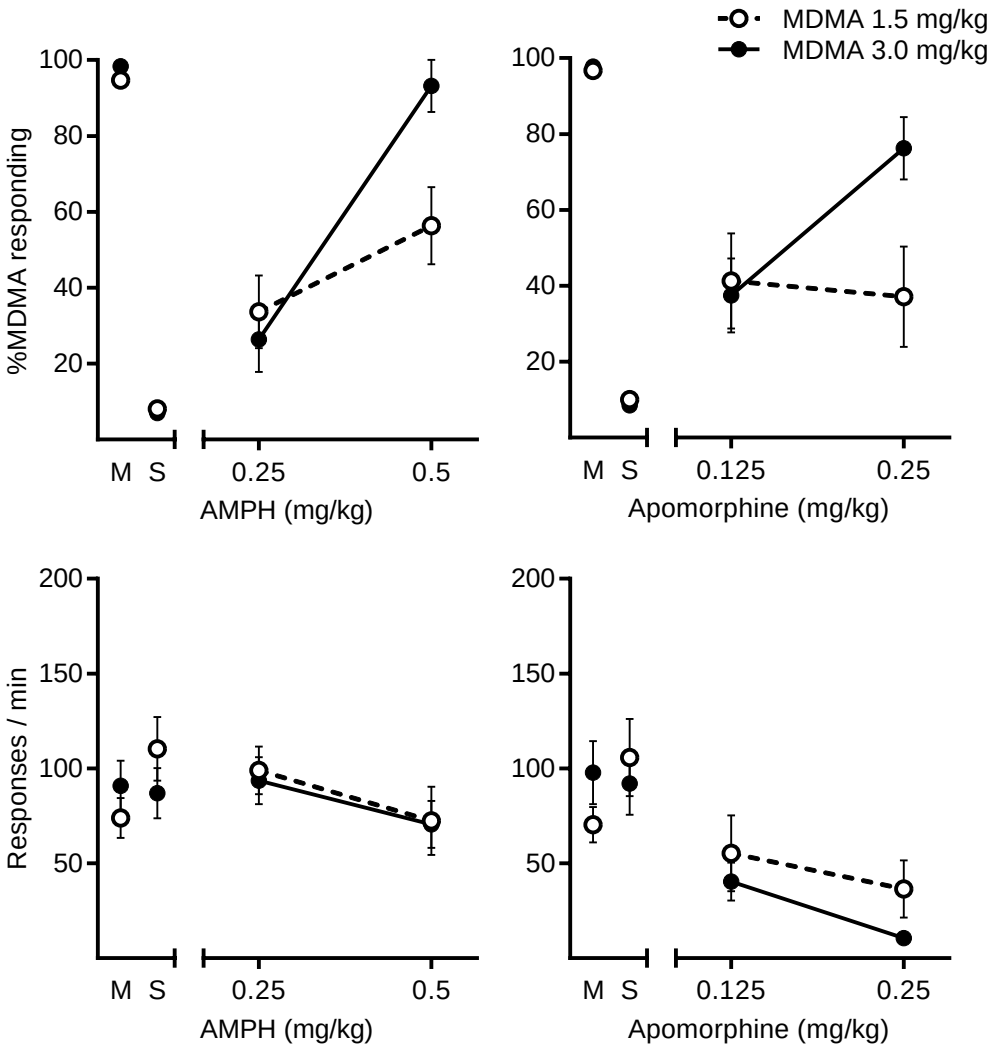

Fig 5.1 Top panels display the mean (±SEM) percentage of responses on the MDMA lever following administration of AMPH (left) or apomorphine (right). Bottom panels display the mean (±SEM) number of responses per minute during test sessions. Baseline responding following the training dose of MDMA (M) and saline (S) are also shown.

Selective D_1 / D_2 agonists

Figure 5.2 shows the generalisation functions and response rates produced by the selective D_1 agonist, SKF38393, and the selective D_2 agonist, quinpirole. All doses of SKF38393 failed to generalise to the discriminative stimulus effects of either 1.5 mg/kg or 3.0 mg/kg MDMA. MDMA-lever responding was marginally higher in the low MDMA training dose group however this difference was not statistically significant [$F(1,16) = 3.15$, $p = .095$]. All three doses produced elevations in response rates to levels significantly above baseline [$F(3,48) = 7.06$, $p < .001$]. This effect appeared to be more prominent in the low training dose group however ANOVA revealed no main effect of group.

The D_2 agonist produced moderate levels of MDMA-responding in the 1.5 mg/kg MDMA training dose group but this effect was not dose-dependent. In contrast, only the two highest doses produced notable MDMA-lever responding in the high training dose group however ANOVA revealed no significant interaction or main effects. Interpretation of the data from tests with quinpirole should be made with caution since the drug produced visible disruptive effects in a number of rats. Three rats in the low training dose group failed to complete 10 responses following administration of the highest dose (0.3 mg/kg), but were included in the analysis since >5 responses were made. Quinpirole produced significant dose-dependent decreases in response rates in both groups [$F(3,48) = 6.64$, $p < .001$].

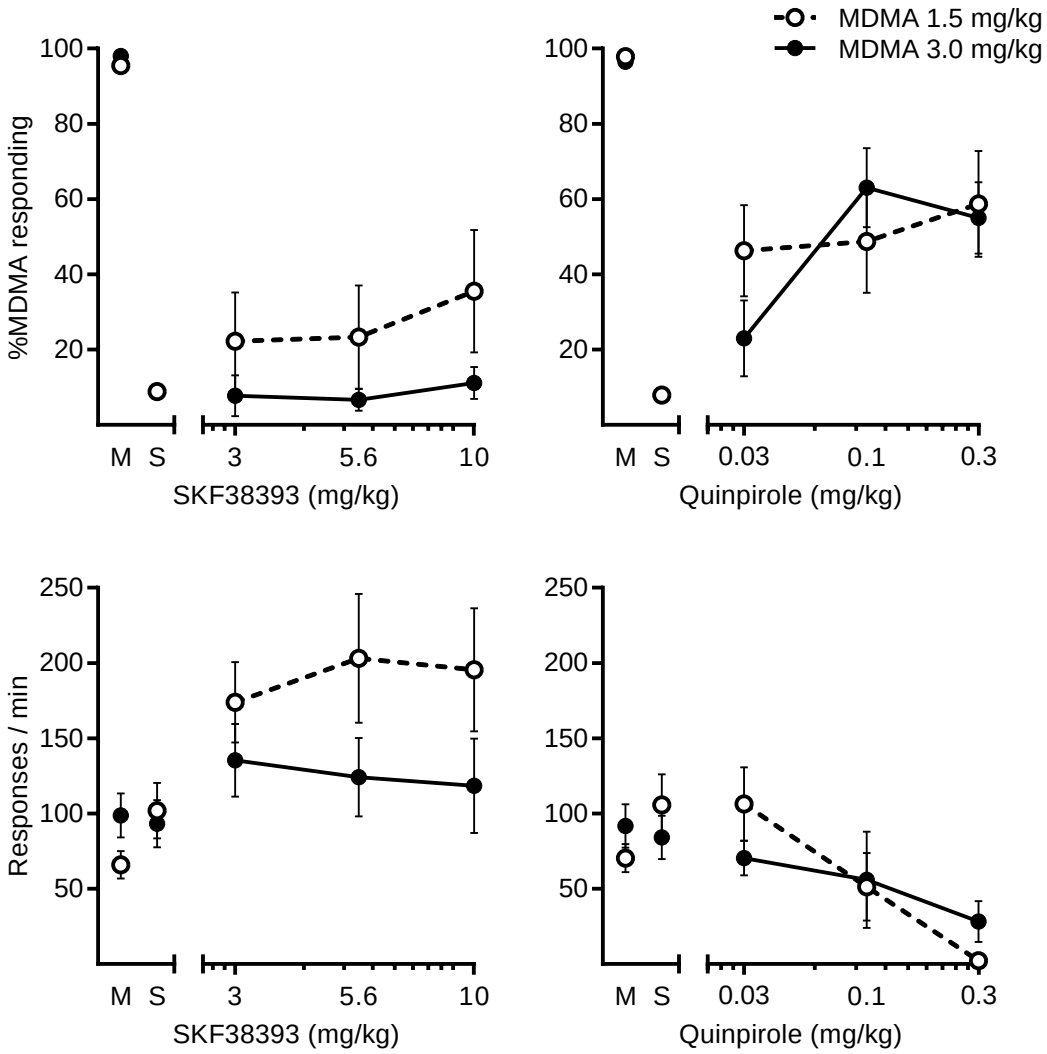

Fig 5.2 Top panels display the mean (±SEM) percentage of responses on the MDMA lever following administration of SKF38393 (left) or Quinpirole (right). Bottom panels display the mean (±SEM) number of responses per minute during test sessions. Baseline responding following the training dose of MDMA (M) and saline (S) are also shown.

Discussion

The DA releasing stimulant, AMPH, and the non-selective D_1/D_2 agonist, apomorphine, either fully, or partially generalised, respectively to the discriminative stimulus effects of the high but not low training dose of MDMA. These findings support the hypothesis that the discriminative stimulus effects produced by the high but not low dose of MDMA involve the recruitment of dopaminergic mechanisms.

A number of studies have compared the effects of MDMA with its parent compound, AMPH, in drug discrimination experiments. AMPH either failed to generalise or only partially generalised to the discriminative stimulus effects of 1.5 mg/kg MDMA in MDMA-trained rats (Baker & Makhay, 1996; Glennon & Misenheimer, 1989; Oberlender & Nichols, 1988; Schechter, 1988) whereas 3.0 mg/kg MDMA generalised to the discriminative stimulus effects of AMPH in AMPH-trained pigeons (Evans & Johanson, 1986). The former findings were replicated in the present study insofar as AMPH failed to generalise to the discriminative stimulus effects of 1.5 mg/kg MDMA. This result may come as no surprise since the discriminative stimulus effects of 1.5 mg/kg MDMA are primarily mediated by serotonin whereas the discriminative stimulus effects of AMPH have been attributed to dopaminergic (Callahan et al., 1991) rather than serotonergic mechanisms (Arnt, 1992; Moser, 1992; Moser et al., 1995; Silverman & Ho, 1980). The non-selective D_1/D_2 agonist, apomorphine, also failed to generalise to the discriminative stimulus effects of 1.5 mg/kg MDMA suggesting that the subjective effects of the low MDMA training do not involve dopamine receptor mechanisms.

