A Three-way Drug Discrimination Study:
A Role for DA-mediated Effects in MDMA's Discriminative cue properties

by

Anna-Lena Langen

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While 3,4-methylenedioxymethamphetamine (MDMA) shares many similarities with amphetamine, previous two choice drug discrimination procedures have shown that substitution between the two substances is inconsistent. Three choice drug discrimination procedures have revealed that MDMA can be discriminated from amphetamine, due to MDMA's primary influence in releasing 5-HT. Neurochemical evidence had previously suggested that at doses >3.0mg/kg MDMA-induced dopamine (DA) release will increase significantly. In the current study rats were trained to discriminate MDMA from amphetamine and saline. As the dose of MDMA increased beyond the training dose (>1.5mg/kg) MDMA-appropriate responding decreased, while the proportion of amphetamine lever responding increased and eventually surpassed MDMA-appropriate responding at the highest dose (4.5mg/kg). This would indicate an important role for DA mediated influences in MDMA's discriminative cue properties. Further evidence for this conclusion comes from tests with the D1 antagonist SCH23390 and the D2 antagonist eticlopride which attenuated this effect and also led to a nonsignificant increase in the proportion of saline lever responding. Subsequent tests with the 5-HT2c antagonist RS102221resulted in no significant dose dependent changes, but appeared to reduce MDMA-appropriate responding especially at the training dose. The current findings would suggest that low doses of MDMA are discriminable from amphetamine, however with increasing doses MDMA will be perceived as more "amphetamine-like". These findings could suggest that at relatively high doses MDMA produces effects that are typically associated with dopamine-releasing drugs, such as high abuse potential.

MDMA

Ecstasy is a drug that is commonly used and abused recreationally and the main ingredient of Ecstasy is (+/-) 3,4 methylenedioxymethamphetamine (MDMA). MDMA is a structural analogue to amphetamine, but also possesses structural similarities to mescaline. This has led to the classification of MDMA as an entactogen (Greek-producing a touching within), which differentiates MDMA from standard stimulants and hallucinogens (Nichols, 1986).

While MDMA was first synthesized in 1912 by German pharmaceutical company Merck, research concerning its effects only began in the Fifties and Sixties (Freudenmann, Özler, Bernschneider & Reif, 2006). Due to reports of MDMA's subjective effects of facilitating closeness, openness, euphoria and heightened sensory awareness (Morgan, 2000), MDMA began to be used in American psychotherapy in the Eighties (Rochester & Kirchner, 1999). Any findings or data of MDMA use in clinical settings was never formally published (Shulgin, 1990). Additionally, increased mishandling as well as a rise in the reporting of unpredictable side effects in the general public led to MDMA's classification as a schedule A drug in 1986. This classification indicates that MDMA has a high abuse potential with no established medical use (Cole & Sumnall, 2003).

Studies in New Zealand and elsewhere have indicated that Ecstasy is one of the most popular illegal drugs consumed, especially for the student population (Strote, Lee & Wechsler, 2002, Wilkins & Sweetsur, 2008). Ecstasy is generally taken in pill form, however, some users have begun to snort and inject it as well

(Morgan, 2000). Its popularity has also been increasing over recent years, which has led to a growing number of first time users and rising consumption by those experienced with the drug (MoH, 2010). Wilkins and Sweetsur (2008) showed that lifetime prevalence of Ecstasy use in the general population increased by 5% from 3% in 1998 to 8% in 2006 in those aged 15 to 45. Interestingly, in the same time frame, the prevalence of use in the previous 12 month period more than doubled from 1.2 % to almost 4% in the same population.

Amphetamine

Amphetamine ((±)-1-phenylpropan-2-amine) is a psychostimulant drug and was first synthesized in 1887 in Berlin, Germany (Sulzer, Sonders, Poulsen & Galli, 2005). However, it was not before the 1930's that amphetamine began to catch the interest of pharmaceutical companies. During the early years of amphetamine's pharmacological use, it was prescribed for a multitude of diseases and disorders; for example narcolepsy, mild to moderate depression and as a nasal congestant (Berman, Kuczenski, McCracken & London, 2009). While success rates of amphetamines in ameliorating these disorders and diseases were debatable, the pharmaceutical company Smith, Kline and French were persistent in finding a use for this new drug (Rasmussen, 2006). Amphetamine's popularity grew quickly and it was commonly used by night workers, soldiers and athletes to facilitate alertness and peak performances. Similarly, its use as a weight loss agent increased. However, at the same time its addictive potential and apparent ability to induce psychosis like symptoms became more apparent, which led to amphetamine becoming a controlled substance in 1971 (Rasmussen, 2006). Today, amphetamine is used as a last resort

treatment for narcolepsy and ADHD, reflecting its accepted medical usefulness but also its high dependence potential (Morgan, 2000).

Studies in New Zealand show that amphetamine abuse, especially in the form of its derivative methamphetamine, is a serious concern. However, the national drug survey (2010) showed an increase in amphetamine use from 1998 to 2001 (7.6% to 11%), followed by a subsequent stabilisation and slight decline. Yet in the same time frame, the quantity consumed by a single user has increased, with 1 in 5 amphetamine users consuming a dangerous quantity in a single session alone (Wilkins, Pledger, Battha & Casswell, 2002). A 2006/2007 survey in New Zealand indicated that lifetime use of amphetamine type drugs was 7.2% with 2.1 % of those surveyed reporting having used amphetamine in the last year (MoH, 2010).

Effects of MDMA and Amphetamine on Behaviour

Generally, there has been a limited amount of research with human subjects on MDMA's and amphetamines behavioural and cognitive effects. Conclusive data of the effects of MDMA and amphetamine are scarce as most research done with humans has been of a retrospective nature with polydrug users (Vollenweider, Liechti, Gamma, Greer & Geyer, 2002).

Nevertheless, the acute subjective effects of MDMA have been described as encouraging closeness, euphoria and heightened sensory awareness; such as tactile hallucinations (Morgan, 2000). This has led to the description of MDMA as an empathogen (Tancer & Johanson, 2001; Parrot, 2007). Overall, however, human

research has focused on long term effects of MDMA use because of ethical constrains placed on studies that focus on acute effects (Vollenweider et al. 2002). Furthermore, in regards to cognitive effects, there is more concern about longer lasting health changes following chronic or high dose binge exposure (Harper, Wisnewski, Hunt & Schenk, 2005). Studies have suggested that prolonged Ecstasy use can lead to cognitive changes, such as declines in verbal memory performances, episodic memory and semantic word fluency difficulties (Reneman, Lavalaye, Schmand, de Wolff, van Den, den Heeten & Booji, 2001; de Sola Llopis et al, 2008).

In contrast to MDMA, and mostly due to amphetamines' widespread usage in the past, there has been more information on amphetamines effects in humans. Generally, the acute effects of amphetamine have been identified as wakefulness, increased focus, anorexia, hyperactivity and feelings of bliss, as well as nervousness, emotional instability and insomnia (Johanson & Uhlenhuth, 1980; Seiden, Sabol & Ricaurte, 1993). However, although amphetamines is a clinically approved treatment for ADHD and narcolepsy and has a long history of use and abuse, most research has been unsatisfying as it has focused mostly on short time periods, low doses and small controlled sample sizes. (Berman et al, 2009). The focus on studies of long-term amphetamine use has been on its addictive potential, due to a fast development of tolerance. This in turn has been linked to subsequent psychological difficulties (Seiden et al. 1993) and the occurrence of neurotoxicity (Berman et al, 2009).

In general, most of the restrictions encountered in human MDMA and amphetamine research can be effectively addressed in animal research. In terms of acute behaviour effects after MDMA administration, it has been shown that

moderate doses of MDMA in rats are expressed through hyperactivity. Specifically, this is reflected in a dose dependent increase in peripheral activity and rearing (Bankson & Cunningham, 2002; Herin, Liu, Ullrich, Rice & Cunningham, 2005). Higher doses of MDMA generally elicit the 5-HT syndrome; which in rats is expressed through low body posture, abducted hind limbs, salivation and piloerection (Marston, Reid, Lawrence, Olvermann & Butcher, 1999).

Repeated MDMA exposure also leads to the development of sensitisation and tolerance in a variety of effects. For example Balogh, Molnar, Jakus, Quate, Olverman et al. (2004) showed that while a single injection of MDMA (15mg/kg) alters the pattern of circadian rhythms for the following 5-6 days, the duration of this physiological change declines in length for rats that had previously been exposed to MDMA; indicating the development of tolerance. Similarly, behavioural tests conducted in an acute MDMA phase show that skilled motor ability is severely impaired at first exposure, however, tolerance to these effects develops quickly (Marston et al. 1999).

In line with this, it has been argued that the neuroadaptations that take place in the development of sensitisation and tolerance may be critical to the subsequent onset of drug abuse or dependence (Schenk, 2011). A variety of studies have reported that animals quickly learn to self-administer amphetamine, (e.g., Lyness, Friedle & Moore, 1979) as well as MDMA (e.g., Beardsley, Balster & Harris, 1986, Schenk, 2011).

Similarly to MDMA's behavioural effects, amphetamine also increases locomotor activity after acute exposure and often elicits subsequent behavioural sensitisation after chronic exposure (Schenk, 2011). However, unlike MDMA, amphetamine increases locomotor activity throughout the entire activity box and not just in the periphery (Bankson & Cunningham 2002). An in-depth observation of locomotor behaviour after amphetamine administration revealed that low and medium doses (0.5-1.5 mg/kg) increase all behaviours (sniffing, moving, rearing, grooming, scratching, head swinging and licking) while higher doses (3.6mg/kg) are associated with repetitive behaviours (head swinging and licking) and nearly abolishes behaviour switching (Antoniou, Kafetzopoulos, Papadopoulou-Daifoti & Marselos, 1998).

Long-term MDMA and amphetamine exposure have repeatedly been shown to have adverse effects on psychological functioning. For example, rats that have been treated with MDMA display lasting deficits in social interactions with other rats; which is an established indicator of anxiety (McGregor, Clemens, van der Plasse, Li, Hunt, et al. 2003). Similarly, long-term depressive symptoms have been ascribed to previous MDMA exposure (Montaya, Sorrentino, Lukas & Price, 2002). Specifically, it was found that after increased exposure to stress by repeatedly exposing rats to a forced swim test, MDMA treated rats compared to a control group were more immobile and less likely to attempt climbing; an indication of depressive symptoms (McGregor et al, 2003).

Particularly, a combination of stress and MDMA exposure appears to markedly decrease functioning in a variety of cognitive and behavioural paradigms.

Accordingly, research that has combined a treatment of high doses of MDMA (4*every 2 hrs 7.5 mg/kg) and exposure to stress (CUS, Chronic Unpredicatable Stress) over a period of 10 days found that this paradigm would lead to impairments in spatial learning, even though this treatment did not appear to increase anxiety (Cunningham, Raudensky, Tonkiss & Yamamoto, 2009). Employing the MWMT (Morris Water Maze Task), it was also shown that both groups of rats, those treated with MDMA and those additionally exposed to chronic unpredictable stress would take longer and travel a greater distance to find the escape platform. This effect was enhanced for the rats exposed to the combination of MDMA and CUS, when tested three to four weeks later (Cunningham et al, 2009). However, other research did not find the same results one week after MDMA exposure, indicating perhaps delayed neurotoxic effects to MDMA (Sprague, Preston, Leifheit & Woodside, 2003).

Similarly, long-term exposure to amphetamines also has detrimental effects on psychological functioning. For example, due to amphetamine's sensitisation effects, repeated exposure can elicit a paranoid psychosis. Generally these episodes are not lasting and end after approximately 10 amphetamine free days in humans (Berman et al, 2009). This has led researchers to utilise repeated amphetamine administration and its resulting sensitization as an animal model for schizophrenia. Classical symptoms of schizophrenia are often hypothesized to arise due to attentional deficits. To test whether amphetamine can induce attentional deficits in rats Kozak et al. (2007) exposed rats to an amphetamine challenge, in which rats were given injections daily with ever increasing amount of amphetamine for 2 months (from 1-10mg/kg). It was found that only rats that had been exposed to the amphetamine pretreatment regimen and were additionally exposed to amphetamine

(1.0mg/kg) were significantly impaired in the DMTS task. The deficit observed in these rats was a direct result of their inability to discriminate between signal and nonsignal trials. The authors interpreted this as an impairment in cognitive task control, which can be a symptom of schizophrenia (Kozak et al. 2007).

In line with this, it has been argued that difficulties in working memory tasks that become apparent after acute and chronic administration of MDMA can also be interpreted as a result of a deficiency in general attention processes or impairments in executive functioning (Marston, 1999). Specifically, research utilising the delayed matching to sample (DMTS) paradigm investigated whether memory deficits from acute MDMA exposure were indicative of specific impairments in memory processes or more general deficits in attentional processes (Harper et al. 2005). It was found that exposure to MDMA (and amphetamine) resulted in encoding-specific or attention-specific deficits, which is reflected in a general delay-independent decrease in discriminability. In addition they found that MDMA produced an increased proactive interference effect; which was larger at longer delay trials. That is rats showed a tendency to base their current-trial choice response on the response made in the immediately preceding trial. Thus, it was argued that MDMA exposure results in increased confusion about the order of required responses (Harper et al. 2005). To further clarify these results, an additional study conducted using the Radial Arm Maze confirmed that MDMA was more likely to impair reference memory (indicated by the ability to remember general rules to complete the task-which is a trial independent occurrence) than working memory (remembering the events that have already occurred vs those that need to be completed within a single trial) (Kay, Harper & Hunt, 2010).

Research concerning amphetamine's effects on memory function has yielded mixed results. Some research has shown that Amphetamine, like MDMA, produces significant amounts of proactive interference in a DMTS paradigm, indicating increased confusion about task order, and thus pinpointing deficits in attention rather than working memory (Harper et al. 2005). However, research with low doses of amphetamine (0.42mg/kg) in humans has observed no deficits in driving related cognitive processes. In fact there was some evidence to suggest enhancement in attentional, perceptual and psychomotor procedures during acute exposure. The enhancement was due to increased speed of performance and accuracy in detection (Silber, Croft, Papafotion & Stough, 2006). Similarly, using the T-maze, Shoblock, Maisonneuve and Glick (2003) revealed that acutely administered low doses of amphetamine (0.5 mg/kg) improved performance compared to a control group. However, higher doses still led to performance impairments, especially at the longest delays (10s).

Thus, long term exposure to amphetamine and MDMA leads to a variety of deficits; which are influenced by the drugs mechanisms to elicit either tolerance or sensitisation to different effects according to varying treatment regimes. The deficits observed in the rats behaviour parallel those encountered in some psychological deficits, which has enabled researchers to utilise amphetamine exposed rats as animal models of some mental illnesses, such as schizophrenia and bipolar disorder. Overall, amphetamine and MDMA are similar in regards to their effects on behaviour and cognition, while some differences, such as specific locomotion patterns and subjective perceptual experiences persist.