In contrast, AMPH fully generalised to the discriminative stimulus effects of 3.0 mg/kg MDMA suggesting that dopaminergic mechanisms may contribute to the subjective effects of the higher dose of MDMA. This finding is consistent with the significant increase in extracellular DA produced by 3.0 mg/kg MDMA in microdialysis studies (Baumann et al., 2005; Baumann, Clark, Franken, et al., 2008; Kankaanpaa et al., 1998). The non-selective D_1/D_2 agonist produced near full generalisation in the high MDMA training dose group. These findings are consistent with the idea that the discriminative stimulus effects of 3.0 mg/kg MDMA involve the activation of dopamine receptors. Apomorphine binds with comparable affinity to D_1 (K_i = 87 nM) and D_2 (K_i = 98) receptors (Andersen, Grønvald, & Jansen, 1985) however the discriminative stimulus effects of the drug were effectively blocked by

the D_2 antagonist, haloperidol (Colpaert, Niemegeers, Kuyps, et al., 1975). Furthermore, while the D_1 agonist, SKF38393, and the D_2 agonist, quinpirole, each failed to generalise to the discriminative stimulus effects of MDMA, quinpirole produced substantially more MDMA-lever responding in the high training dose group. Taken together these findings might indicate that D_2 rather than D_1 receptors play a more important role in the discriminative stimulus effects of 3.0 mg/kg MDMA.

One explanation for the failure of SKF38393 and quinpirole to generalise to the discriminative stimulus effects of 3.0 mg/kg MDMA is that selective activation of either D_1 or D_2 receptors is insufficient to produce MDMA-like effects and that simultaneous activation of both receptor types is required. D_1 and D_2 receptor mechanisms are able to interact with each other in either opposing or synergistic fashion (Clark & White, 1987; Rahman & McBride, 2001). One example is the 'enabling' type interaction between D_1 and D_2 receptors that has been proposed as an underlying mechanism of the discriminative stimulus effects of AMPH. The D_2 agonist, quinpirole, but not the D_1 agonist, SKF38393, generalised to the discriminative stimulus effects of 1.0 mg/kg AMPH suggesting a critical role of D_2 rather than D_1 receptor mechanisms (Callahan et al., 1991). However, the discriminative stimulus effects of AMPH were successfully blocked by the selective D_1 antagonist, SCH23390, or the D_2 antagonist, haloperidol. Thus, D_1 activation may not be sufficient to produce AMPH-like discriminative stimulus effects, but it may be necessary to 'enable' D_2 mediated subjective effects. It would be helpful, therefore, to assess whether selective DA antagonists are able to attenuation the discriminative stimulus effects of 3.0 mg/kg MDMA. In particular, if a significant attenuation is produced by blockade of D_1 receptors then this may suggest that the subjective effects of MDMA involve a synergistic interaction of DA receptor mechanisms similar to that of its parent compound, AMPH.

Chapter 6: Antagonism of the MDMA stimulus

Generalisation experiments test the ability of selective agonists to generalise to the discriminative stimulus effects of MDMA. If generalisation occurs, this may indicate that the test compound shares some underlying neurochemical mechanisms. It is also possible that the test compound produces discriminative stimulus effects that resemble those of MDMA but does so indirectly via mechanisms that are secondary to its main pharmacological action. For example, administration of the D_1/D_2 agonist, apomorphine, produces a range of dopaminergic effects, but also increases 5-HT levels via the activation of post-synaptic D_2 receptors on 5-HT neurons in the dorsal raphe (Ferré & Artigas, 1993). Therefore the possibility that 5-HT release, rather than DA receptor activation, contributed to the generalisation of apomorphine to the discriminative stimulus effects of 3.0 mg/kg MDMA cannot be ruled out. One way to clarify this issue is to conduct antagonism tests. In this type of experiment, selective antagonists are administered in conjunction with the training dose of MDMA in order to determine whether the discriminative stimulus effects of MDMA can be attenuated via blockade of a particular type of receptor.

In experiments described in previous chapters, a number of 5-HT and DA receptor subtypes have been identified as having a potential role in the subjective effects of 1.5 mg/kg and 3.0 mg/kg MDMA. 5-HT_{1A}, 5-$HT_{1B/1A}$, and 5-HT_{2A} agonists generalised to the subjective effects of both the low and high training dose suggesting that these receptor subsystems may constitute a core component of the MDMA stimulus. In contrast, the non-selective D_1/D_2 agonist, apomorphine, partially generalised to the subjective effects of the high but not the low training dose suggesting that 1.5 mg/kg and 3.0 mg/kg MDMA can be dissociated in terms of some underlying dopamine receptor mechanisms. The following experiments were designed to investigate these possibilities by testing the ability of selective 5-HT and DA antagonists to attenuate the discriminative stimulus effects of 1.5 mg/kg versus 3.0 mg/kg MDMA.

An advantage of this type of experiment is that antagonists tend to be more selective than their agonist counterparts. For example, the 5-HT_{2A} agonist, DOI, displays an approximately two-fold greater affinity for 5-HT_{2A} over 5-HT_{2C} receptors, but also produces some weak activity at 5-HT_{1A}, and 5-HT_{2B} receptors (Knight et al., 2004). In comparison, the 5-HT_{2A} antagonist, ketanserin, displays a

>10-fold greater affinity for 5-HT_{2A} over 5-HT_{2C} receptors and displays negligible affinity at other CNS binding sites (Boess & Martin, 1994). Accordingly, the interpretation that the generalisation of DOI to the discriminative stimulus effects of MDMA reflects 5-HT_{2A} receptor mechanisms is strengthened if the discriminative stimulus effects of MDMA are also blocked by ketanserin.

The use of selective antagonists can also help to clarify the results from generalisation tests with non-selective agonists such as RU-24969 and apomorphine. The mixed 5-$HT_{1B/1A}$ agonist, RU-24969, generalised to the subjective effects of 1.5 mg/kg and 3.0 mg/kg MDMA. However, the same pattern of generalisation was produced by the more selective 5-HT_{1A} agonist, 8-OH-DPAT, and so it is unclear whether the generalisation observed in tests with RU-24969 can be attributed to 5-HT_{1B} or 5-HT_{1A} receptor mechanisms. The relative contribution of 5-HT_{1A} versus 5-HT_{1B} receptor mechanisms to the discriminative stimulus effects of MDMA were further examined by testing the ability of the 5-HT_{1A} antagonist, WAY100635, and the 5-$HT_{1B/1D}$ antagonist GR-127935 to attenuate these effects. WAY100635 is highly selective for 5-HT_{1A} (K_i = 0.24 nM) with practically no affinity for 5-HT_{1B} (K_i > 1,000 nM) receptors whereas GR-127935 shows an approximately 60-fold greater affinity for 5-HT_{1B} (K_i = 1.00 nM) over 5-HT_{1A} (K_i = 63.0 nM) receptors (Mos, Van Hest, Van Drimmelen, Herremans, & Olivier, 1997; Price et al., 1997). The efficacy of these compounds in attenuating 5-HT mediated discriminative stimulus effects has also been established in previous drug discrimination experiments (Kleven & Koek, 1998; Maurel, Schreiber, & De Vry, 1998).

Apomorphine, but not SKF38393 or quinpirole, generalised to the discriminative stimulus effects of 3.0 mg/kg MDMA. One interpretation of these findings is that simultaneous activation of both D_1 and D_2 receptor subtypes were required to produce MDMA-like effects. In this case, administration of either the selective D_1 antagonist, SCH23390, or the selective D_2 antagonist, eticlopride, would be expected to attenuate the discriminative stimulus effects of 3.0 mg/kg MDMA. These two compounds are highly selective for D_1 (K_i = 0.35 nM) and D_2 (K_i = 0.04 nM) receptors, respectively, and have been used extensively in previous drug discrimination studies (Bubar, Pack, Frankel, & Cunningham, 2004; Callahan et al., 1991; Melia & Spealman, 1991; Munzar & Goldberg, 2000; Neumeyer et al., 2003; Tang, Todd, Heller, & O'Malley, 1994)

Methods

Subjects were the two groups of 12 rats from two-lever experiments described in previous chapters. One group was trained to discriminate 1.5 mg/kg MDMA from saline and the other group was trained to discriminate 3.0 mg/kg MDMA from saline. These experiments were carried out after all generalisation experiments described in the previous chapters were completed. As a result, due to the limited lifespan of rat subjects, time restrictions meant that only a single dose for each drug could be tested. Therefore, in an effort to ensure that any potential effects could be detected, drugs and doses were selected that produced significant effects in other behavioural paradigms in our laboratory such as locomotor activity and self-administration (Schenk et al., 2016; Schenk et al., 2011). Thus, antagonist test sessions (see General Methods for full procedure) were carried out in which the training dose of MDMA (1.5 or 3.0 mg/kg) was administered in combination with the 5-HT_{2A} antagonist, ketanserin (3.0 mg/kg), the 5-HT_{1A} antagonist, WAY100635 (1.0 mg/kg), the 5-HT_{1B} antagonist, GR127935 (3.0 mg/kg), the D_1 antagonist, SCH23390 (0.04 mg/kg), or the D_2 antagonist, eticlopride (0.3 mg/kg).