Neurochemical effects of MDMA

Many of the acute effects of MDMA are thought to be governed via its influence on 5-HT release (Bankson & Cunningham, 2002; Schechter, 1990). However at sufficiently high doses (above 3.0 mg/kg) MDMA's behavioural effects may be mediated by increased synaptic dopamine in a variety of brain regions (Baumann, Clark & Rothman, 2008). In vivo studies have shown that both extracellular levels of 5-HT and DA increase after MDMA administration. However, a relatively low dose of MDMA (1.0 mg/kg) increases 5-HT levels five times whereas DA levels increase only twice that of the baseline amount (Baumann et al. 2008).

As evidenced by studies with the SSRIs (serotonin reuptake inhibitors), 5-HT increases because MDMA binds or reverses SERT, the 5-HT transporter. Combined administration of SSRIs and MDMA attenuates the rise in extracellular 5-HT (Rudnick & Well, 1992). Finally, SSRIs also block hyperactivity usually associated with MDMA administration (Callaway, Wing & Geyer, 1990). Similarly, 5HT2A antagonists also attenuate MDMA associated behavioural effects such as peripheral hyperactivity and rearing (Herin et al. 2005). Similarly, treatment with a chronic 5HT2A or 5HT1B/1D agonists in conjunction with MDMA impacts on potentiated behavioural responses that mirrors the sensitization associated with psychostimulants (Ross, Herin, Frankel, Thomas & Cunningham, 2006; McCreary, Bankson & Cunningham, 1999).

Acute neurochemical effects of MDMA administration are a decrease in 5-HT tissue level and tryptophan hydroxylase activity, however while these are reversible, chronic administration of MDMA can cause irreversible damage (Schmidt & Taylor, 1987). The neurotoxicity at the 5-HT neuron is also characterized by a loss of 5-HT transporters (Battaglia, Yeh & de Souza, 1988; Koch & Galloway, 1997).

The rise in extracellular DA is due to at least two mechanisms; direct action of MDMA at the DAT (dopamine transporter) and indirect action through endogenously released 5-HT (Baumann et al. 2008). Studies have shown that DA release can be decreased by DA specific antagonists as well as SSRIs, indicating an important role for intact 5-HT release in MDMA's effects on DA (Koch and Galloway, 1997). Furthermore, the 5-HT agonist and antagonists that have shown to effect hyperactivity have shown an affinity for receptors that are situated within the mesocorticoaccumbens and nigrostriatal DA circuits, that is also associated with addictive behaviours (Bankson & Cunningham, 2002; Ross et al, 2006). DA antagonists also decrease MDMA associated locomotor activity. It has also been shown that at 3mg/kg of MDMA, D1 antagonists also decrease rats abilities to discriminate MDMA from saline (Bubar, Pack, Frankel & Cunningham, 2004). Research has suggested that MDMA's effects on DA and 5-HT release elicit different behaviours. Thus, increased 5-HT levels in the nucleus accumbens and caudate nucleus is associated with stereotypic behaviours. On the other hand, increased DA levels in these areas as a result of MDMA administration is correlated with ambulation (Baumann et al, 2008).

Less research has been focused on MDMA's influence on norepinephrine release, although some in vitro studies found that norepinephrine release was enhanced to the same extent as serotonin release after MDMA administration (Rothman, Baumann, Dersch, Romero, Rice et al. 2001). Recent behavioural studies have also indicated that parzosin (a norepinephrine antagonist) reduces MDMA induced locomotor activity by 55% (Selken & Nichols, 2007).

Neurochemical effects of Amphetamine

Amphetamine's behavioural effects are mainly a result of its effects on DA release, although increased 5-HT and norepinephrine release are also associated with the administration of amphetamine (Fleckenstein, Volz, Riddle, Gibb & Hanson, 2007). Amphetamine predominantly amplifies extracellular DA by increased DA release and by blocking DA reuptake. The increased release of DA at low and medium doses is a result of amphetamine binding to DAT and thus achieving a reversal of the transporter which usually transports DA out of the neuron (Pifl, Drobny, Reither, Hornykiewicz & Singer, 1995). The specific mechanism by which this occurs is suggested to be by the exchange diffusion model. Consequently, cellular AMPH replaces DA and is thus transported into the cells by the dopamine transporter (simultaneously blocking DA reuptake), which in turn increases cytosolic DA's binding to the DAT, thus eliciting DA release. At higher doses AMPH can also elicit DA release from intraneuronal binding sites, and thus allowing DA to depart the terminals using DAT (Fleckenstein et al, 2007).

Acutely administered amphetamine and MDMA are very similar in their effects on cognition and behaviour, as well as their effects on the neurotransmitters 5-HT, DA and norepinephrine. However, MDMA at relatively low doses (<3.0mg/kg, i.p.) has a significant impact on 5-HT enhancement, whereas amphetamine at all doses has less effect on 5-HT release (Baumann et al, 2008; Schenk, 2011). While this difference may account for some of the behavioural variances found, the significant enhancement of DA release at higher doses of MDMA (>3.0mg/kg) could suggest that MDMA at these doses is indistinguishable to amphetamine.

Drug Discrimination

Drug Discrimination is one of the main tools of behavioural pharmacology. The drug discrimination procedure is used to discriminate different subjective effects of different drugs and doses (Colpaert, 1999; Young, James & Rosecrans, 2001; Schenk, 2011). To this end, the paradigm constitutes an in vivo method of neuropharmacology. Specifically, it allows for the analysis of molecular, behavioural and cell-physiological data. This permits an insight into a variety of subjects, such as drug abuse through an evaluation of receptor mechanism and the stability of stimulus control (Colpaert, 1999). The drug discrimination procedure has also been used in order to assist new drug discoveries. For example, the antipsychotic drug pirenpirone was discovered because of its ability to fully antagonize LSD's discriminative effects (Colpaert & Janssen, 1983).

Usually in a drug discrimination procedure, rats are trained to discriminate between a specific dose of a drug and vehicle (saline), which constitutes the absence of any drug. Operant boxes are used in which rats are trained to associate a certain lever either with the drug or vehicle. Whenever the rat chooses the appropriate lever and makes a predetermined amount of responses on this (different FR, VI, and VR schedules have been employed), reinforcement will be delivered (Colpeart et al. 1999; Young et al. 2001; Schenk, 2011; Stolerman, 1993). Whenever the rat presses the wrong lever, the counter will be reset and reinforcement will only be delivered after the correct continuous amount of responses have been made on the appropriate lever (Stolerman, 1993).

Training is completed when rats make 80% or more responses on the correct lever 10 sessions in a row. Frequently stimulus generalization studies are undertaken following this. To this end, higher and lower doses of the training drug are administered. The first predetermined amount of responses that are made continuously on one lever indicate to what extent the different doses generalize to the training dose (Young et al. 2001). A quantal measurement in which responses made on a particular lever are divided by total amount of responses made and then multiplied by 100 reveals the percentage of lever choice. This in turn also provides a dose/response curve for the drug evaluated and if sufficient different doses were tested, the potencies of different drugs can be calculated along with ED 50 measures. (Colpaert et al. 1999). However, it has been argued that depending on the dose of the training drug used, dose/response curves can vary. Thus using a low dose as the training dose, will likely result in a steeper curve, but may result in generalisation to dissimilar compounds. Likewise higher doses tend to enhance cue specificity, but

decrease sensitivity of subjective effects. Most of these observations stem from opioid discrimination studies, and there has been some evidence that suggests different doses of stimulant drugs vary from the above mentioned training dose effects. Thus it was found that a low dose of amphetamine elicits a dose response curve that is flatter than the one found with a higher training dose (Stadler et al. 2001).

Often drug discrimination research also employs a test drug in order to assess whether the stimulus effects of this drug are similar to those of the training drug. If the test drug fully substitutes for the training drug, 80-100% of responses will be made on the drug appropriate lever, which indicates that the drugs elicit comparative subjective effects. Partial substitution occurs when 40-70% of the responses are made on the drug appropriate lever, possibly an indication of some similar pharmacological action. If less than 40% of responses are made on the drug appropriate lever, this indicates that the test drug elicits completely different (or no) subjective effects than the training drug (Colpaert, 1999).

Another frequently used procedure in drug discrimination research is the combined administration of the drug and an antagonist. This can block the action of the drug completely, resulting in saline appropriate responding. Commonly, combined antagonist and different doses of the training drug will move the dose response curve to the right, indicating that a higher dose is needed to obtain the same subjective effects (Stolerman, 1993). Similarly, saline-appropriate responding indicates that the antagonist blocks all the stimulus effects associated with the drug.

When this occurs, it can be concluded that the subjective effects of the drugs occur as a result of the neurotransmitter or receptor that is blocked due to the antagonist.

<u>Evidence from Drug Discrimination studies with MDMA</u>

Drug Discrimination studies have proven useful in differentiating drugs according to their subjective effects. To this end drug discrimination procedures between MDMA or AMPH and vehicle have shown inconsistent results. It was found that when rats are taught to discriminate between amphetamine and vehicle, MDMA would generalize to AMPH (Glennon & Young, 1984). This has also been replicated with pigeons. Specifically in this instance it was observed that amphetamine appropriate responding increased with increased MDMA doses (Evans & Johanson, 1986). Yet, other researchers were unable to replicate these findings (Oberlander & Nicholls, 1988), but instead reported that when rats are trained to discriminate between MDMA and vehicle, AMPH would substitute for MDMA. Again, however, other research has not been able to replicate this finding (Baker & Makhay, 1996) or found that AMPH only partially substituted for MDMA (Schechter, 1989). Substitution tests with other compounds have been more conclusive. Thus, it has been shown that serotonergic agents, such as fenfluramine consistently substitute for MDMA and vice versa (Schechter, 1986). Furthermore, partial generalization with LSD (serotonin agonist) has also been reported (Baker & Taylor, 1997).

Additionally, studies with recreational human MDMA users have shown generalization with amphetamine (this was especially the case for the most practiced

drug users) and m-CPP, a serotonin agonist (Tancer & Johanson, 2001). A follow up study specifically attempted to identify the subjective effects of MDMA. Utilising a variety of questionnaires it was found that effects paralleled those identified after d-amphetamine administration but appeared to be shorter in duration and have hallucinogenic effects. Moreover, the study used different doses but could not find that any effects increased in a dose dependent manner, as was expected (Tancer & Johanson, 2003).

Initially, it was argued that the inconsistent research findings were a result of the differential use of MDMA's optical isomers. Specifically, it was observed that S(+)-MDMA's appeared to be more potent than R(-)-MDMA in releasing DA, and induces behavioural effects that mimic those found with d-amphetamine, whereas R(-)-MDMA binds with greater affinity to 5-HT2 receptors (Johnson, Hoffman and Nichols, 1986; Lyon & Glennon, 1986). However, subsequent research that trained rats to discriminate between a stimulant (d-amphetamine) and a hallucinogen (LSD or mescaline) found that neither MDMA isomer substituted for d-amphetamine, but R(-)-MDMA almost fully substituted for mescaline, and both MDMA isomers showed partial generalization with LSD (Baker & Taylor, 1997). On the other hand, research that trained mice to discriminate between the two isomers found that d-amphetamine would fully substitute for (+)-MDMA (Murnane, Murai, Howell & Fantegrossi, 2009). This shows that the inconsistencies in MDMA's discriminative effects are not explained through the differential effects of MDMA's optical isomers.

Research teaching rats to discriminate in a two lever drug-drug (d-amphetamine vs. norfenfluramine) discrimination study found that MDMA would

generalize to the serotonergic drug norfenfluramine (Schechter, 1997). However, while it was found that the generalization occurred as MDMA dose increases, this does not indicate that lower doses of MDMA substitute for amphetamine. Drug-drug discrimination studies are often difficult to interpret due to a lack of vehicle discrimination (Stolerman, 1993).

Generally, most two lever discrimination studies have failed to fully account for MDMA's complex stimulus effects. It is believed that three lever drug discrimination paradigms are more sensitive and especially suitable to use with drugs that have multiple pharmacological actions (Goodwin et al. 2000; Schenk, 2011; Stolerman, 1993).

Subsequent research trained rats to discriminate between MDMA and LSD in order to further explore the role of serotonin and dopamine in the stimulus properties of MDMA (Goodwin et al. 2003). Amphetamine substitution tests showed a dose dependent increase to MDMA appropriate responding until partial generalization at the highest dose (2.0mg/kg). There was also a slight dose dependent increase with LSD appropriate responding (highest dose- 20%). On the other hand fenfluramine does not substitute for LSD whereas it completely generalizes to MDMA, indicating that stimulus properties of MDMA and fenfluramine are extremely similar. Haloperidol, a dopamine antagonist does not block MDMA appropriate blocking (Goodwin at al, 2003). These results support the notion that serotonin plays a dominant role in MDMA's stimulus property effects. Specifically, it was shown that drug discrimination in this paradigm did not rely on dissociations between dopaminergic and serotonergic effects. This is also evidenced by the fact that

fenfluramine (5-HT) substituted for MDMA but not LSD. Furthermore, blocking DA

had no impact on subsequent discrimination of MDMA and LSD. Thus, even

though rats had to learn to discriminate between two serotonergic agents, the

discrimination was not based on MDMA's additional effects on dopamine alone.

Goodwin & Baker (2000) also directly compared MDMA and amphetamine

discrimination in a 3 lever drug discrimination procedure. Rats learned to

discriminate between MDMA, amphetamine and saline. The fact that the rats learned

this discrimination indicates that the stimulus properties of both drugs are

sufficiently different. Specifically, when the rats were given MDMA hardly any

responses were made on the amphetamine lever, and this pattern appeared to be dose

independent. Similarly, the training dose of amphetamine produced barely any

responses on the MDMA lever. However, at the lowest dose (0.25 mg/kg) of

amphetamine responses were almost equally divided between saline, MDMA and

amphetamine response choices. Yet, only about 30% of responses occurred on each

lever, indicating that this dose of amphetamine does not generalize to any response

choice. Overall, these results would suggest that when MDMA is experienced at a

low dose (1.5mg/kg) it is sufficiently different from amphetamine. Further tests with

higher doses of MDMA would have been of interest, as this could clarify whether a

high dose of MDMA may generalize to amphetamine, thus indicating increased

activity in the dopamine system. Subsequent substitution tests with dopamine and

serotonin agonists again highlight the complex nature of MDMA. Thus, cocaine (a

DA agonist) showed a dose-dependent increase in amphetamine lever responding;

with the highest dose (10.0 mg/kg) entirely substituting for amphetamine.

Conversely, LSD almost entirely (78%) substituted for MDMA at a medium dose

(0.08mg/kg). A higher dose (0.16 mg/kg) did not produce any further increases in generalization. Also, a medium dose of fenfluramine (2.0 mg/kg) generalized to MDMA. On the other hand DOM (2,5 dimethoxy-4-bromoamphetamine) a serotonin agonist at 5-HT2A receptors produced only partial generalization to MDMA. This could be explained by MDMA's effects on other receptors. However, pirenpirone a serotonin antagonist administered in conjunction with MDMA produced only partial blocking of MDMA responding (Goodwin et al. 2000). Therefore, the results clearly indicate a dominant role for serotonin in the stimulus properties of MDMA, however, the role that dopamine plays remains unclear.