Preliminary testing established that the optimal pre-treatment time for Ketanserin was 60 minutes prior to the injection of MDMA. Pre-treatment times for WAY100635 (15 min), GR127935 (60 min), and eticlopride (30 min) were selected based time course data from the aforementioned locomotor activity experiments. SCH23390 was administered 15min prior to MDMA since this protocol produced significant effects in other drug discrimination experiments (Callahan et al., 1991).

Results

Low MDMA training dose

Figure 6.1 shows results from antagonist tests with the 5-HT_{2A} antagonist, ketanserin (3.0 mg/kg), the 5-HT_{1A} antagonist, WAY100635 (1.0 mg/kg), and the 5-HT_{1B} antagonist, GR127935 (3.0 mg/kg) in rats trained to discriminate MDMA (1.5 mg/kg) from saline. Only ketanserin reduced MDMA-responding below the 80% generalisation threshold, however a paired samples *t*-test found that this decrease was not statistically significant [$t(6)=1.55$, $p = .173$]. Neither WAY100635 nor GR127935 attenuated the discrimination of 1.5 mg/kg MDMA ($p > .05$)

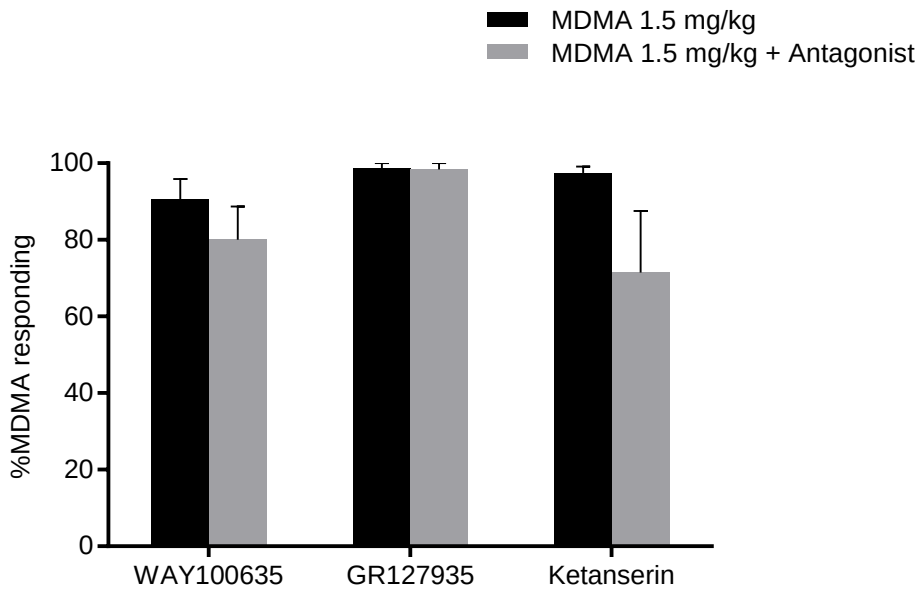

Fig 6.1 The effect of 5-HT antagonists on the discrimination of MDMA (1.5 mg/kg). Bars depict mean (+SEM) percentage of MDMA-lever responses following co-administration of MDMA with an antagonist (grey) versus MDMA alone (black)

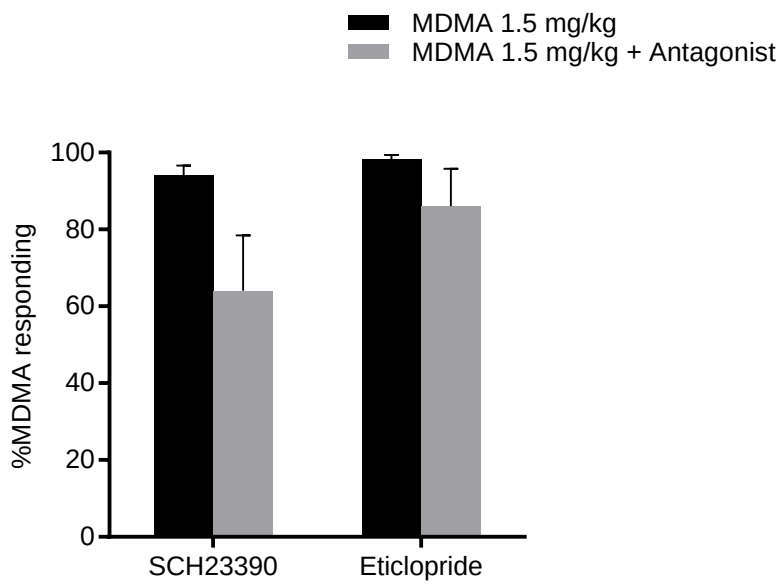

Fig 6.2 The effect of DA antagonists on the discrimination of MDMA (1.5 mg/kg). Bars depict mean (+SEM) percentage of MDMA-lever responses following co-administration of MDMA with an antagonist (grey) versus MDMA alone (black)

Figure 6.2 shows the results from antagonist tests with the selective D_1 antagonist, SCH23390 (0.04 mg/kg) and the selective D_2 antagonist, eticlopride (0.3 mg/kg). SCH23390 produced a moderate attenuation in MDMA responding however this decrease did not reach statistical significance [$t(9) = 2.13$, $p = .061$]. Eticlopride did not significantly attenuate the discrimination of 1.5 mg/kg MDMA.

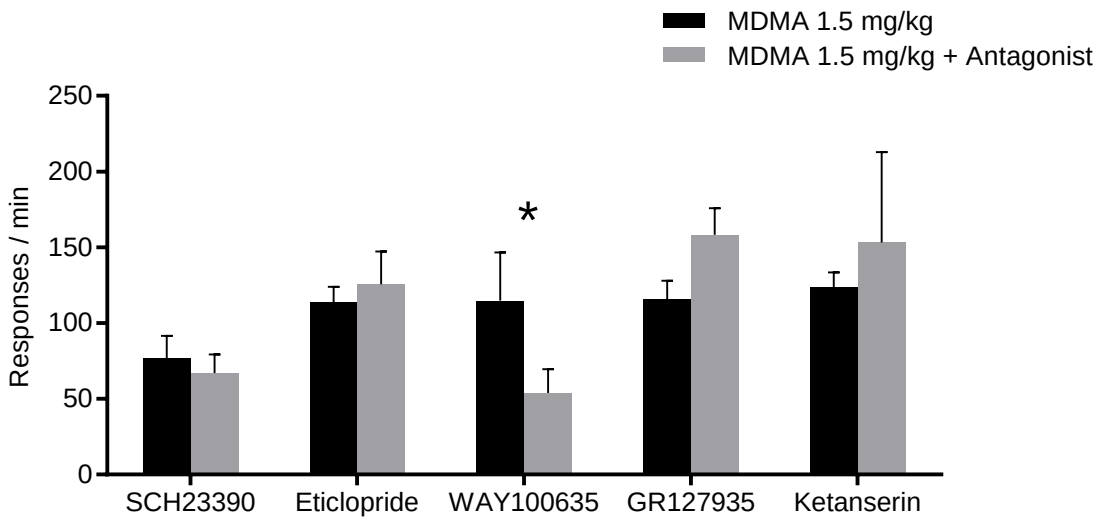

Fig 6.3 Bars depict the mean (+SEM) number of responses per minute following co-administration of MDMA (1.5 mg/kg) with an antagonist (grey) versus MDMA alone (black). * Indicates a significant difference from baseline

Figure 6.3 shows response rates during antagonist test sessions for all tested compounds in the low MDMA training dose group in comparison to baseline. The 5-HT_{1A} antagonist, WAY100635, significantly reduced rates of responding [$t(10) = 2.41$, $p = .037$]. No other compound produced significant changes in response rates although a high degree of variability was observed following administration of the 5-HT_{2A} antagonist, ketanserin.

High MDMA training dose

Figure 6.4 displays the results from antagonist tests with the 5-HT_{2A} antagonist, ketanserin (3.0 mg/kg), the 5-HT_{1A} antagonist, WAY100635 (1.0 mg/kg), and the 5-HT_{1B} antagonist, GR127935 (3.0 mg/kg) in rats trained to discriminate MDMA (3.0 mg/kg) from saline. None of the 5-HT antagonists tested produced significant changes in MDMA-lever responding compared to administration of the MDMA training dose alone.

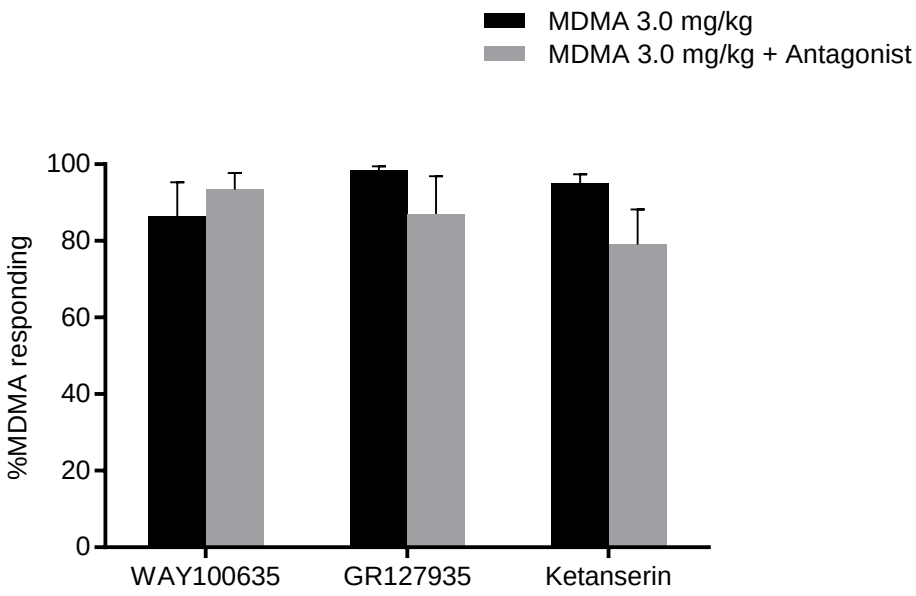

Fig 6.4 The effect of 5-HT antagonists on the discrimination of MDMA (3.0 mg/kg). Bars depict mean (+SEM) percentage of MDMA-lever responses following co-administration of MDMA with an antagonist (grey) versus MDMA alone (black)