A subsequent study by Baker and Makhay (1996) investigated the neurotoxic regimen of fenfluramine on rats trained to discriminate MDMA from vehicle. Again, a significant decrease in MDMA appropriate responding was observed for the neurotoxically treated rats. Following the initial generalization tests, rats were retrained to discriminate between MDMA and vehicle. Subsequent MDMA test sessions showed that lower doses of MDMA were actually discriminated better from vehicle than before the neurotoxic regimen. Furthermore, after retraining, fenfluramine treated rats showed increased substitution of amphetamine for MDMA. This is in stark contrast to rats that did not undergo the neurotoxic regimen and substitution tests undertaken prior to the neurotoxic regimen, which all showed no substitution. In fact, for the fenfluramine treated rats the highest dose of amphetamine (1.0mg/kg) resulted in complete generalization (Baker & Makhay, 1996). These results indicate that the neurotoxic regime of fenfluramine was successful in depleting serotonin neurons. Furthermore, the fact that amphetamine now substitutes for MDMA indicates that the stimulus properties of MDMA have

changed from a dominant role for serotonin to a dominant role in dopamine, which is in contrast to the study by Goodwin et al. (2003) which found no role for MDMA's effects on dopamine in its discriminative effects.

A study that utilised a different neurotoxic regime (twice daily 20mg/kg MDMA for four days) found that this regime led to a shifting in the dose response curve (Schechter, 1991). Accordingly, MDMA-appropriate responding decreased with all doses of MDMA, but the only significant decrease was found at 1.0mg/kg MDMA. It was hypothesized that the neurotoxic regime depleted serotonin which led to the shifting of the dose response curve. However, conditioned place preference tests were also undertaken and the neurotoxic regime did not change preference for MDMA associated chambers (Schechter, 1991). It has been suggested that place preference is mediated by dopaminergic influences. Similarly, even though MDMA appropriate responding decreased, it still occured, which could be argued to be mediated more dominantly by dopaminergic influences as well. However, it is unclear to which end 5-HT or perhaps even dopamine release were inhibited in the brain.

It has been argued that the most effective way to significantly decrease 5-HT is to utilise p-CPA (para-chlorophenylalanine), which effectively inhibits tryptophan enzyme synthesis (Koe & Weissman, 1966). To this end, Schechter (1991) demonstrated that rats previously able to discriminate between MDMA (1.5mg/kg) and saline showed that MDMA appropriate responding significantly decreased after pCPA treatment which returned to normal by day 9 at which stage tryptophan enzyme synthesis resumed. Therefore, it was shown that at a low dose of MDMA

(1.5mg/kg or less) the interoceptive cue of MDMA relies predominantly on its effects on serotonin (Schechter, 1991).

It has thus been suggested that many of the acute (and even post-exposure) behavioural effects of MDMA are thought to be governed via its influence on 5-HT release (Bankson & Cunningham, 2002; Schechter, 1990). However at sufficiently high doses (above 3.0 mg/kg) MDMA acts as a dopamine agonist, therefore at relatively higher doses some of MDMA's behavioural effects may be a product of increased dopamine in a variety of brain regions (Baumann et al. 2008).

Aim of the current study

The aim of the current study is to show that standard training doses of MDMA (1.5 mg/kg) and AMPH (0.5 mg/kg) can be differentiated from each other and vehicle (saline) in a three lever drug discrimination procedure. This discrimination is theorized to occur through MDMA's effects on 5-HT release and AMPH's effects on DA release. Furthermore it is hypothesized that higher doses of MDMA (3.0 mg/kg and 4.5mg/kg) will result in increased DA release, which will be determined by increased responding on the AMPH lever.

Experiment 1 effectively replicates earlier published work (Goodwin et al. 2000) by assessing the ability of rats to discriminate between MDMA, saline and AMPH; but extends this work to examine discrimination performance at MDMA doses higher than those originally trained on.

Experiment 2 is divided into three parts and involves further tests of discrimination performance with the concurrent presence of DA or 5-HT antagonists. In Part A and B of Experiment 2 the D1 antagonist SCH 23390 or the D2 antagonist eticlopride were administered just prior to MDMA exposure in order to further clarify the role of DA in MDMA's subjective effects. Previous research has documented a rightward shift in MDMA's dose response curve when MDMA is administered in conjunction with the D1 antagonist SCH23390. In contrast, there was no effect on MDMA's discriminative effects when MDMA was given in conjunction with the D2 antagonist eticlopride (Bubar et al, 2004). However, the previous study utilised a low dose of MDMA (1.0mg/kg) to assess the MDMA's discriminative effects in combination with the DA antagonist. Contrary to this, significant behavioural effects were found when 3.0mg/kg MDMA was given in conjunction with both DA antagonists. Therefore, it is expected that only the D1 antagonist will have a small impact on MDMA's discriminative effects at low doses <1.5mg/kg. This is because; at low doses MDMA significantly enhances 5-HT but has only minimal impact on DA (Baumann et al, 2008). Of interest is, whether as the MDMA dose increases and a significant enhancement of DA release (dose >3.0mg/kg) occurs, can this be observable with both DA antagonists (SCH23390 and eticlopride). This would indicate that both D1 and D2 receptors play an important role in MDMA's discriminative cue properties. Specifically, as high doses of MDMA are administered in conjunction with a DA antagonist, the increase in synaptic DA that occurs usually at these doses should be attenuated through the simultaneous presence of the DA antagonists. Alternatively an increase in saline appropriate responding would also indicate that the subjective effects of higher doses of MDMA are significantly mediated by its influence on DA. Therefore, there should

be a significant difference in proportion of responding between Experiment 2A + B and Experiment 1. Utilising two DA antagonists allows for a more specific understanding of MDMA's exact influence on the DA receptors.

In Part C of Experiment 2 the 5HT2c antagonist RS102221 was administered in conjunction with MDMA in order to further explore the role that 5HT plays in MDMA's subjective effects. As opposed to Experiment 2A+ B, it is expected that RS102221 will have a significant impact on MDMA's discriminative effects at all doses. However, it has been shown that RS102221 primarily increases MDMA induced locomotion due to 5HT2c receptors typically inhibitory effects on DA release (Filip & Cunningham, 2002). Therefore antagonising the 5HT2c receptors should have two different effects in mediating MDMA's discriminative cue properties. Firstly, the antagonist should directly decrease 5HT release, which could result in increased saline and/or amphetamine lever responding. Secondly, successful 5HT2c blockage should lead to increased DA release and therefore impact on MDMA's discriminative effects in that it is experienced similar to amphetamine.

Experiment 1

<u>Introduction</u>

Research with two-lever drug discrimination studies have shown inconsistencies in whether MDMA substitutes fully, partially or not at all for amphetamine (Baker & Makhay, 1996; Goodwin & Baker, 2000; Oberlander & Nichols, 1988; Schechter, 1986, 1989). Additionally, some neurochemical studies have indicated that MDMA's relative effects on the 5-HT and on the DA system appear to be dose-dependent. Baumann et al. (2008) showed that low doses of MDMA produce only minimal DA release, while doses above 3.0mg/kg MDMA tend to result in proportionally greater increases in extracellular DA than 5-HT.

Drugs, like MDMA with complex neurochemical and subjective effects are better suited to three-choice drug discrimination procedures (Stolerman & d'Mello 1981). In line with this, Goodwin and Baker (2001) utilised a procedure in which rats were trained to discriminate between 1.0mg/kg amphetamine, 1.5mg/kg MDMA and vehicle. Their study demonstrated that the three substances can be discriminated from one another, which appeared to be due to MDMA's influence on 5-HT and amphetamine's effects on DA. The possibility that DA release becomes more important at mediating MDMA's subjective effects at doses above 3.0mg/kg was not explored.

The first experiment in this thesis will explore the effects that different doses of MDMA (0.5-4.5mg/kg) have on the rat's ability to discriminate MDMA from amphetamine and saline vehicle using a three-lever discrimination procedure. To this

end, dose response curves for MDMA and amphetamine will be constructed. The AMPH dose response curve will allow for an exploration of subjective DA effects with minimal 5-HT involvement. An additional MDMA dose response curve will allow an exploration of 5-HT effects with lesser DA involvement. Utilising separate dose response curves essentially permits an insight into the mechanisms of two different monoamine systems through its influence on the subjective effects. A classic generalisation gradient is expected, in that at doses below the target training dose of either drug (1.5mg/kg for MDMA and 0.5mg/kg AMPH) the drugs will be increasingly treated like saline. On the other hand, with higher doses of MDMA (>3.0 mg/kg) it is expected that MDMA will be increasingly treated like amphetamine, consistent with an increase in DA release at these higher doses.

Method

Subjects

Subjects were 14 Norwegian Hooded rats. Rats had previously been exposed to operant training on a single lever in an undergraduate lab but were drug naïve. Of the 14 rats, 8 were female and the remaining 6 male. The female rats weighed between 210 and 260 grams at the start of the study. The male rats weighed between 320 and 400 grams. All the rats were approximately 6 months old. Subjects were housed in pairs in a room maintained on a 12h light (0700-1900)/12h dark cycle and kept at temperatures between 20-22°C. Subjects were allowed free access to water while commercial rat food was rationed to maintain body weights of 85-90% of free feeding weights.

Apparatus

All training and testing sessions took place in 14 commercially available rodent operant chambers (ENV221M: MED Associates Inc., Georgia, VT) containing three retractable levers. A food pellet delivery machine was located at the centre of the front panel, while one lever was situated to the right and one to the left of it, respectively. The third lever was located at the centre of the back panel of the operant chamber. Standard 100mA white lights were situated above every lever. MED-PC instrumentation and software were used to run experimental events and data collection.

Drugs

(+)-MDMA hydrochloride was attained from ESR (Porirua, New Zealand) while *d*-amphetamine sulphate was attained from Sigma Aldvich (Australia). Drugs were dissolved in 0.9% physiological saline and were administered intraperitoneally (i.p.) 15 min prior to training or testing with an injection volume of 1ml/kg. Doses are expressed as the salt form of each drug.

Training procedures

Training began with 14 sessions of autoshaping. Prior to the autoshaping session no substance was administered. Following the autoshaping sessions discrimination training commenced. Rats were administered amphetamine (0.5mg/kg), MDMA (1.5 mg/kg) or vehicle i.p. at the start of discrimination training sessions. Training doses were selected on the basis of previous research (Glennon & Higgs, 1992; Goodwin et al., 2001 & 2002; Schechter, 1989; 1991 & 1998).

However, initial pilot studies showed that 1.0mg/kg of amphetamine suppressed responding too much, and thus a training dose of 0.5mg/kg was chosen (unpublished data). After drug delivery rats were placed in their designated operant chamber and 15 minutes later the training began. The first 15 sessions consisted of errorless discrimination, in which only the drug appropriate lever was present. A single response on this lever resulted in delivery of a single food pellet. A session ended after the rat had collected 60 reinforcers or 45 minutes had elapsed.

Following this phase all three levers were presented simultaneously, which is how the setup remained until the end of study. Training began with a FR1 schedule which was slowly increased to a FR10 schedule, as responding became stable. Training conditions were administered in a pseudo random order in which the only requirement was that the same condition would not be run twice in a row. Reinforcement was only delivered if the rat made 10 consecutive responses on the drug appropriate lever. Responses made on any other levers reset the response counter. All the rats were run at the same time and drug-lever selection was kept uniform across subjects. Training sessions lasted 30 minutes and were administered 4-6 times a week. Subjects were required to meet a discrimination criterion of 80% of responses on the condition-appropriate lever, prior to delivery of the first reinforcer; this standard had to be met in at least 8 out of 10 sessions.

Testing procedures

After subjects met criterion, generalization tests with four different MDMA doses (0.5-4.5mg/kg) and three different amphetamine doses (0.125-0.75mg/kg)

were performed. The probe sessions were run in a pseudo randomized order two to
six times with at least 5 days between the same probe sessions. The data was
collapsed across different days for the same probe session. Generalization sessions
were similar to training sessions, with the exception that no reinforcers were
delivered after the first 10 responses were made and the session was terminated
following 10 responses (i.e. a single FR 10).

Table 1: Summary of drug/dose and number of sessions run in each condition.

Condition (Drug/Dose)	No. of sessions
Saline	6
MDMA 1.5mg/kg	6
AMPH 0.5mg/kg	6
MDMA 0.5mg/kg	2
MDMA 1.0mg/kg	2
MDMA 3.0mg/kg	3
MDMA 4.5mg/kg	2
AMPH 0.125mg/kg	3
AMPH 0.25mg/kg	4
AMPH 0.75mg/kg	3

Data Analysis

Two measures of performance were obtained: 'percentage of responses made
on each lever' and 'overall response rate' for each rat at each drug/dose condition.

Percent responses was obtained by examining how many responses a rat made to each of the three levers (MDMA, AMPH and saline) during the 10 responses of the probe FR10 generalisation session. Response rate (number of responses per second) was obtained by examining how long each rat took to complete the FR10 requirement during the probe session (irrespective of lever choice). Dose response curves illustrated overall group mean, total responses and response rates for each drug tested.

Analyses were one way repeated ANOVAs unless otherwise noted.

Results

The data of 11 subjects was utilised in the following analyses. The mean number of sessions to criterion was 59. One rat died due to reasons unrelated to the study. Two rats did not respond following administration of amphetamine, therefore their data was not included in any of the analyses.

Previous research has demonstrated that females are more sensitive to certain effects of MDMA (Liechti, Gamma & Vollenweider, 2001). To test whether similar gender differences occur in drug discrimination studies, between groups t-tests were conducted comparing male and female rat's response percentages at all different doses of AMPH and MDMA. It was found that there were no significant gender differences in any of the responses with any given MDMA dose. However, there was a significant gender difference between male and female rats when rats were given the training dose of amphetamine (0.5mg/kg). That is, females were more likely to respond on the amphetamine-appropriate lever with 95% compared to 72% for the

males [$t(3)=3.671$, $p=.04$]. This in turn contributed to a higher percentage of saline

lever responding for the male rats (with 14% compared to 4% for the females),

however this just missed significance ($p=.058$). In line with this, there was also a

noteworthy discrepancy in response rates when 0.5mg/kg amphetamine was

administered. Male responding was faster than female responding, but again this just

missed significance ($p=.052$). However, gender differences were not observed in

regard to MDMA's subjective effects in any condition, which is the main focal point

of the current study. Additionally, there were no significant gender differences

observed in any other condition. Therefore the two genders were grouped together

for the remainder of the analyses.

Figure 1: Dose response curves for MDMA *(n/N=11/11).* The mean percentage of the total responses on each of the three levers is plotted on the top graph; median overall response rate is displayed in the bottom graph. The training dose of MDMA is 1.5mg/kg.

Proportion of Responses-MDMA Probe sessions

The top part of Figure 1 demonstrates the results of the generalization tests with MDMA (0-4.5mg/kg), which revealed a significant dose dependent increase in MDMA responding [$F(5, 45)= 21.48$, $p=.000$]. Increasing doses of MDMA, up to

the training dose of 1.5mg/kg (0.5-1.5mg/kg) produced significant dose dependent

increases in the proportion of responses made on the MDMA lever during probe test

sessions [$F(2,18)=16.55$, $p=.001$]. Specifically, responding at 0.5mg/kg is

significantly lower (35%) than responding at 1.0mg/kg (75%) and at the training

dose of 1.5mg/kg MDMA (89%). Higher doses (3.0-4.5mg/kg), however, resulted in

dose dependent decreases on the MDMA lever [$F(2,18)=40.457$, $p=.001$].

Specifically responding decreased from 89% on the MDMA lever (at 1.5mg/kg) to

58% (at 3.0mg/kg) and 28 % (at 4.5mg/kg).

In comparison, responses on the AMPH lever remained low across doses less

than, and up to, the training dose of 1.5mg/kg ($p=.64$) but increased significantly for

doses 3.0 mg/kg and 4.5 mg/kg [$F(2,18)=68.068$, $p<0.01$] from 3% at the training

dose of MDMA to 61% at 4.5mg/kg (and 28 % at 3.0mg/kg).

In contrast to amphetamine, saline responding decreased significantly from 0.5mg/kg

(62%) to 1.0mg/kg (23%) to 1.5mg/kg (7%) [$F(2,18)=15.75$, $p=.001$] and remained

low at 3.0mg/kg (16%) and 4.5mg/kg (14%) MDMA ($p=.14$).

Response Rate-MDMA Probe sessions

The bottom part of Figure 1 depicts the overall response rate. The response

rate significantly decreased as the dose of MDMA increased [$F(4,36)=35.52$,

$p=.001$]. In fact only 6 rats made the full 10 responses during the generalization test

with 4.5 mg/kg. Additionally, 2 rats failed to complete 10 responses during the probe

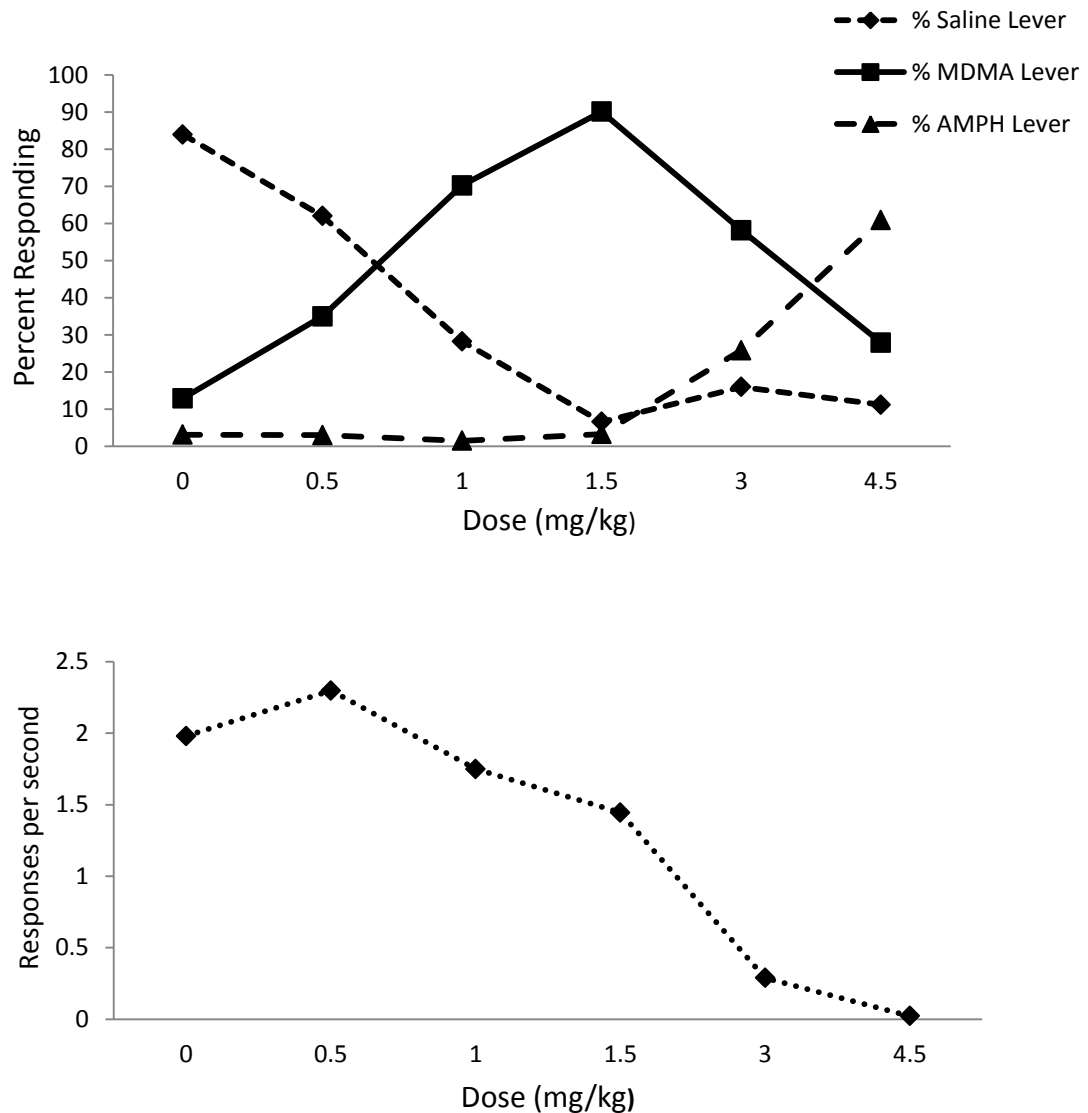session with 3.0mg/kg in the required time frame (15min).
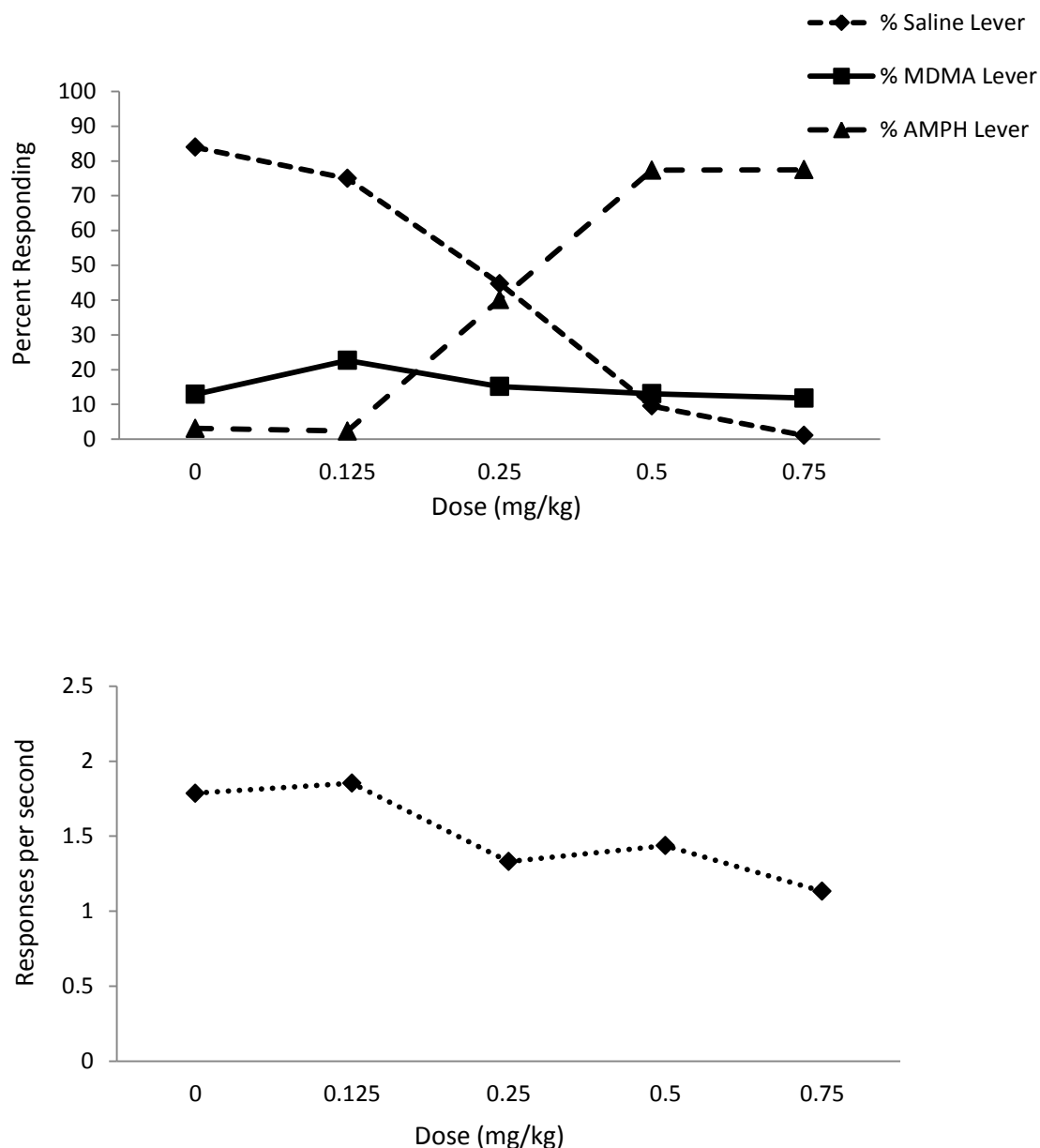
Figure 2: Dose response curves for AMPH *(n/N=11/11).* The mean percentage of the total responses on each of the three levers is plotted on the top graph; median overall response rate is displayed in the bottom graph. The training dose of AMPH is 0.5mg/kg.

Proportion of responses-Amphetamine Probe sessions

The top part of Figure 2 shows the proportion of responses made on the

levers at each dose. As the dose of AMPH increases (0.125-0.5mg/kg) there is a

significant dose dependent increase in the proportion of AMPH appropriate responding [$F(2,18)=229.41$, $p=.001$] with only 3% of all responses made on the AMPH lever at the lowest dose, 42% at 0.25mg/kg and 84% at 0.5mg/kg. There is no additional increase in percent responding with the highest dose of amphetamine.

In contrast, the proportion of responses made on the saline lever decreased significantly in a dose dependent manner [$F(2,18)=77.81$, $p=.001$] while amphetamine increases. With the lowest dose of amphetamine, saline lever selection lies at 75% it then decreases to 46% at 0.25mg/kg and to 8% at the training dose of 0.5mg/kg. There is only a small, nonsignificant increase to 11% at 0.75mg/kg AMPH.

Although, percent responding on the MDMA lever decreased in a consistent manner, the changes were far smaller. Nevertheless, overall there was also a significant effect with higher doses of AMPH resulting in decreased MDMA lever responding [$F(3,27)=5.28$, $p=.005$] with 22% at 0.125mg/kg and 5% at 0.75mg/kg.

Response Rate-Amphetamine Probe sessions

The bottom part of Figure 2 depicts the mean response rate from all probe sessions for every dose of AMPH. Overall response rate significantly decreased with increasing dose of AMPH [$F(3,27)=6.241$, $p=.002$]. However the lowest response rate of 1.2 responses per second was exhibited at the training dose of 0.5mg/kg (the highest response rate of 1.9 responses per second occurred at 0.25mg/kg). Therefore

the current doses of AMPH did not interrupt responding. In contrast to the MDMA response rates, all the rats finished each amphetamine probe FR10.

Summary

A classic generalisation gradient was produced following administration of doses lower than the training dose for both MDMA and AMPH, showing increased saline lever responding as the doses of either drug decreased. The novel finding here was that higher doses of MDMA (>3.0mg/kg) were increasingly treated like amphetamine, consistent with the possibility that at higher doses MDMA is subjectively more like AMPH because of increased DA release at these doses.

Experiment 2

Introduction

In order to further explore the different roles the DA and the 5-HT system play in regards to MDMA's subjective effects, a variety of antagonist tests were undertaken. To this end, low and high doses of MDMA (1.5-4.5mg/kg) were given in conjunction with the different antagonists.

Previous studies have demonstrated that the ability to discriminate MDMA from saline is significantly reduced when MDMA (0.375-1.0mg/kg) is administered with a D1 antagonist (SCH23390), resulting in a significant decrease in MDMA-appropriate responding (Bubar et al. 2004). Conversely, although D1 and D2

antagonists both decreased MDMA-induced hyperactivity, the selective D2

antagonist eticlopride did not have any significant effects on MDMA's

discriminative cue properties (Bubar et al. 2004). A different study found that

combined administration of SCH23390 and MDMA (5.0mg/kg) would result in a

later onset of MDMA induced locomotion, which was mirrored by slowed and

decreased excitation of motor related neurons in the striatum. The D2 antagonist

eticlopride, on the other hand, completely blocked MDMA induced locomotion,

which was paralleled by an inactivity of the motor related neurons in the striatum

(Ball, Budreau and Rebec, 2003).

Animal studies have shown that 5-HT2 postsynaptic receptors play a

dominant role in the behavioural and subjective effects of MDMA (Fletcher, Sinyard

& Higgins, 2006; Ross, Herin, Frankel, Thomas & Cunningham, 2006) although the

exact effects the different receptors play remain unclear. For example, Smithies and

Broadbear (2011) showed that co-administration of MDMA with 5-HT1a and 5-

HT2a/c antagonists WAY and ritanserin impaired the ability of rats to discriminate

MDMA (1.5mg/kg) from amphetamine and saline. Specifically, both of these

antagonists produced a shift in responding away from MDMA-appropriate towards

saline lever responding. This finding is consistent with the possibility that at

relatively low doses the ability of rats to discriminate MDMA from AMPH and

saline arises because of MDMA's effect in predominantly increasing 5-HT. Since

low doses of MDMA have only little effect in increasing DA (Bauman et al. 2008),

blocking MDMA's effects at any 5-HT2 receptor sites may change MDMA's

subjective effects to be more saline-like.

Alternatively, it has been shown that drug induced (cocaine, amphetamine and MDMA) activity of the 5-HT2c receptor has an inhibitory effect on DA release (Filip & Cunningham, 2002). Consistent with this conclusion, previous research has indicated that 5-HT2c antagonists (e.g. SB248404) increase MDMA-induced locomotion. This effect is argued to be mediated by indirect DA stimulation via an attenuation of the normally inhibitory role of the 5-HT2c receptors (Fletcher et al. 2006).

Another indication for an important role of the 5-HT2c receptor in DA mediated effects comes from research that has used the D1 antagonist SCH23390. It was shown that the ability to block MDMA induced behavioural sensitisation is primarily a result of SCH23390's affinity for 5-HT2c receptors. Using the 5-HT2c receptor antagonist RS102221 attenuates SCH23390's effect in suppressing sensitisation (Ramos, Gońi-Allo & Aguirre, 2005).