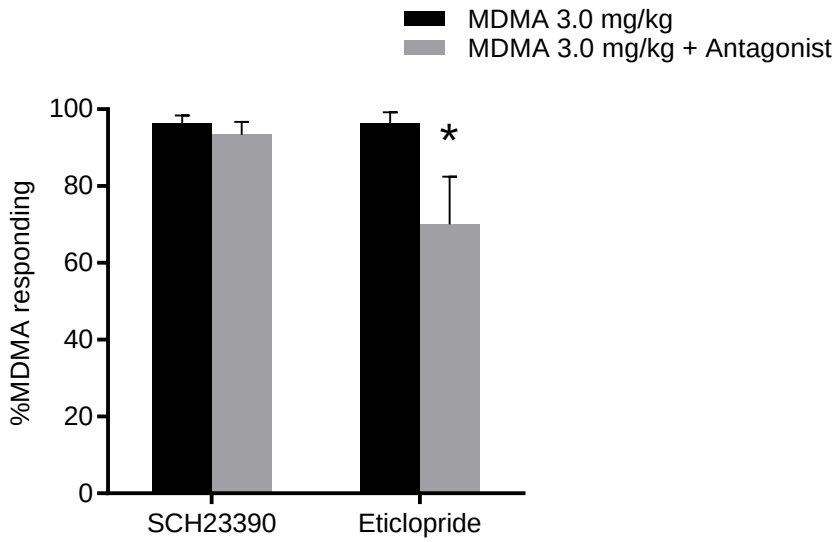

Fig 6.5 The effect of DA antagonists on the discrimination of MDMA (3.0 mg/kg). Bars depict mean (+SEM) percentage of MDMA-lever responses following co-administration of MDMA with an antagonist (grey) versus MDMA alone (black). * Indicates a significant difference from baseline

Figure 6.5 shows the effects of co-administration of 3.0 mg/kg MDMA with the D_1 antagonist, SCH23390 (0.04 mg/kg), and the D_2 antagonist, eticlopride (0.3 mg/kg). Administration of SCH23390 had no effect of the 3.0 mg/kg MDMA discrimination. The D_2 antagonist, eticlopride, produced a moderate decrease in MDMA-lever responding. A paired-samples *t*-test confirmed this to be a significant difference compared to baseline [$t(9) = 2.43$, $p = .038$].

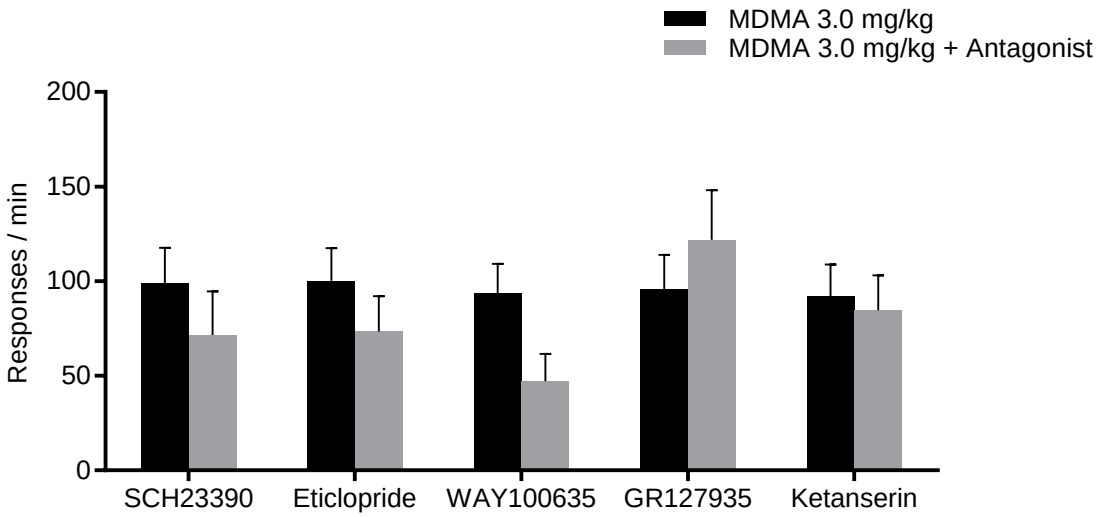

Fig 6.6 Bars depict the mean (+SEM) number of responses per minute following co-administration of MDMA (3.0 mg/kg) with an antagonist (grey) versus MDMA alone (black)

Figure 6.6 shows the response rates during antagonist test sessions compared to baseline for all tested compounds. Rates of responding were not noticeably affected by SCH23390, eticlopride, GR127935, or Ketanserin. As was the case in the low MDMA training dose group, a moderate decrease was observed following co-administration of MDMA 3.0 mg/kg with the 5-HT_{1A} antagonist, WAY100635, however this change was not statistically significant (p = .07).

Discussion

The purpose of this experiment was to determine the role of specific 5-HT and DA receptor subtypes in the discriminative stimulus effects of a low versus high training dose of MDMA. Selective 5-HT antagonists did not attenuate MDMA-lever responding when co-administered with either the 1.5 mg/kg or 3.0 mg/kg training dose of MDMA. Dopamine antagonists had no effect on the discrimination of the 1.5 mg/kg training dose of MDMA, whereas a moderate, but statistically significant, attenuation of the 3.0 mg/kg MDMA stimulus was produced by the D_2 antagonist, eticlopride. The failure of DA antagonists to attenuate the discriminative stimulus effects of the low MDMA training dose is consistent with previous studies (Harper et al., 2014; Schechter, 1988) and strengthens the hypothesis that the discriminative stimulus effects of 1.5 mg/kg MDMA are mediated by serotonergic, rather than dopaminergic, mechanisms.

In generalisation experiments described in the previous chapter, the non-selective D_1/D_2 agonist, apomorphine, partially substituted for 3.0 mg/kg MDMA, but the relative contribution of D_1 versus D_2 receptor mechanisms could not be determined. In the present experiment, The D_2 antagonist, eticlopride, but not the D_1 antagonist, SCH23390, produced a moderate attenuation of the discriminative stimulus effects of 3.0 mg/kg MDMA. These findings suggest that 3.0 mg/kg MDMA-like subjective effects may be dependent on the activation of D_2 rather than D_1 receptors. Furthermore, these findings do not support the 'enabling' hypothesis proposed in the previous chapter, since SCH23390 had no impact on the discriminative stimulus effects of the high MDMA training dose.

D_2 receptors are expressed on 5-HT neurons in the dorsal raphe nucleus (DRN) and so it is possible that the present findings may reflect an interaction between D_2 receptor activation and 5-HT neurotransmission. The selective D_2 antagonist, raclopride, prevented the increase in extracellular 5-HT produced by local infusion of apomorphine (Ferré & Artigas, 1993). Therefore it is possible the attenuation of the 3.0 mg/kg MDMA stimulus by eticlopride in the present study may reflect a D_2-mediated decrease in serotonergic activity rather than a decrease in dopaminergic activity *per se.* However, given that eticlopride had no effect on the primarily serotonergic discriminative stimulus effects of 1.5 mg/kg MDMA, this explanation seems unlikely.

In generalisation experiments described in chapter four, 5-HT_1, and 5-HT_2 agonists fully generalised to the discriminative stimulus effects of 1.5 mg/kg. In previous studies, the 5-HT_{1A} antagonist, NAN-190, and the non-selective 5-HT_2 antagonist, pirenperone, partially attenuated the discriminative stimulus effects of 1.5 mg/kg MDMA in a two lever task (Glennon et al., 1992). Similarly, the discrimination of 1.5 mg/kg MDMA from saline and AMPH in a three-lever task was significantly disrupted by pirenperone (Goodwin & Baker, 2000), as well as the 5-$HT_{2A/2C}$ antagonist, ritanserin (Smithies & Broadbear, 2011). The failure of all three 5-HT antagonists to produce a significant effect in the 1.5 mg/kg MDMA group in the present experiment was therefore surprising, and accordingly, may necessitate caution when interpreting the present results.

Drugs are metabolised at different rates, and therefore produce maximal effects at different times following their administration. The failure of antagonists to attenuate the discriminative stimulus effects of MDMA might therefore be explained by the use of inappropriate pre-treatment times. Some measures were taken to address this possibility. In most cases, pre-treatment times were based on time-course data collected from locomotor activity experiments in our laboratory. Thus, a pre-treatment window was selected for each drug that corresponded to the time at which maximal effects on locomotor activity were observed (Schenk et al., 2016). In other cases, parametric testing was carried out in which the pre-treatment time was systematically varied. Figure 6.7 shows preliminary data from pilot test sessions with ketanserin (left) and WAY100635 (right). Statistical analyses were not carried out for these data due to the small number of rats in each sample (n = 6) and it should be emphasised that the data presented are strictly preliminary.

When administered simultaneously (i.e. pre-treatment time of 0 mins) with MDMA (1.5 mg/kg), ketanserin had no effect on the percentage of MDMA-lever responses. However, when the pre-treatment time was increased to 30 and then 60mins, a moderate attenuation in MDMA-lever responses was observed. Thus a 60min pre-treatment window was selected for future test sessions. Pilot tests were also carried out in which the 5-HT_{1A} antagonist, WAY100635, was administered 15mins prior to an injection of the 5-HT_{1A} agonist, 8-OH-DPAT. WAY100635 (1.0 mg/kg) fully attenuated the MDMA-lever responding produced by 8-OH-DPAT suggesting that the antagonist was active after this 15min delay.

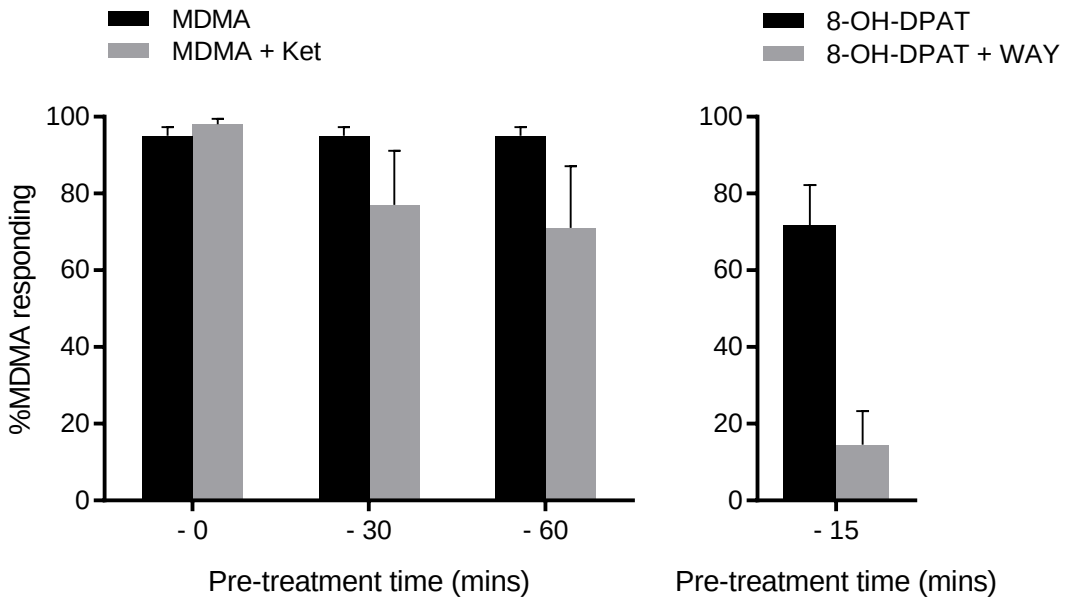

Fig 6.7 Preliminary data from tests with Ketanserin (left) and WAY100635 (right) determining suitable pre-treatment times

Unfortunately, due to the high cost of test compounds as well as the limited time available for systematic testing, these time-course experiments could not be completed for all combinations of antagonists and training drugs. Instead it was decided to prioritise the testing of a broad range of compounds in order to identify the specific 5-HT and DA receptor subtypes that are critical to the discriminative stimulus effects of MDMA. Separate investigations are warranted in which the temporal and dosing parameters of each compound are systematically examined.

An obvious limitation was the testing of only a single dose of each antagonist against a single dose of MDMA. In some cases, the dose of antagonist may simply have been insufficient to produce attenuations in the discriminative stimulus effects of MDMA. In other cases, antagonism of a particular receptor may have in fact produced an enhancement of the discriminative stimulus effects of MDMA which could not be detected since maximal MDMA-responding was already being

produced by each group's respective training dose. A more thorough investigation might test a behaviourally relevant dose of a given antagonist in conjunction with a range of doses of MDMA. Thus, an attenuation of the discriminative stimulus effects of MDMA would be apparent as a rightward shift in the MDMA-dose response curve, whereas an enhancement would be apparent as a leftward shift. Unfortunately, this type of parametric testing could not be carried out as part of the present thesis due to limited time and resources.

Another significant limitation was that, with respect to the other experiments that constitute this thesis, the antagonism tests described in the current chapter were the last to be carried out. Thus, at the time of these experiments, rats had been in drug discrimination training for close to 18 months and were nearing the end of their natural lifespan. Consequently, baseline discrimination performance of some subjects was beginning to deteriorate. This deterioration may be related to significant cognitive deficits that have been reported in 24-month old Sprague-Dawley rats (Fischer, Gage, & Björklund, 1989; Fukui et al., 2002).

General Discussion

Summary of Rationale

Over the last few decades ±3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") has gained worldwide popularity as a recreational 'party drug'. MDMA is sometimes considered to be less harmful than other illicit substances, such as cocaine and methamphetamine, despite the fact that there are several reports of MDMA dependence and abuse. MDMA users report a variety of subjective effects including euphoria, closeness to others, and altered sensory perception, in addition to stimulant-like effects. For this reason, it is typically distinguished from chemically related stimulant and hallucinogenic compounds such as AMPH and mescaline. Studying the subjective effects of potentially harmful drugs in human subjects can be problematic, but substantial knowledge has been gained from experiments conducted with laboratory animals. The discriminative stimulus effects produced by psychoactive drugs in animals are thought to be analogous to subjective effects in humans, and the nature of these effects and their underlying neurochemical mechanisms can be investigated using drug discrimination procedures.

The majority of drug discrimination studies, employ a training dose of 1.5 mg/kg MDMA which is roughly equivalent to the amount of MDMA contained in a typical 'ecstasy' pill (Brunt et al., 2012). The pharmacological action of 1.5 mg/kg MDMA is to selectively increase 5-HT levels, and the discriminative stimulus effects have often been attributed to serotonergic mechanisms (Glennon et al., 1992; Schechter, 1988). Training dose, however, can markedly impact the results of drug discrimination experiments, and different doses of the same drug can sometimes produce qualitatively distinct discriminative stimulus effects involving different neurochemical mechanisms (Stolerman et al., 2011). In addition to increases in 5-HT levels, higher doses of MDMA (>3.0 mg/kg) also significantly increased extracellular DA (Baumann et al., 2005; Kankaanpaa et al., 1998). Since few studies employ a training dose of MDMA in excess of 1.5 mg/kg, the impact of this additional DA response on the subjective effects of MDMA may have been overlooked. This gap in the literature was addressed in the present thesis by investigating the role of 5-HT as well as DA in the subjective effects of a low versus high training dose of MDMA.

The Value of the Drug Discrimination Paradigm

Any exogenous compound that produces changes in physiological or psychological state might be considered a drug, however only a fraction of these compounds are abused by humans. Drugs of abuse are notable for their ability to produce pleasant or desirable subjective effects (e.g. euphoria, hallucinations, increased energy). The drug discrimination paradigm offers the unique ability to measure these otherwise inaccessible subjective experiences in laboratory animals.

In a typical drug discrimination experiment, various compounds are tested for their ability to generalise to the subjective effects of a training drug. Thus, a drug can be rapidly screened for abuse potential by comparing its subjective effects to a known drug of abuse (Holtzman, 1990; Solinas et al., 2006). For example, a novel compound with potential for use as an antidepressant, URB597, also increased levels of the endogenous cannabinoid, anandamide. In drug discrimination experiments, URB597 did not, however, produce tetrahydrocannabinol (THC)-like discriminative stimulus effects suggesting that it did not produce rewarding effects resembling commonly abused cannabis derivatives (Solinas et al., 2006). While this screening technique represents a reasonably straightforward way to positively identify abuse-related subjective effects, there are some situations in which these types of results may be misleading.

SSRIs such as fluoxetine, and clomipramine produce discriminative stimulus effects in rats, but are not commonly abused by humans (Dekeyne & Millan, 2003). MDMA fully generalised to the discriminative stimulus effects of both of these compounds which might suggest that MDMA has a similarly low potential for abuse. On the other hand, human MDMA users often report stimulant-like subjective effects resembling those produced by AMPH (Cohen, 1995; Nichols, 1986). AMPH and other psychostimulants readily substituted for each other in drug discrimination studies (Desai et al., 2010; Schechter, 1997; Stolerman & D'Mello, 1981), however MDMA did not generalise to the discriminative stimulus effects of AMPH, or cocaine (Glennon & Misenheimer, 1989; Khorana et al., 2004; Kueh & Baker, 2007; Schechter, 1986). Again, these findings might suggest that MDMA does not share abuse-related effects with the frequently abused psychostimulants. Nevertheless, MDMA remains one of the most widely-used recreational drugs and a number of users meet DSM-IV criteria for substance abuse (Cottler et al., 2001; Uosukainen et

al., 2015). The drug discrimination paradigm may therefore be limited in terms of its ability to assess abuse potential *per se*.

A more common, and perhaps more valuable, application of the drug discrimination paradigm is in the investigation of the underlying neurobiological mechanisms of centrally active drugs. Discrimination procedures allow the action of psychoactive drugs to be analysed at the whole-organism level, while maintaining a high level of pharmacological specificity. Early studies in which rats were trained to discriminate the opiate, fentanyl, from saline demonstrated that orderly dose-dependent generalisation functions could be generated by testing various doses of the training drug, or equivalently, by compounds with known agonist activity at opiate receptors (Colpaert, Lal, Niemegeers, & Janssen, 1975; Colpaert, Niemegeers, Lal, & Janssen, 1975). Importantly, generalisation to the discriminative stimulus of fentanyl and morphine was not produced by drugs which acted on CNS sites other than opioid receptors (Colpaert, 1978). Since these seminal studies, drug discrimination experiments have been carried out with compounds from nearly every conceivable pharmacological class, and as a result it has been demonstrated that effects mediated by practically any neurotransmitter system or molecular mechanism are open to investigation (Colpaert, 1999; Solinas et al., 2006). These qualities of versatility and sensitivity made the drug discrimination paradigm an ideal tool for investigating the neurochemical mechanisms underlying the subjective effects of MDMA.