Therefore MDMA induced activation of 5-HT (at low levels) and both 5-HT and DA (at relatively higher levels) may contribute to the ability of subjects to discriminate MDMA from AMPH and saline. The alterations in 5-HT and DA are not independent in that activation of 5-HT2c receptors in particular appears to have an inhibitory effect on MDMA's ability to activate DA. Blockade of these inhibitory effects through a 5-HT2c antagonist should result in increased activity of the DA system and an increased tendency for subjects to treat MDMA like AMPH.
In Experiment 2A and 2B probe tests were conducted in which MDMA was administered concurrently with the D1 antagonist SCH23390 (Experiment 2A) or the D2 antagonist eticlopride (Experiment 2B) in order to further explore the role that

DA plays in MDMA's subjective effects. Previously it has been shown that low doses of MDMA (0.75-1.0mg/kg) in conjunction with SCH23390 will reduce MDMA-appropriate responding (Bubar et al. 2004). In the current experiment it is also expected that SCH23390 will reduce MDMA-appropriate responding at all doses and may have even more obvious effects at higher doses of MDMA that are associated with large increases in DA activity (>3.0mg/kg). In particular, it is anticipated that SCH23390 would produce a significant change from MDMA-appropriate responding towards saline responding. Additionally, it is expected that the previous shift from MDMA-appropriate responding to AMPH lever responding at higher doses (see Experiment 1) will be reduced.

In terms of the D2 antagonist eticlopride, previous studies have found that eticlopride will attenuate MDMA-induced hyperactivity (0.375-5.0mg/kg) (Ball et al. 2003; Bubar et al. 2004). On the other hand, there has been no evidence to show that eticlopride has an effect on MDMA's discriminative cue properties (0.395-1.0mg/kg) (Bubar et al. 2004). However, eticlopride has so far not been used in a three-lever discrimination paradigms or in conjunction with higher doses of MDMA (>1.0mg/kg). As discussed previously, three lever drug discrimination paradigms are more sensitive in terms of assessing stimulus cue properties (Goodwin & Baker, 2000, Stolerman & d'Mello, 1981). Similarly, as has been indicated in Experiment 1 DA plays a more important role in terms of MDMA's discriminative properties at higher MDMA doses (>3.0mg/kg), therefore it is possible that the D2 receptor will also become more relevant at higher doses.

In Experiment 2C the effect of a 5-HT2c antagonist on the ability of subjects to discriminate MDMA from saline and amphetamine will be examined. Previous research has shown that the 5-HT2b/2c antagonist SB206553 significantly increases MDMA-induced locomotion, which mirrors locomotor behaviour otherwise only seen at higher doses of MDMA (Bankson & Cunningham, 2002). The current study utilised the 5HT2c antagonist RS 102221, in order to explore the role of the 5-HT2c receptor on the ability of subjects to discriminate MDMA from saline and AMPH at different doses of MDMA. It is hypothesized that a dose dependent increase in AMPH lever responding will occur, due to the direct and indirect DA system stimulation.

Method 2A

Subjects

Subjects were the same Norwegian Hooded rats as used in Experiment 1. Apparatus and Training procedures were the same as in Experiment 1.

Drugs

SCH233390 was acquired through TOCRIS (UK).

Testing procedures

Antagonist tests were similar to generalization tests in Experiment 1, however 15min prior to the administration of MDMA (1.5-4.5mg/kg) SCH 23390 (0.02 or 0.04) was administered i.p.

Table 2: Number of sessions run in each condition.

Condition (Dose/Antagonist; Dose/Drug)	No. of Sessions
0.02mg/kg SCH23390 + Saline	2
0.02mg/kg SCH23390 + 1.5mg/kg MDMA	2
0.02mg/kg SCH23390 + 3.0 mg/kg MDMA	2
0.02mg/kg SCH23390 + 4.5mg/kg MDMA	2
0.04 mg/kg SCH23390 + Saline	1
0.04 mg/kg SCH23390 + 1.5mg/kg MDMA	1
0.04 mg/kg SCH23390 + 3.0mg/kg MDMA	1
0.04 mg/kg SCH23390 + 4.5mg/kg MDMA	1

Data Analysis

As in Experiment 1, but only MDMA dose response curves were created.

Results 2A

After Experiment 1, an additional 16 training sessions were undertaken to ensure discrimination criterion continued to be met. During these training sessions, another rat reached the criterion. Hence, the data of 12 rats were used in the following analyses. In the weeks that the antagonists tests took place, training sessions were randomly interspersed in order to ensure stimulus control remained stable.

Initial t-tests proved that no significant gender differences were present when MDMA was given in conjunction with SCH23390. Furthermore, paired samples t-

tests confirmed that saline in conjunction with SCH23390 and saline administered by

itself (Experiment 1) do not differ significantly (paired samples t-test, all $p < .05$).

Therefore, SCH23390 on its own did not impact on performance.
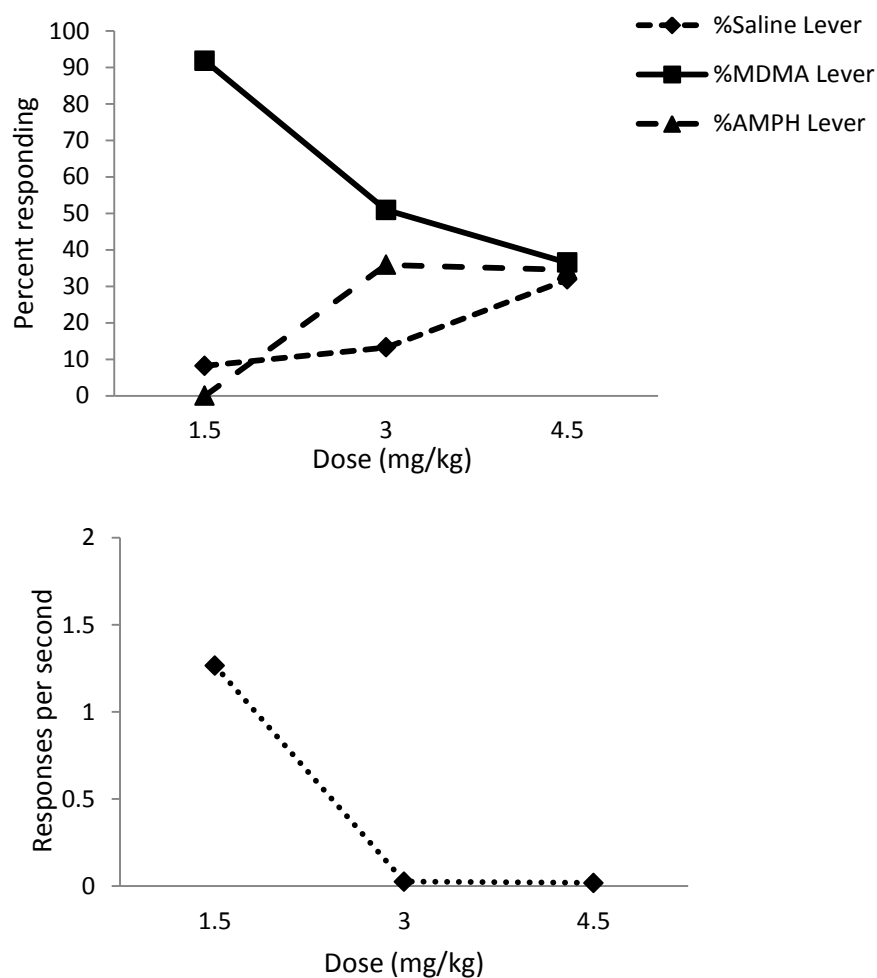
Figure 3: Results of antagonist tests with 0.02 mg/kg SCH 23390 and MDMA (1.5-4.5mg/kg). The mean percentage of total responses is plotted in the top graph and the median overall response rate is displayed in the bottom graph.
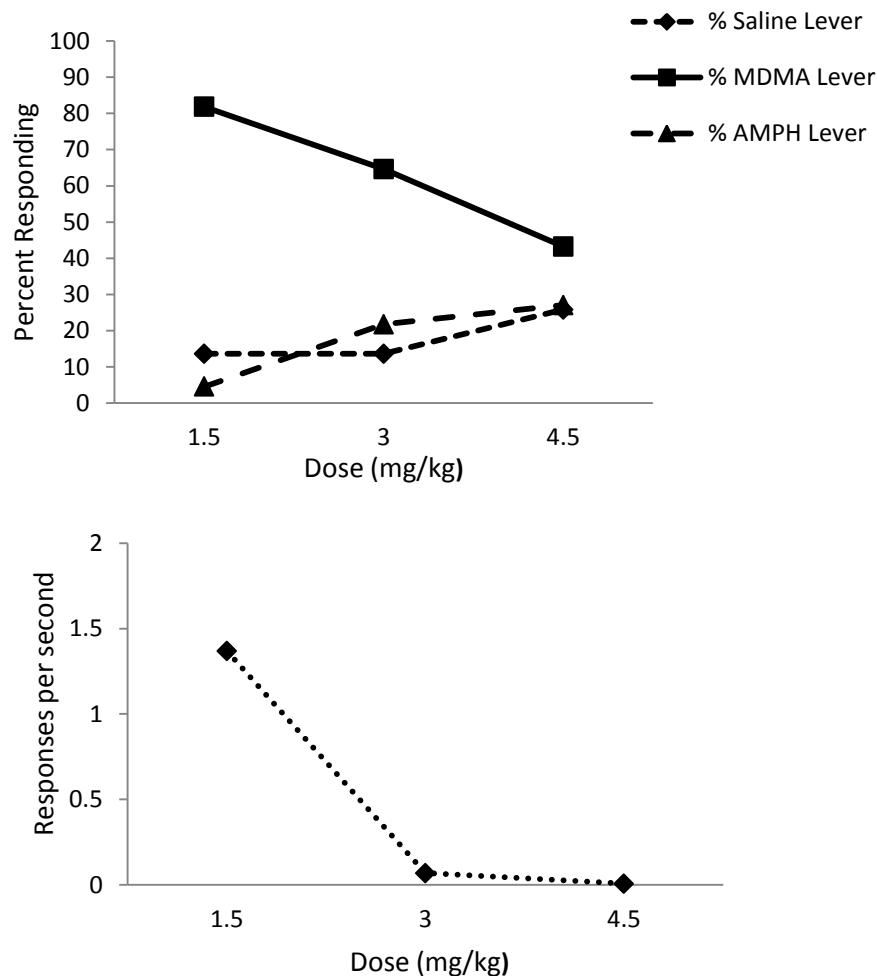
Figure 4: Results of antagonist tests with 0.04mg/kg SCH 23390 and MDMA (1.5-4.5mg/kg). The mean percentage of total responses is plotted in the top graph and the median overall response rate is displayed in the bottom graph.

Proportion of Responses

Figure 3 and 4 show the results of the antagonist tests. Two different doses of the D1 antagonist SCH 23390 were used. MDMA-appropriate responding decreased significantly as the dose of MDMA increased following pre-treatment with SCH 0.02 [Greenhouse Geisser $F(1.33,13.29)=10.84$, $p=.004$] and with SCH0.04 [$F(1,20)=4.77$, $p=.02$]. Here and elsewhere the more conservative Greenhouse Geisser or Huynh Feldt correction were used, if Mauchly's test of sphericity was

significant. With the lower dose of SCH23390 (0.02mg/kg) MDMA-appropriate responding decreased as the dose of MDMA increased from 92% at the training dose (1.5mg/kg MDMA) to 51% at 3.0mg/kg to only 36% at the highest MDMA dose (4.5mg/kg). While MDMA-appropriate responding in conjunction with 0.04mg/kg also decreased significantly as the dose of MDMA increased, this effect was attenuated. Accordingly, MDMA-appropriate responding at 4.5mg/kg MDMA in conjunction with 0.04mg/kg SCH23390 was at 47%.

AMPH lever responding also increased significantly when MDMA was given in conjunction with the lower dose of SCH (0.02mg/kg) [$F(2,20)= 9.88$, $p=.001$], that is AMPH lever responding increased from 0% at the training dose to 36% at 3.0mg/kg. There is no further increase in AMPH lever responding from 3.0mg/kg to 4.5mg/kg MDMA. In contrast the higher dose of SCH23390 (0.04 mg.kg) resulted only in a non-significant increase of AMPH lever responding as the dose of MDMA increased. This trend extended further to the proportion of saline lever responding, which also increased in a nonsignificant manner with both doses of SCH23390.

Comparison to Experiment 1

On the other hand, the proportion of saline responding is significantly different when 4.5mg/kg MDMA is used in conjunction with 0.02mg/kg SCH23390 (in Experiment 2A) compared to saline responding without it (Experiment 1) [$t(9)=-2.576$, $p=.03$]. As can be seen in Figure 5, the proportion of saline lever responding lies at 11% when 4.5mg/kg MDMA is given by itself and increases to 32% when MDMA is given in conjunction with 0.02mg/kg SCH 233390. Contrary to this, there

is no significant difference in saline lever responding between 4.5mg/kg by itself or in conjunction with 0.04mg/kg SCH23390.

Conversely, amphetamine lever responding is at 61% when 4.5mg/kg MDMA is administered by itself, but the same dose administered in conjunction with 0.02mg/kg SCH 23390 results in only 33% of amphetamine lever responding or 28% with 0.04mg/kg SCH233390 of amphetamine lever responding (Figure 5). These differences are significant [$t(9)=2.857$, $p=.019$ with 0.02mg/kg SCH23390; $t(9)=2.61$, $p=.028$ with 0.04mg/kg SCH23390].

Additionally the lower antagonist dose (0.02mg/kg SCH 23390) also resulted in a lower proportion of amphetamine lever responding at the training dose (1.5mg/kg MDMA) compared to amphetamine lever responding without the antagonist (0% compared to 4%). This difference is significant ($p=.016$).