Acquisition of the MDMA Discrimination

A number of studies have reported reliable drug discrimination maintained by a 1.5 mg/kg dose of MDMA (Goodwin & Baker, 2000; Goodwin et al., 2003; Oberlender & Nichols, 1988; Schechter, 1986, 1988, 1991). The criterion used to define reliable discrimination in those studies, however, was less stringent (>80% correct for 8 out of 10 sessions) than those used in studies investigating other drugs, such as cocaine or AMPH, in which a response accuracy of >80% was required for 10 consecutive sessions (Callahan et al., 1991; Colpaert et al., 1980; Kantak et al., 1995; Mantsch & Goeders, 1999; Picker et al., 1990). In two-lever acquisition experiments described in chapter three, nearly all subjects in the 1.5 mg/kg MDMA training dose group had met the standard ‘8 out of 10 sessions’ criterion after 40 sessions whereas less than 50% of these subjects were able to respond accurately for

8 or more *consecutive* sessions by the end of the experiment. These findings suggested that 1.5 mg/kg MDMA may in fact produce discriminative stimulus effects that are not easily discriminated, and that the 3.0 mg/kg MDMA training dose may be better suited for experiments of this nature. The decision was made, however, to maintain the use of a 1.5 mg/kg MDMA training dose in subsequent experiments, partly due to the wealth of previous literature that has employed this dose. It was also hoped that the consistency of accurate discrimination by the low training dose group would improve following additional training sessions throughout the course of subsequent experiments. This decision appears to have been justified given the orderliness and consistency of generalisation data collected in subsequent test sessions with these same subjects.

Nevertheless, an important implication of the initial findings described in chapter three is that the criterion employed by a large number of studies might not be adequate to accurately determine whether the 1.5 mg/kg MDMA discrimination has been acquired. Reliable baseline behaviour is critical for interpreting data in subsequent test sessions, and these findings suggest that tests carried out immediately following the completion of the standard criterion would risk falsely attributing effects to poor stimulus control which might have been otherwise apparent during normal training. While no acquisition criteria can provide an absolute guarantee regarding a subject's subsequent behaviour, future experiments might be able to estimate the level of stimulus control that can be expected once a given criterion has been met.

A simple measure of stimulus control is whether a subject meets the 80% threshold for responses on the stimulus-appropriate lever during the first trial of a session. Thus, failure to reach this threshold could be defined as a discrimination error. Figure 7.1 represents a schematic diagram of a basic experiment which could be used to test the reliability of a given acquisition criterion. Subjects would be trained in a typical drug discrimination procedure until a given criterion was met, then the number of errors made in sessions subsequent to meeting criterion would be measured. In an ideal drug discrimination procedure, once the discrimination is learned, as defined by a given acquisition criterion, subjects should always select the correct lever following the administration of either MDMA or saline. In such a case, subjects would display no, or very few, errors in subsequent sessions. If, on the other hand, a large number of errors are made after an acquisition criterion has been met,

then the validity of that criterion would come into question. Assessing the strength of different criteria in this way would allow future experimenters to more confidently determine when to proceed from drug discrimination training to test sessions.

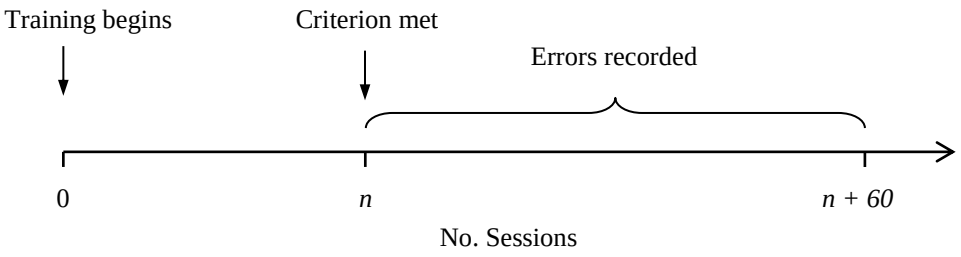

Fig 7.1 A hypothetical experiment measuring the number of discrimination errors made after meeting a given acquisition criterion

Dose-dependent Neurochemical Mechanisms

Results from generalisation experiments described in chapters four and five suggest that the subjective effects of 1.5 mg/kg MDMA are primarily mediated by increases in extracellular serotonin and likely involve the activation of 5-HT_1 and 5-HT_2 receptors. The neurochemical mechanisms underlying the discriminative stimulus effects of 3.0 mg/kg MDMA, however, are less clear. The present results are the first to suggest that the role of 5-HT in producing MDMA-like subjective effects may be dose-dependent and that the subjective effects of higher doses of MDMA may involve the recruitment of dopaminergic mechanisms.

These findings may have important implications with respect to the potential of MDMA as a drug of abuse. Commonly abused psychostimulants such as AMPH, cocaine, and methamphetamine act as powerful reinforcers in operant procedures

such as self-administration (Richardson & Roberts, 1996), and as conditioned reinforcers in conditioned place preference (CPP) experiments (Bardo, Rowlett, & Harris, 1995). The strength of these reinforcing effects roughly corresponds to the ability of each compound to activate mesocorticolimbic dopaminergic pathways (Di Chiara et al., 2004). Accordingly, reliable self-administration of the potent dopamine increasing stimulants, cocaine and AMPH, is acquired rapidly within a small number of training sessions (Carroll & Lac, 1997). In contrast, the self-administration of low doses of MDMA (1.0 mg/kg), which preferentially increases 5-HT, is acquired only after a considerable number of training sessions (>15) and then only by approximately 50% of subjects (Schenk, Gittings, Johnstone, & Daniela, 2003; Schenk et al., 2007), suggesting that the initial reinforcing effects of MDMA are comparatively weak. It has been proposed that the acquisition of self-administration is, therefore, reliant on changes in the neurochemical response to MDMA that occur after extended self-administration training sessions, such as a decrease in the 5-HT response leading to a disinhibition of DA release (Bradbury et al., 2014; Do & Schenk, 2013; Schenk & Bradbury, 2015).

In the present thesis, dopamine-mediated discriminative stimulus effects only became apparent when a higher training dose of MDMA was used. While the drug discrimination paradigm does not directly measure reinforcing effects, these findings raise the possibility that an increase from 1.5 mg/kg to 3.0 mg/kg MDMA may be associated with an increase in the acute reinforcing properties of the drug. In the context of recreational use by humans, the potential of MDMA to lead to abuse and dependence may therefore depend on the amount of drug consumed per session. This may be particularly important given reports of users ‘stacking’ multiple pills over the course of an evening (Parrott, 2001). It would be interesting to determine the ability of 3.0 mg/kg MDMA to support self-administration in future experiments. Since this dose appears to produce behaviourally relevant dopaminergic effects, it is possible that the acquisition of 3.0 mg/kg MDMA self-administration would more closely resemble that of cocaine and AMPH. Self-administration experiments, however, are also susceptible to disruption by the rate-suppressing effects of higher doses of MDMA. In the present thesis, administration of 3.0 mg/kg MDMA disrupted lever-pressing even in rats that had been repeatedly exposed to 1.5 mg/kg MDMA. Self-administration of 3.0 mg/kg MDMA by drug-naïve rats is therefore likely to be similarly affected.

While the discriminative stimulus effects of the two training doses of MDMA used in the present experiments could be dissociated in terms of 5-HT_2 receptor mechanisms, 5-$HT_{1A/1B}$ agonists generalised to the discriminative stimulus effects of 1.5 mg/kg and 3.0 mg/kg MDMA with equal potency. This might suggest that 5-HT_1 receptor mechanisms may underlie a central component of the MDMA stimulus. 5-HT_{1A} receptors are distributed throughout the CNS but are particularly abundant in the raphe nucleus where they function primarily as somatodendritic autoreceptors (Ito, Halldin, & Farde, 1999; Sharp & Hjorth, 1990). Activation of 5-HT_{1A} autoreceptors results in a decrease in the release of 5-HT (Hjorth & Sharp, 1991). Considering that SSRIs, which produce a general increase in 5-HT levels, fully generalised to the discriminative stimulus effects of 1.5 mg/kg MDMA, it seems unlikely that generalisation of the 5-HT_{1A} agonist, 8-OH-DPAT, reflects the inhibition of 5-HT via autoreceptor activation. A more likely explanation is that 8-OH-DPAT produced MDMA-like subjective effects that were produced via post-synaptic mechanisms involving other neurotransmitter systems.

For example, stimulation of 5-HT_{1A} receptors by 8-OH-DPAT significantly increased the release of the hormone and neuropeptide, oxytocin (Bagdy & Kalogeras, 1993). Oxytocin has been associated with range of social behaviours (Blaicher et al., 1999; Domes, Heinrichs, Michel, Berger, & Herpertz, 2007; Heinrichs & Domes, 2008) and is thought to play a role in the pro-social subjective effects which are so characteristic of MDMA (Dumont et al., 2009; Thompson, Callaghan, Hunt, Cornish, & McGregor, 2007). Importantly, drug discrimination studies have shown that antagonism of oxytocin receptor sites via the administration of atosiban attenuated the discriminative stimulus effects produced by1.5 mg/kg MDMA (Broadbear et al., 2011). Therefore, the substitution of 8-OH-DPAT for 1.5 mg/kg MDMA observed in the present study may reflect 5-HT_{1A} receptor-mediated oxytocin release. Interestingly, the 5-HT_{2A} agonist, DOI, also produced significant increases in oxytocin (Van de Kar et al., 2001). The fact that DOI and 8-OH-DPAT were the only selective ligands to generalise to the discriminative stimulus effects of both training doses of MDMA raises the possibility that oxytocin release may represent a shared component of the discriminative stimulus effects of 1.5 mg/kg and 3.0 mg/kg MDMA. Future experiments could explore this idea by examining the effect of oxytocin agonists and antagonists on the discriminative stimulus effects of 3.0 mg/kg MDMA.

In two-lever generalisation experiments, the dopamine releasing stimulant, AMPH, fully generalised to the discriminative stimulus effects of 3.0 mg/kg MDMA suggesting that these two compounds produced similar subjective effects. This finding was difficult to reconcile with results from acquisition experiments in which rats were trained to discriminate 3.0 mg/kg MDMA from AMPH and saline in a three-lever task. If the higher training dose of MDMA produced dopamine-mediated discriminative stimulus effects that could not be distinguished from those of AMPH in a two-lever task, then a question arises as to how rats were able to acquire the three-lever discrimination with reasonable success.

An important difference between the two experiments is that rats in the three-lever task were repeatedly exposed to AMPH during drug discrimination training sessions. Therefore, it is possible that a differences in drug exposure led to different neuroadaptations in subjects in the three-lever compared to the two-lever experiment. Repeated exposure to AMPH produces enhanced behavioural responses to subsequent challenge injections of AMPH (Pierce & Kalivas, 1997; Robinson & Becker, 1986). These changes likely reflect neuroadaptations in DA circuitry and some evidence suggests that activation of D_1 but not D_2 receptors might be critical to the development of this sensitised response (Bjijou, Stinus, Le Moal, & Cador, 1996). Repeated administration of MDMA also produced a sensitised behavioural response to subsequent MDMA injections (Kalivas et al., 1998; Schenk & Bradbury, 2015; Spanos & Yamamoto, 1989). In contrast to sensitisation produced by AMPH, however, these changes appear to be dependent on D_2 rather than D_1 mechanisms. Co-administration of a D_1 receptor antagonist with an MDMA pre-treatment regimen failed to attenuate the enhanced locomotor activating effect (Ramos, Goñi-Allo, & Aguirre, 2004). Furthermore, challenge injections of the D_1 agonist, SKF81297, produced a sensitised locomotor activity response in AMPH- but not MDMA- pre-treated rats whereas the opposite pattern of results was produced by the D_2 agonist, quinpirole (Bradbury, Gittings, & Schenk, 2012). These findings suggest that the both the development and the expression of behavioural sensitisation produced by AMPH and MDMA may be differentially mediated by D_1 and D_2 receptor subtypes, respectively.

It is unclear whether intermittent exposure to relatively low doses of MDMA and AMPH during drug discrimination training would be sufficient to produce sensitisation. If comparable changes in D_1 and D_2 receptor function did occur,

however, it might be case that AMPH and 3.0 mg/kg MDMA became distinguishable based on differences in D_1 and D_2 receptor mediated subjective effects.

Limitations

One weakness of the drug discrimination paradigm is that it is sensitive to rate-suppressing effects associated with higher doses of some drugs. Several of the compounds tested in the present thesis (e.g. mCPP, 8-OH-DPAT, apomorphine, quinpirole, and WAY100635) significantly decreased response rates. On the one hand, these disruptive effects are not likely to have affected the overall pattern of results since interpretation of data was primarily based on whether a rat chooses to respond on the MDMA versus the saline lever. This choice should be independent of any motor impairments or stereotyped behaviour which may interfere with the physical act of pressing a lever (Colpaert et al., 1976; Overton, 1971).

On the other hand, in some cases severe disruption of lever-pressing prohibited the testing of higher doses in generalisation experiments. For example, the 5-HT_{2C} agonist, mCPP, failed to generalise to the discriminative stimulus effects of 3.0 mg/kg MDMA suggesting that 5-HT_{2C} receptor activation did not produce MDMA-like effects in this group of rats. It is possible, however, that 5-HT_{2C} mediated MDMA-like subjective effects may have been produced by higher doses of mCPP, but that these effects could not be detected due to the inability of rats to complete 10 lever responses. A similar situation may have occurred in generalisation test sessions with quinpirole. The D_2 agonist appeared to produce a dose-dependent increase in MDMA-lever responding in rats trained with 3.0 mg/kg MDMA until higher, behaviourally disruptive, doses of quinpirole were tested. It might have been possible to address these issues by attempting to produce tolerance to some of these disruptive effects. Repeated administration of mCPP produced tolerance to the anxiogenic (Griebel et al., 1994) and locomotor inhibiting (Sills, Lucki, & Frazer, 1985) effects of the drug and so it is possible that a similar pre-treatment regime may have attenuated the rate-suppressing effects of mCPP observed in the present experiments. However, such a manipulation would be counterproductive since any mCPP-produced neuroadaptations might be expected to impact the discriminative stimulus effects of the training dose of MDMA as well.

Another limitation of experiments in the present thesis is that temporal parameters such as the delay between the administration of a drug and the onset/duration of its peak effects could not be assessed. Once administered, all drugs are metabolised by various physiological mechanisms, resulting in the gradual decay of any behavioural or psychoactive effects. The term 'half-life' refers to the amount of time taken for the peak effects of a drug to decay by 50%. When carrying out drug discrimination experiments, it is important to consider the half-life of the training, and test compounds, to ensure that both are active during the brief window in which a subject's responses are recorded. For example, a drug with a very short half-life may no longer be active in the CNS if the delay between administration and testing is too long. Similarly, pharmacokinetic factors such as route of administration can affect how long it takes for a drugs peak effects to appear.

The choice of pre-treatment times may have impacted results from tests of agonists and antagonists (see chapter six), but the time-course of MDMA-produced subjective effects must also be considered when interpreting the results. In experiments described in the present thesis, discriminative stimulus effects were measured 15 min after the administration of MDMA, since it is around this time that peak effects are observed. However, the drug remains active for up to 4-hrs (Schechter, 1987) and there Is some evidence that the discriminative stimulus effects at later time points may reflect different neurochemical mechanisms. For example, DA antagonists had no effect on the discriminative stimulus effects of 1.5 mg/kg MDMA at 20mins post injection but produced a moderate attenuation of MDMA-appropriate responding after a longer delay of 105 mins (Schechter, 1988). Future experiments could extend findings from the present thesis by testing the ability of 5-HT and DA ligands to alter the discriminative stimulus effects of MDMA after a range of post-MDMA-injection times.

Future directions: The Impact of 5-HT Neurotoxicity

Evidence from human drug discrimination studies suggest that extended MDMA use may alter the subjective effects of the drug itself. Experienced users perceived MDMA to be AMPH-like whereas less experienced users perceived it to be more similar to the 5-HT agonist, mCPP, (Johanson et al., 2006). Changes in self-reported subjective effects have also been reported with typical empathogenic effects

gradually giving way to AMPH-like stimulant effects (Peroutka, 1990). This apparent shift in the subjective effects as a function of experience may reflect neuroadaptations as a result of drug exposure.

Functional impairments in 5-HT neurotransmission have been observed in laboratory animals following repeated exposure to MDMA (Commins et al., 1987; Jensen et al., 1993; Schmidt, 1987; Stone et al., 1987). In human subjects, Positron Emission Tomography (PET) revealed significant global reductions in SERT binding in patients with a history of MDMA use compared to a control group, and these reductions were positively correlated with the extent of MDMA experience (McCann, Szabo, Scheffel, Dannals, & Ricaurte, 1998). Deficits in 5-HT function in MDMA users was correlated with behavioural changes including memory disturbances (Reneman, Booij, Schmand, van den Brink, & Gunning, 2000) as well as changes in mood and cognition (Thomasius et al., 2006). An important avenue for future research is to determine whether MDMA-produced neurotoxicity also impacts the subjective effects of the drug itself.

A handful of studies have examined the effect of 5-HT neurotoxicity on the discriminative stimulus effects of MDMA. In a three-lever drug discrimination procedure, exposure to an MDMA 'binge' (3 injections of 10 mg/kg MDMA administered every two hours) significantly disrupted the discrimination of 1.5 mg/kg MDMA from AMPH and saline (Smithies & Broadbear, 2011). Similarly, repeated administration of a neurotoxic dose of MDMA (20 mg/kg twice a day for four days) led to a flattening of the MDMA dose response function in rats trained to discriminate 1.5 mg/kg MDMA from saline (Schechter, 1991), suggesting a decrease in sensitivity to the discriminative stimulus effects of MDMA. An identical neurotoxic pre-treatment regimen disrupted the discrimination of the stereoisomer, S(+)-MDMA, but co-administration of the SSRI, fluoxetine, during the pre-treatment phase protected against this effect (Virden & Baker, 1999) suggesting that neurotoxic effects may be specific to 5-HT neurons.

The training dose of 1.5 mg/kg MDMA used in these studies produces discriminative stimulus effects which are primarily mediated via serotonergic mechanisms (see chapters four and five). It is therefore unsurprising that disruption to 5-HT neurotransmission would impair the ability of subjects to discriminate this dose of MDMA from saline. In another study, injections of AMPH (1.0 mg/kg) which initially elicited only saline-responding, fully generalised to the discriminative

stimulus effects of 1.5 mg/kg MDMA following a neurotoxic dosing regimen of fenfluramine. These findings raise the possibility that 5-HT neurotoxicity leads to alterations in the discriminative stimulus effects of MDMA so that they gradually become more AMPH-like; a pattern that resembles the shift in subjective effects reported by human users described earlier. Human users often initially consume MDMA in small amounts and at irregular intervals before escalating to a pattern of binge use (Parrott, 2001, 2013). The relevance of neurotoxic effects in laboratory animals might therefore be limited since the non-contingent administration of very high doses of MDMA used in these studies may not accurately reflect the patterns of use in humans.

A way to model a more 'human' pattern of MDMA intake in animal subjects is with the self-administration paradigm (Do & Schenk, 2013). Briefly, self-administration experiments involve the surgical implantation of an intravenous catheter so that animal subjects are able to press a lever in order to receive an automatic infusion of MDMA. When this procedure is carried out over several sessions, rats typically self-administer small amounts of MDMA at first, but some gradually escalate their intake until consumption increases to as much as 20 mg/kg/session (Schenk et al., 2003; Schenk et al., 2007). Significant decreases in 5-HT tissue levels were observed in rats that self-administered a total of at least 315 mg/kg MDMA, and these deficits were still apparent following 2 weeks of abstinence (Do & Schenk, 2013). Thus an experiment was designed, as part of the present thesis, to determine the impact of these self-administration produced 5-HT deficits on the subjective effects of MDMA.