Figure 5: (top panel) Percent responding when1.5mg/kg of MDMA is administered by itself or in conjunction with 0.02mg/kg or 0.04mg/kg SCH23390. (middl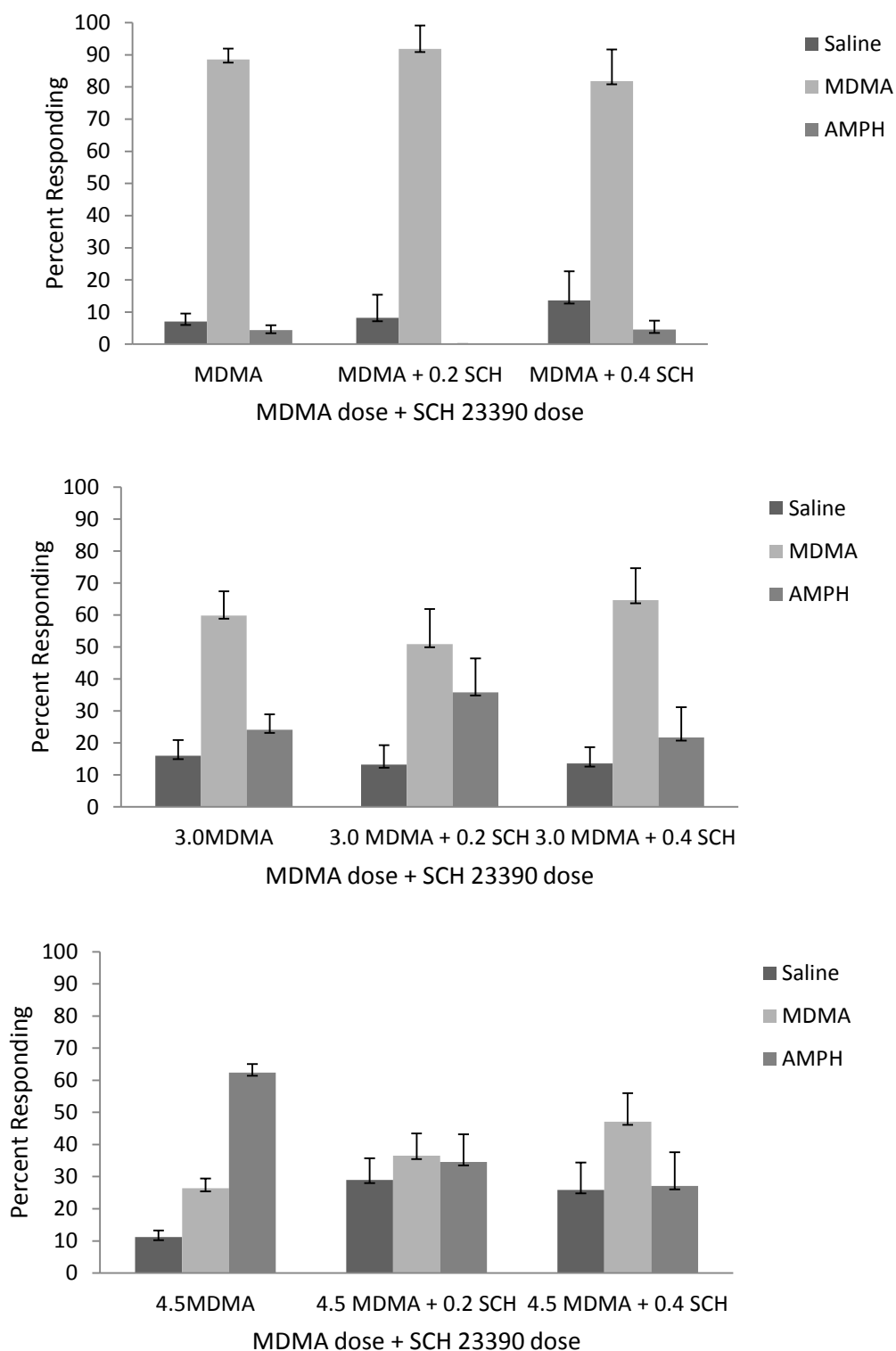e panel) Percent responding when 3.0mg/kg of MDMA is administered by itself or in conjunction with 0.02mg/kg or 0.04mg/kg SCH23390. (bottom panel) Percent responding when 4.5mg/kg of MDMA is administered by itself or in conjunction with 0.02mg/kg or 0.04mg/kg SCH23390. Bars represent response choices.

Response Rate

The bottom part of Figure 3 and 4 depicts the overall response rate. With both doses of SCH23390 responding significantly decreased as the dose of MDMA increased [Greenhouse Geisser, $F(1.0, 10.7) = 17.71$, $p = .001$ with 0.02mg/kg SCH23390; $F(2,20) = 19.40$, $p = .001$ with 0.04mg/kg SCH23390]. In line with this, responding was disrupted at 4.5mg/kg MDMA in conjunction with 0.02mg/kg (only 8 rats completed 10 responses in the allocated time frame). Two of these rats also failed to complete 10 responses when 3.0mg/kg MDMA was given in conjunction with 0.02mg/kg SCH23390. Responding was even more severely disrupted when 4.5mg/kg MDMA was administered in conjunction with 0.04mg/kg SCH23390. Only 2 (out of 12) rats completed ten responses. Five rats also failed to finish 10 responses at the higher SCH23390 dose when administered in conjunction with 3.0mg/kg MDMA.

Summary

As the dose of MDMA increases there is a change away from MDMA-appropriate responding. While there is a significant increase in AMPH lever responding with the lower dose of SCH23390 (0.02mg/kg), the increase that occurs with the higher dose of SCH23390 (0.04mg/kg) does not meet significance. Overall, as MDMA dose increases saline responding increases. This also results in a significant increase in saline lever responding at 4.5mg/kg MDMA compared to Experiment 1. Consequently, the trend found in Experiment 1 in which AMPH lever responding is enhanced with increasing MDMA dosage is attenuated in this

experiment. Additionally, it appears that when MDMA is given in conjunction with SCH23390 saline lever responding will increase.

Method 2B

Subjects

Subjects were 14 new drug and experimentally naïve female Norwegian Hooded rats. The rats weighed between 180 and 210 grams at the start of the study and were approximately 4 months old. The rats were kept in the same room and under the same conditions as the rats in the previous experiments. Apparatus and Training procedures were the same as in Experiment 1.

Drugs

Eticlopride was acquired through TOCRIS (UK).

Testing procedures and Data analysis were the same as in Experiment 2A

Table 3: Summary of the number of sessions run in each condition.

Condition (Dose/Antagonist; Dose/Drug)	No. of Sessions
Saline + 0.5mg/kg eticlopride	1
1.5mg/kg MDMA + 0.5mg/kg eticlopride	1
3.0 mg/kg MDMA + 0.5mg/kg eticlopride	2
4.5mg/kg MDMA + 0.5mg/kg eticlopride	2

Results 2B

Only 8 out of 14 female rats acquired the discrimination. Six rats failed to learn the discrimination due to frequent equipment failures. The equipment failures and extended break periods resulted in a lengthy acquisition period. Altogether 84 training sessions were undertaken before criterion was met consistently (80% of drug-appropriate responding in 8 out of 10 sessions).

Paired samples t-test showed no significant differences when saline was administered by itself or in conjunction with eticlopride. Therefore, eticlopride on its own has had no impact on performance.
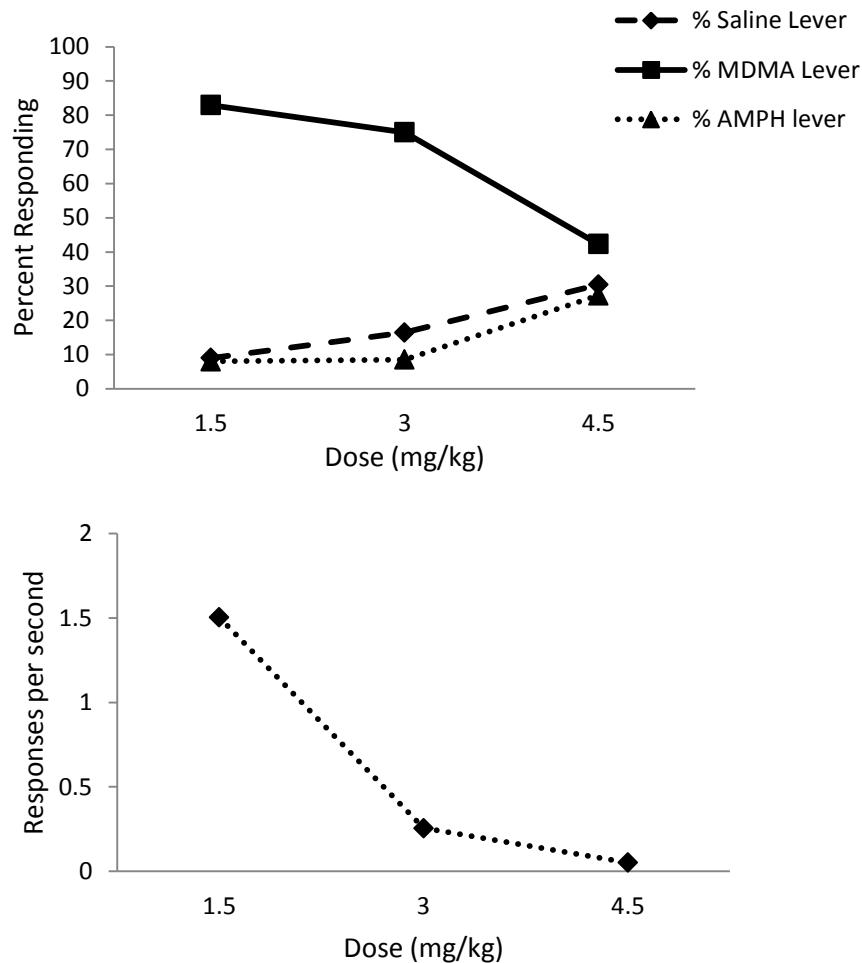
Figure 6: Results of antagonist tests with 0.05mg/kg eticlopride and MDMA (1.5-4.5mg/kg). The mean percentage of total responses made on each response option (MDMA vs. AMPH vs. Saline lever) during probe sessions is plotted in the top graph. The median overall response rate made across all response options during the probe FR10 is displayed in the bottom graph.

Proportion of Responses

Figure 6 shows the results of the antagonist tests with the D2 antagonist eticlopride. MDMA responding decreased significantly with increases in MDMA dose [Greenhouse Geisser correction, $F(1.2,8.4)=6.041$, $p=.03$]. On the other hand, the proportion of saline lever responding did not change significantly in a dose dependent manner when MDMA was given in conjunction with eticlopride. In line

with this, the increase in AMPH lever responding also just missed significance when MDMA was given in conjunction with eticlopride ($F(2,14)=3.507, p=.06$).

Comparison to Experiment 1

This represents a small, but noteworthy difference from Experiment 1, in which AMPH lever responding increased significantly with MDMA dosage (≥ 3.0mg/kg). In line with this, paired samples t-test indicate a significant difference in the proportion of amphetamine responding at 3.0mg/kg MDMA administered in conjunction with eticlopride and 3.0mg/kg MDMA administered by itself [$t(8)=-2.56$, $p=.38$]. Hence, the proportion of amphetamine lever responding changed from 26% (3.0mg/kg MDMA by itself) to 8% (3.0mg/kg MDMA in conjunction with 0.05mg/kg eticlopride) (also see figure 7). There is also a significant difference when 4.5mg/kg MDMA is administered in conjunction with eticlopride or without it [$t(8)-4.70, p=.002$]. Here, the proportion of amphetamine responding changes from 61% (4.5mg/kg MDMA by itself) to 27% when 4.5mg/kg MDMA was given in conjunction with eticlopride (see Figure 7). On the other hand, there were no significant differences in saline and MDMA-appropriate responding at any of the MDMA doses tested between MDMA by itself or in conjunction with eticlopride.

Figure 7: (top panel) Percent responding when 1.5mg/kg of MDMA is administered by itself or in conjunction with 0.05mg/kg eticlopride. (middle panel) Percent responding when 3.0 mg/kg of MDMA is administered by itself or in conjunction with 0.05mg/kg eticlopride. (bot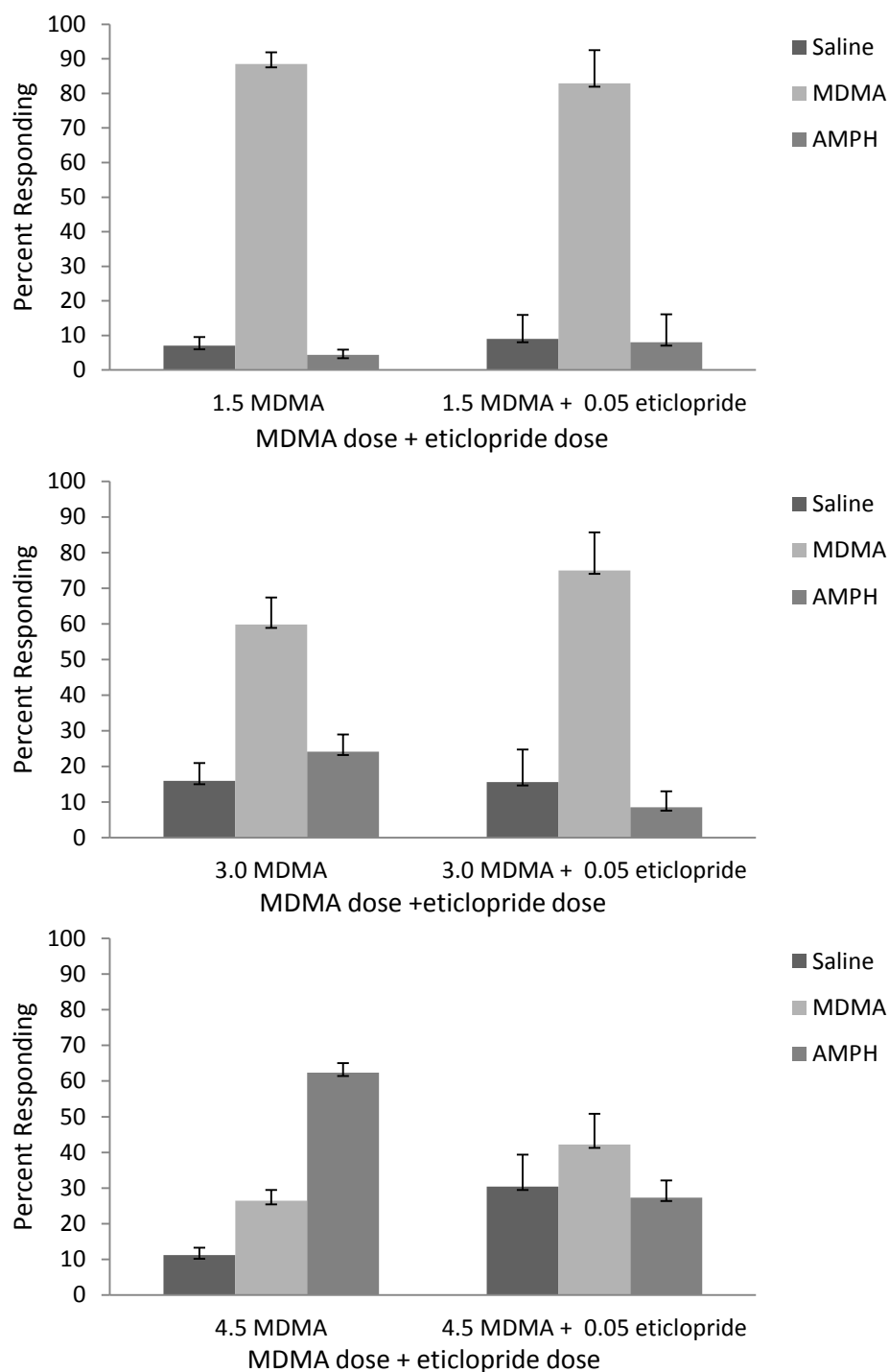tom panel) Percent responding when 4.5mg/kg of MDMA is administered by itself or in conjunction with 0.05mg/kg eticlopride. Bars represent response choices.

Response Rate

Response rates were similar to previous experiments and decreased in a dose dependent manner [Greenhouse Geisser, F (1.1,7.8) =9.85, p=.013). Accordingly, two rats failed to respond 10 times in one instance when 4.5mg/kg MDMA was given in conjunction with 0.05mg/kg eticlopride.