If the impaired 5-HT function associated with chronic self-administration of MDMA leads to changes in the subjective effects of the drug, then these changes should be detectable in drug discrimination experiments. To test this idea, a three-phase experiment was proposed and is outlined in Figure 7.2. In phase one, forty rats were trained to discriminate MDMA (1.5 mg/kg) from AMPH (0.5 mg/kg) and saline in a standard three-lever drug discrimination procedure. Once the discrimination had been acquired, generalisation test sessions were carried out in order to determine dose effect curves for MDMA and AMPH. These dose-effect curves were generated so that comparisons could be made between the discriminative stimulus effects of MDMA and AMPH before and after the self-administration phase.

Immediately following these test sessions, rats were surgically implanted with an intravenous catheter. Surgery was carried out under deep anaesthesia produced by an injection of a ketamine/xylazine mixture (90 mg/kg / 9 mg/kg). An area on the chest was shaved and cleaned using a solution of ethanol and iodine and a small incision was made in order to expose the right jugular vein. Once exposed, a Silastic catheter was implanted and fixed in place with surgical thread. The distal end of the catheter was passed subcutaneously to an exposed portion of the skull where it was attached to a 3 cm length of stainless steel tubing (22 gauge, BD Needles). The tube was fixed onto the skull using screws and embedded in a layer of dental acrylic (Ostron 100). Subcutaneous injections of the analgesic, carprofen (5.0 mg/kg) and Hartman's solution (10 mL) were administered at the completion of the surgery in order to facilitate recovery. Post-operative care involved daily 2 mL infusions of heparinised saline (3U) to ensure catheter patency as well as additional injections of carprofen, once per day for 2 days.

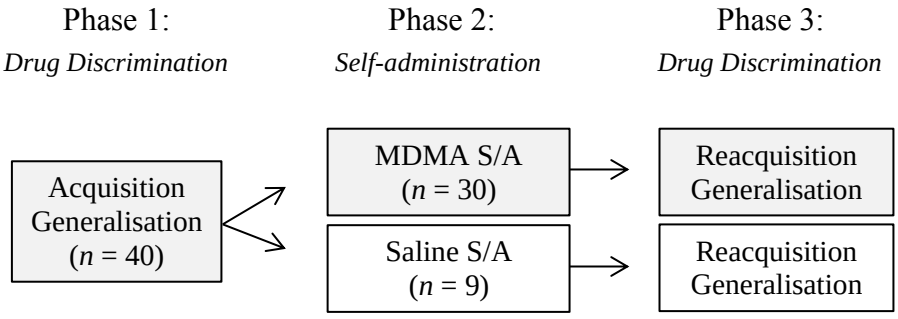

Fig 7.2 Diagram outlining the initial design of an experiment examining the role of MDMA self-administration on the discriminative stimulus effects of MDMA

For phase two, rats were divided into two separate groups. In previous self-administration experiments in our laboratory, approximately 40% of rats failed to acquire MDMA self-administration with 25 sessions (Schenk et al., 2007). In order to ensure that sufficient number of rats reached the third phase of the experiment, 30 rats were assigned to the MDMA self-administration group. The remaining 9 rats were assigned to a saline self-administration control group. Self-administration sessions were carried out after a 7 day recovery period following surgery. Briefly, rats were placed in an operant chamber and the exposed portion of the catheter was connected via a length of microbore tubing to a computer controlled syringe pump. Depression of the lever resulted in a 12-second automatic infusion of MDMA (1.0 mg/kg). The plan was to allow all rats to self-administer a total intake of 350 mg/kg before returning them to drug discrimination training for phase three of the experiment. New, 'post-self-administration' dose response curves were to be generated to test whether chronic exposure to MDMA impacted the drug's discriminative stimulus effects.

Unfortunately, unforeseen complications were encountered during the self-administration phase of this experiment. A number of rats self-administered large quantities of MDMA during the first week of self-administration sessions and several fatal overdoses occurred. In an attempt to protect the remaining subjects, a forced timeout period was employed between drug infusions. Despite this added precaution, after several weeks of self-administration training an unacceptable number of overdoses had occurred and the experiment was abandoned.

These problems might be attributed to the fact that subjects had prior experience in a lever-pressing operant task. In typical MDMA self-administration experiments in our laboratory, drug-naïve rats complete few lever presses during the first few sessions and only approximately 60% of rats acquire self-administration behaviour within 25 sessions (Schenk et al., 2003). Thus a fixed ratio of one response per infusion (FR1) is used to facilitate the acquisition of MDMA self-administration. In the present experiment, rats had extensive lever-pressing experience following months of drug discrimination training on a FR10 schedule. This may have led some rats to attempt 10 lever presses and thus receive 10 automatic infusions (approximately 10 mg/kg) over a short time period. Another factor that may have contributed to the high attrition rate was the age and size of the rats. Decreased body weight from restraint induced stress provided significant protection from the

neurotoxic effects of MDMA in mice (Johnson, Sharp, & Miller, 2000). It is therefore possible that the impact of MDMA-produced toxicity was more severe in the present attempted experiment in which older and larger rats were used.

While no useable data was generated from self-administration procedures, drug discrimination testing in phase 1 of the experiment yielded some valuable results. Figure 7.3 displays the allocation of lever responses following administration of various doses of MDMA in generalisation tests carried out during the phase one of the experiment.

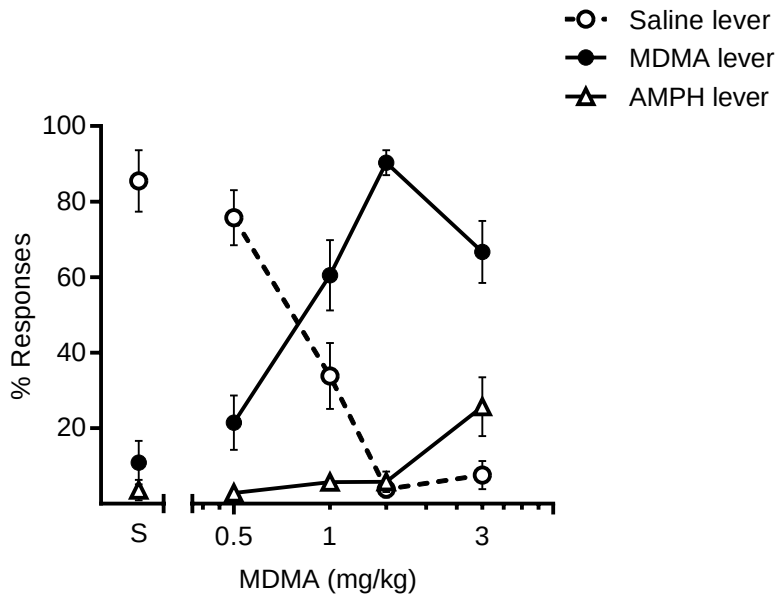

Fig 7.3 Mean percentage (±SEM) of responses on saline (open circles), MDMA (close circles), and AMPH (triangle) levers following administration of MDMA (0 – 3.0 mg/kg) in rats trained to discriminate MDMA (1.5 mg/kg) from AMPH (0.5 mg/kg) and saline.

Injections of MDMA, up to the training dose of 1.5 mg/kg, dose-dependently increased the percentage of responses on the MDMA-lever [$F(4, 80) = 27.87$, $p < .001$]. Administration of a higher 3.0 mg/kg dose of MDMA, however, led to a significant (Bonferroni post-hoc comparisons) decrease in MDMA-lever responding and a corresponding increase in AMPH-lever responding. These findings are consistent with the hypothesis that 3.0 mg/kg MDMA produces discriminative stimulus effects that resemble the dopaminergic stimulant AMPH, and provide an important replication of previous results which have been frequently cited throughout the present thesis (Harper et al., 2014).

Further attempts to carry out this experiment in full will be extremely valuable. In future experiments, the present complications that were encountered during the self-administration phase might be avoided by employing a different operant task instead of lever-pressing. For example, several self-administration experiments have been carried out in which rats are able to trigger an automatic drug-infusion via a nose-poke response (Moody & Frank, 1990; Schindler, Thorndike, & Goldberg, 1993; Welzl, Kuhn, & Huston, 1989). If self-administration sessions could be continued until a cumulative intake of ~ 350 mg/kg MDMA had been reached, then rats could be returned to drug discrimination training in order to test the effect of chronic MDMA exposure on the subjective effects of the drug.

In previous experiments, extensive MDMA self-administration was associated with a decrease in 5-HT tissue levels (Bradbury et al., 2014) as well as an enhanced DA-response to subsequent challenge injections of MDMA (Colussi-Mas, Wise, Howard, & Schenk, 2010). It is possible that these changes could lead to the discriminative stimulus effects of 1.5 mg/kg MDMA becoming difficult to distinguish from the primarily dopaminergic discriminative stimulus effects of AMPH. Thus in subsequent drug discrimination training sessions, one might expect rats that self-administered MDMA to be less able to reacquire the three-way discrimination than rats that self-administered saline. Such findings might imply that sustained MDMA-use could increase the risk of the development of abuse, since the initial ‘entactogenic’ subjective effects may gradually give way to those that more closely resemble a commonly abused psychostimulant.

Conclusions

The neurochemical mechanisms underlying the subjective effects of MDMA change as a function of dose. The subjective effects of 1.5 mg/kg MDMA appear to be primarily mediated by the release of 5-HT and the subsequent activation of 5-HT_1 and 5-HT_2 receptors. The subjective effects of higher doses appear to involve the additional recruitment of dopaminergic mechanisms, and may begin to resemble those produced by the commonly abused psychostimulant, amphetamine. These findings suggest that the amount of MDMA consumed at one time may have a significant impact on the potential for the development of substance-abuse symptoms. Future research is warranted to examine whether chronic exposure to MDMA produces changes in the subjective effects of the drug which also contribute to its potential for abuse.

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