Summary

As the dose increased, MDMA appropriate responding decreased, and a nonsignificant increase in saline lever responding occurred. In contrast to Experiment 1 and 2A, the dose dependent increase of amphetamine lever responding did not meet significance. Specifically, there was a significant reduction in AMPH responding at 4.5 mg/kg and at 3.0mg/kg compared to AMPH responding without eticlopride (Figure 7).

Method 2C

Subjects

Experiment 2B and 2C were undertaken in the same time fame, thus the same rats as in Experiment 2B were utilised.

Apparatus and Training procedures were the same as in Experiment 1.

Drugs

RS 102221 was acquired through TOCRIS (UK).

Testing procedures and Data analysis were the same as in experiment 2 A & B.

Table 4. Number of sessions run in each condition

Condition (Dose/Antagonist + Dose/Drug)	No. of sessions
Saline + 0.5mg/kg RS 102221	1
1.5mg/kg MDMA + 0.5mg/kg RS 102221	1
3.0mg/kg MDMA + 0.5mg/kg RS 102221	1
Saline + 1.0mg/kg RS 102221	1
1.5mg/kg MDMA + 1.0mg/kg RS 102221	1
4.5mg/kg MDMA + 1.0mg/kg RS 102221	1

Results 2C

The two different doses of 5-HT2c antagonist RS 102221 were given in conjunction with MDMA and vehicle on different days. However, due to equipment failure only two separate MDMA conditions were recorded with each RS102221 dose.
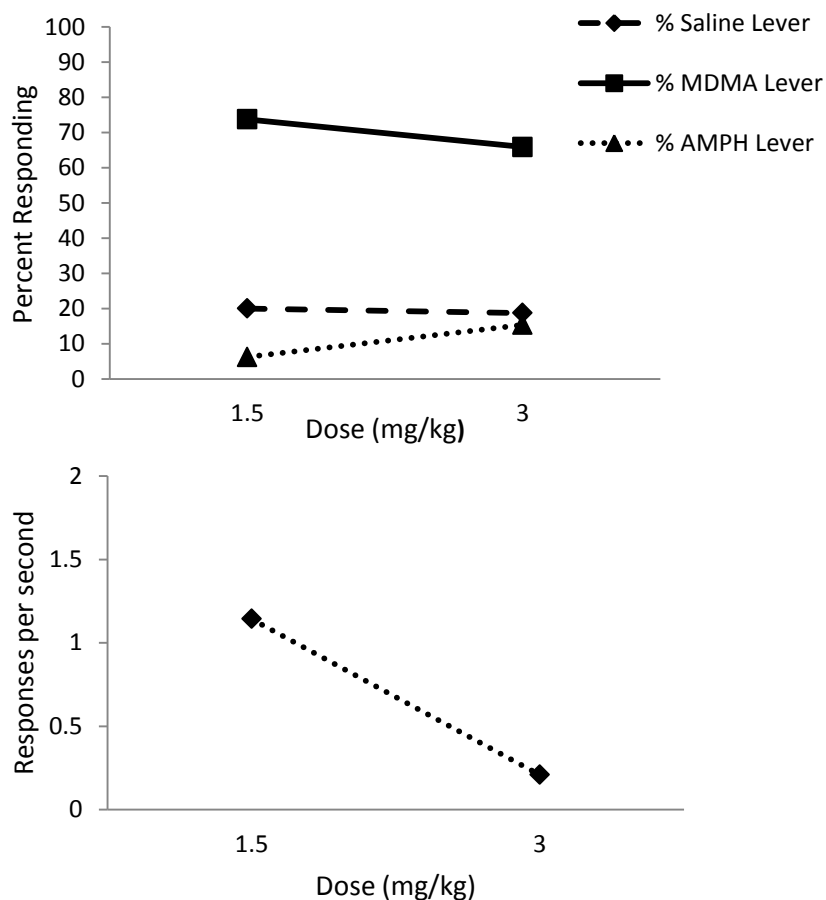
Figure 8: The top graph displays percentage responding for each lever at 1.5mg/kg and 3.0mg/kg MDMA in conjunction with 0.5mg/kg RS 102221. The bottom graph shows the response rate in responses per second.

Proportion of Responses

There were no significant dose dependent changes in MDMA, AMPH and saline lever responding between the two different doses of MDMA (1.5mg/kg and 3.0mg/kg), when MDMA was given in conjunction with 0.5mg/kg RS 102221.
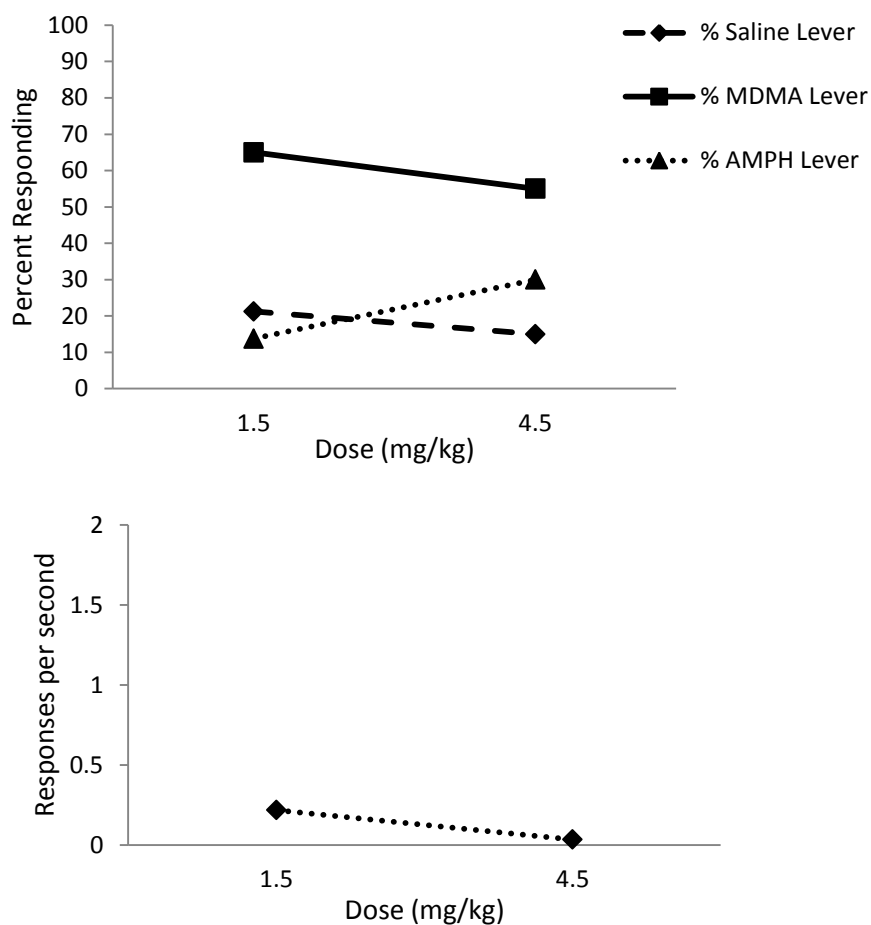
Figure 9: The top graph shows the responses for each of the three levers when 1.5mg/kg and 4.5mg/kg was given in conjunction with 1.0 mg/kg RS 102221. The bottom graph displays median overall response rates.

Again, there were no significant dose-dependent changes in MDMA, AMPH and saline lever responding when MDMA (1.5mg/kg and 4.5mg/kg) was given in conjunction with the higher dose of the antagonist (1.0mg/kg RS102221).

Comparison to Experiment 1

With the lower dose of RS102221 (0.5mg/kg) there was no significant difference between MDMA, AMPH and saline lever responding at 1.5mg/kg amd 3.0mg/kg MDMA compared to responding when MDMA (1.5mg/kg and 3.0mg/kg)

was administered by itself. Similarly, there was also no significant difference in saline lever responding at 1.5mg/kg and 4.5mg/kg MDMA when MDMA was administered in conjunction with 1.0mg/kg RS102221 or by itself.

Furthermore, there was no significant difference in MDMA-appropriate responding when MDMA was given in conjunction with 1.0mg/kg RS102221 or by itself (see Experiment 1) with 1.5mg/kg and 4.5mg/kg MDMA. However, MDMA-appropriate responding decreased from 25% when MDMA is given by itself to 55% when 4.5mg/kg MDMA is given in conjunction with 1.0mg/kg RS 102221. However, this result just missed significance [$t(7)=-2.05$, $p=.08$]. Additionally, at 4.5mg/kg MDMA there is also a significant difference in AMPH lever responding when 4.5mg/kg MDMA is given by itself or in conjunction with 1.0 mg/kg RS102221 [$t(7)=-2.94$, $p=.02$] (also see figure 10). Hence, AMPH lever responding decreased from 62% (MDMA by itself) to 30% when 4.5mg/kg MDMA is given in conjunction with 1.0mg/kg RS 102221.
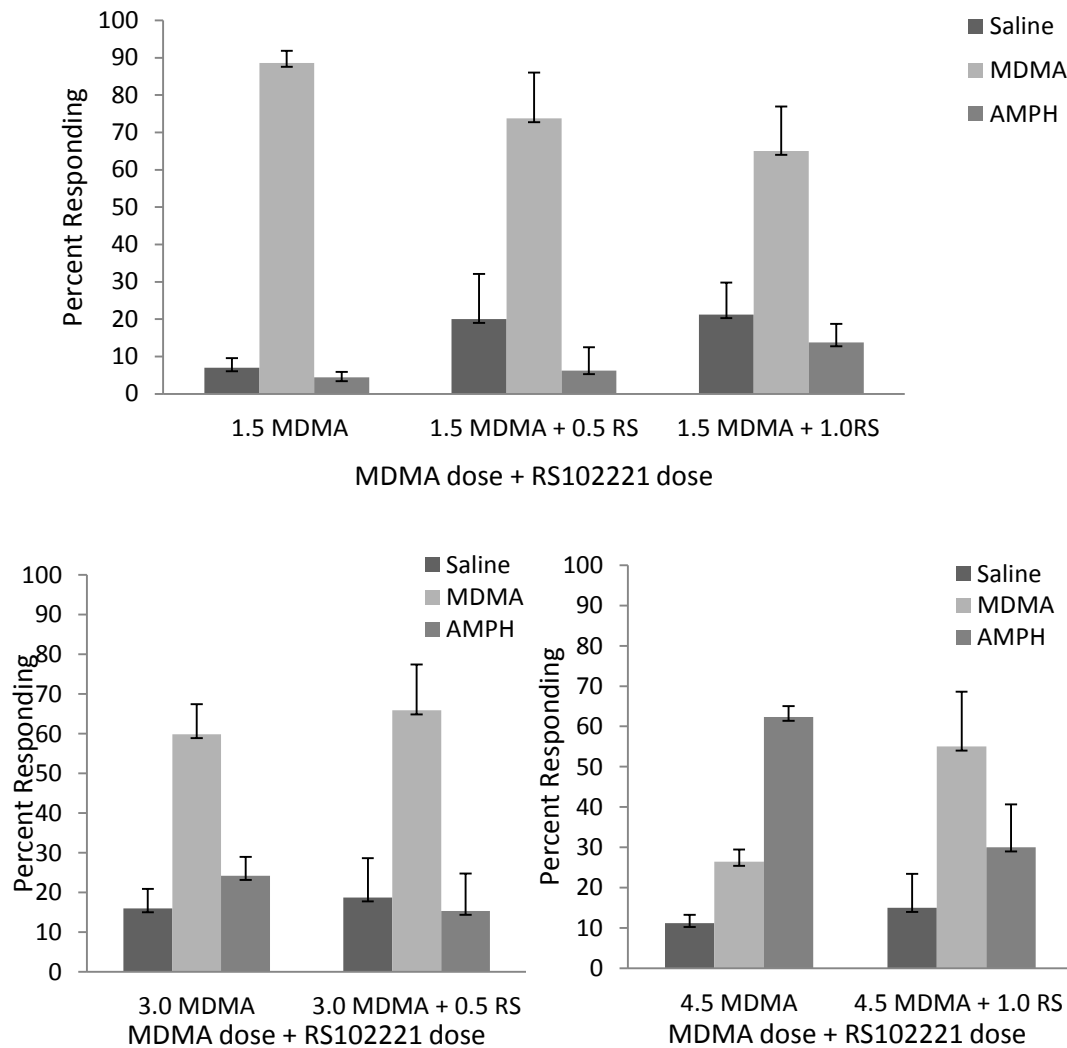
Figure 10: (top panel) Percent responding when1.5mg/kg of MDMA is administered by itself or in conjunction with 0.5mg/kg or 1.0mg/kg RS 102221. (bottom panel) Graph on the left displays percent responding at 3.0mg/kg MDMA when MDMA is administered in conjunction with 0.5mg/kg RS 102221. Graph on the right shows percent responding when 4.5mg/kg of MDMA is administered by itself or in conjunction with 1.0mg/kg RS 102221. Bars represent response choices.

Response Rate

There were no significant differences in response rates between the two doses and whether MDMA was administered by itself (Experiment 1) or in conjunction with 0.5mg/kg RS 102221. However, overall responding was less impaired. Only 2 rats did not finish 10 responses in one instance (at 3.0mg/kg MDMA in conjunction

with 0.5mg/kg RS 102221). Additionally response rate was significantly lower when 1.5mg/kg was given in conjunction with 1.0mg/kg RS 102221 compared to 1.5mg/kg MDMA by itself [$t(7)$=-4.48, p=.003]. This decrease in response rate did not extend to the lower dose of RS 102221.

Summary

MDMA in conjunction with RS 102221 (both doses) resulted in only non-significant changes in percent responding for all response choices. Consequently, increased doses of MDMA did not lead to increases in the proportion of amphetamine lever responding or decreased MDMA-appropriate responding (as in Experiment1).

In line with this, there was a noteworthy increase in MDMA-appropriate responding at 4.5mg/kg MDMA when MDMA was administered with 1.0mg/kg RS 102221 compared to MDMA administered by itself. It is possible that this would have reached significance, if more than one antagonist probe test had been undertaken at this dose. Additionally, there was a significant reduction in AMPH lever responding when MDMA (also 4.5mg/kg) was administered in conjunction with RS102221 compared to MDMA without the antagonist (Figure 10). These findings indicate that the higher dose (1.0mg/kg) of the 5-HT2c anatgonist RS102221 had an effect in attenuating AMPH lever selection at higher MDMA doses.

Of interest also is the significant reduction in response rates with 1.0mg/kg of RS 102221 with 1.5mg/kg. There was no significant change at the same MDMA

dose with 0.5mg/kg of RS 102221. Therefore it is possible that 1.0mg/kg of RS

102221 had a detrimental effect on responding. However contrary to this notion, all

the rats finished 10 responses at any of the probe tests with 1.0mg/kg RS 102221.

Comparing Results of Experiments 2 A, B + C

There were no significant differences between MDMA, AMPH and saline

responding at any dose (1.5mg/kg-4.5mg/kg) between the D2 antagonist eticlopride

and the data in experiment 2A with the D1 antagonist SCH23390. This further

extended to the 5-HT2c antagonist RS102221. The only significant differences that

occurred in response lever selection were between the antagonist tests and MDMA

administered by itself (figure 11).
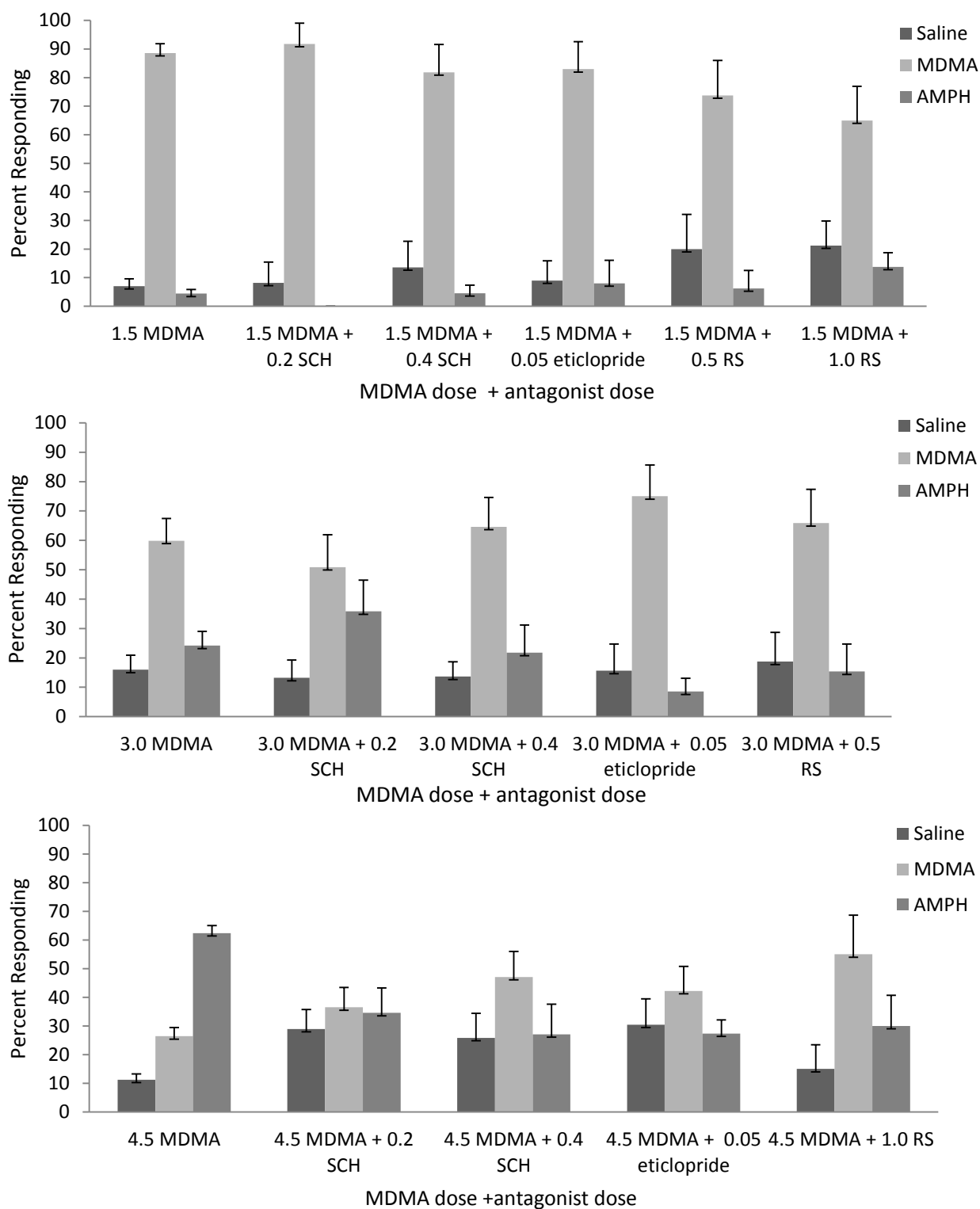
Figure 11: (top panel) Percent Responding at each lever displayed when 1.5mg/kg MDMA is administered by itself or in conjunction with an antagonist.(middle panel) Percentage of responding at each lever when 3.0mg/kg is given by itself or in conjunction with an antagonist.(bottom panel) Percent responses made at each response choice when 4.5mg/kg MDMA was administered by itself or in conjunction with an antagonist.

While there were no significant differences between the different antagonists and the different antagonist doses, a variety of different trends can be seen in Figure 11. For example, the 5-HT2c antagonist RS 102221 appeared to be most effective in sustaining MDMA-appropriate responding even at the highest MDMA dose (4.5mg/kg). The DA antagonist (SCH23390 and eticlopride) on the other hand had the most effect in reducing the proportion of AMPH lever responding as the MDMA doses increased. This was paralleled by the non-significant increase in saline lever responding (>3.0mg/kg MDMA).

Discussion

Experiment 1

In the current study rats were trained to discriminate between MDMA (1.5mg/kg), amphetamine (0.5mg/kg) and saline. The fact that the discrimination was successful implies that MDMA and amphetamine are sufficiently different as stimuli. This is in line with previous research that has also shown that MDMA (1.5mg/kg), amphetamine (1.0mg/kg) and saline vehicle can be differentiated in a three lever drug discrimination study (Goodwin & Baker, 2000). Goodwin and Baker (2000) have suggested that this discrimination occurs due to AMPH's primary influence on DA, while MDMA's stimulus effects are mediated by 5-HT and DA.

MDMA dose response curves

Specifically, at low doses (<3.0mg/kg) MDMA's subjective effects are primarily mediated by its influence on 5-HT release, resulting in the subject's ability to discriminate MDMA from amphetamine. In the current study this can be observed in MDMA dose response curve, which shows a dose dependent increase in MDMA-appropriate responding (0.5-1.5mg/kg), with lower doses of MDMA increasingly treated like saline.

As the dose of MDMA increases (>3.0mg/kg) the proportion of amphetamine lever responding rises, while MDMA-appropriate responding simultaneously decreases. Given that the proportion of amphetamine lever responding goes up

indicates that MDMA becomes gradually more amphetamine-like. Assuming that MDMA-appropriate responding is mediated by MDMA's influence on the 5-HT system and AMPH-appropriate responding is mediated by AMPH's influence on the DA system, it can be presumed that amphetamine lever responding after MDMA injection demonstrates MDMA's influence on the DA system. Therefore, as the dose of MDMA increases, MDMA's discriminative stimulus effects become increasingly mediated by its influence on DA. In fact the proportion of amphetamine lever responding far surpasses MDMA-appropriate responding at 4.5mg/kg.

Changes in Patterns of Responding and MDMA's influence
on the 5-HT and DA system

The pattern of responding seen at higher doses of MDMA is in stark contrast to MDMA-appropriate responding at 1.5mg/kg. At this dose the proportion of amphetamine responding is near zero, which is also the case for 0.5mg/kg and 1mg/kg MDMA. The fact that 0.5mg/kg MDMA resulted in a high percentage of saline responding would indicate that at this dose MDMA's discriminative cue properties are significantly different to the training dose. In this instance, a high proportion of saline lever responding probably indicates that the MDMA drug cue is not sufficiently developed. Interestingly, there appears to be not much difference in the discriminative cue properties of 1.0mg/kg and 1.5mg/kg MDMA. The fact that MDMA-appropriate responding at these doses is very high could indicate that most of the discriminative cue properties at this dose are mediated through MDMA's influence on the 5-HT system.

Only with 3mg/kg of MDMA does the proportion of amphetamine responding become relevant implicating that MDMA's discriminative cue properties have changed. However, at this dose MDMA-appropriate responding is still significantly higher than the proportion of amphetamine lever responding. The proportion of responding reverses at 4.5mg/kg MDMA as amphetamine lever responding surpasses MDMA-appropriate responding in frequency. There are no noteworthy changes in the proportion of saline lever responding after 1.5mg/kg. Consequently, it can be assumed that the significant increase in the proportion of amphetamine responding at this dose along with the significant decrease of MDMA-appropriate responding signals a significant change in terms of the effects of the 5-HT and DA neurotransmitter systems in MDMA's discriminative cue properties. A change away from purely 5-HT mediated influences, implicating a role for DA-mediated influences.

It is possible that because amphetamine and MDMA are very similar in terms of their behavioural and cognitive effects, increasing doses of MDMA may continue to enhance these similarities, while simultaneously decreasing the differences. This can be explored on the example of hyperactivity. MDMA increases hyperactivity through enhanced DA release, which occurs through two mechanisms. Firstly, MDMA directly releases DA and secondly MDMA has indirect effects in increasing extracellular DA through its effects on the 5-HT system. Evidence for this comes from studies that have shown that fluoxetine, which inhibits 5-HT release also significantly decreases DA release (Koch & Galloway, 1997) and hyperactivity (Callaway et al. 1991). With neurochemical studies indicating a significant increase in DA release at higher doses (Baumann et al. 2008) it can be postulated that the two

mechanisms that increase DA release have a more pronounced effect at these doses. This in turn adds to increase MDMA-induced hyperactivity; perhaps to the point of paralleling amphetamine-induced hyperactivity. These alterations in hyperactivity and DA release lead to significant changes in the discriminative cue properties of MDMA, which may mirror those of amphetamine's discriminative cue properties.

Further, in terms of MDMA-induced neurotransmitter release, it has been established that not only does 5-HT have an influence on DA release, but MDMA-induced DA release is a requirement for 5-HT neurotoxicity to occur. It has been shown that blocking DA release can protect 5-HT neurons from neurotoxicity (Schmidt & Taylor, 1987). Many of MDMA's behavioural and cognitive effects also rely on an interaction between DA and 5-HT (Koch & Galloway, 1997). In line with this and the results of Experiment 1, it can be assumed that the same applies for MDMA's subjective cue properties.

Implications for different training doses in the MDMA drug discrimination literature

The observation that the higher doses of MDMA will increase the proportion of amphetamine lever responding also helps to clarify some of the inconsistent findings in previous drug discrimination studies. Generally, previous research found that 5-HT agonists would partially or fully substitute for MDMA; and most DA agonists would fully or partially substitute for amphetamine (e.g., Baker & Makhay, 1996; Oberlander & Nichols; Schechter, 1989, 1998). However, there have been a variety of conflicting results reported when substituting MDMA for amphetamine, or vice versa. For example, Evans and Johanson (1986) trained pigeons to discriminate

between AMPH and saline. They found that the proportion of AMPH lever responding increased as the dose of MDMA increased. The finding that MDMA substitutes for amphetamine has been replicated with rats (Glennon & Young, 1984). However, Oberlander and Nichols (1988) could not corroborate this (1.75mg/kg MDMA). Similarly, discrimination research that has used MDMA as the training drug also contributed some inconsistent findings. Most studies found only partial substitution for amphetamine when MDMA was the training drug (1.5 mg/kg) (e.g., Schechter, 1989). A study that used 3.5 mg/kg of MDMA as the training dose, found no substitution for amphetamine (Baker et al, 1996), while Oberlander and Nichols (1988) found that amphetamine completely substituted for MDMA (1.75mg/kg).

Corresponding to the variation of different MDMA doses that were used (1.0-3.5mg/kg) a number of different amphetamine doses have also been used between different labs (0.8-2.0mg/kg). In the current study 0.5mg/kg amphetamine was used because pilot studies in our lab have indicated that responding was impaired at 1.0mg/kg (unpublished data). It is possible that some of the findings in Experiment 1 are due to differences in amphetamine's stimulus cue properties as a lower dose was used than in previous research (0.5 vs 1.0mg/kg). However, the fact that there were no significant changes in the proportion of MDMA lever responding between 0.125-0.75mg/kg amphetamine and no change at all in the proportion of amphetamine-appropriate responding, argues against this. Additionally, because rats learned to discriminate amphetamine from MDMA and saline and met discrimination criterion at the training dose indicates that amphetamine's discriminative cue properties were sufficiently developed at this dose. Furthermore, it is claimed that smaller training doses enhance sensitivity (Stolermann, 1993)

Generally, it has been argued that substitution and generalization depend on the type of training drug and the specific doses used in the tests (Goodwin & Baker, 2002). For the purposes of the current study the training dose of amphetamine was relatively low (0.5mg/kg AMPH), but the MDMA training dose had been used frequently in past research (e.g.: Schechter, 1989). The fact that only doses above 3.0mg/kg MDMA began to generalize to amphetamine supports the notion that using higher and lower doses of MDMA is essential in generalization tests. Additionally, MDMA's similarities to both hallucinogens and stimulants (Morgan, 2000) argue for the importance of utilising a three-lever drug discrimination procedure when assessing MDMA's discriminative cue properties.

While an assessment of the training and substitution doses of MDMA and AMPH does not immediately clarify the inconsistent results reported in the past literature, it does become apparent that results vary widely when different doses are utilised. Future drug discrimination research should strive to employ consistent drug (and especially training) doses , because a comparison of discriminative cue properties of a complex drug such as MDMA could otherwise be futile. In line with this, Experiment 1 clearly showed that MDMA's discriminative cue properties changed frequently from 0.5-1.0mg/kg (saline-like) to 1.0-3.0mg/kg (MDMA-appropriate) to 3.0-4.5mg/kg (amphetamine-like).

Three-lever drug discrimination studies

Some of the issues that have arisen from two-lever drug discrimination studies have been clarified in previous three-lever procedures. Goodwin and Baker (2000) showed that rats can successfully discriminate amphetamine from MDMA. However, their research did include response proportions above the training dose of 1.5 mg/kg of MDMA. Consequently, these studies have indicated an important role for 5-HT, while the role that DA plays in MDMA's discriminative cue properties has been largely disregarded. This has led to difficulties in explaining some of the results found in subsequent substitution tests. For example, tests with the 5-HT antagonist pirenpirone illustrated that MDMA's influence on 5-HT release is not the sole discriminative cue, as MDMA appropriate responding was only partially blocked. It appears that even at lower doses MDMA's discriminative cue properties are mediated by DA release to some extent. In line with this, another study also showed that rats can successfully discriminate between LSD (5-HT agonist) and MDMA (1.5mg/kg) in a three-lever drug discrimination paradigm (Goodwin et al, 2003). Subsequent substitution tests showed dose dependent increases for both fenfluramine (5-HT agonist) and amphetamine, highlighting MDMA's complex effects on both neurotransmitter systems.

Amphetamine dose response curve

In terms of amphetamine, the subjective cue properties appear to be far less complex. Thus, the dose response curve for amphetamine shows no changes for AMPH-appropriate responding after 0.5mg/kg (training dose). This also indicates a lesser role for dose dependent discriminative cue property changes. Similar to MDMA appropriate responding at the lower MDMA doses (0.5-1.5mg/kg), AMPH-

appropriate responding also increases significantly from 0.125-0.5mg/kg. However, unlike MDMA-appropriate responding there is no additional change in terms of AMPH-appropriate responding at doses above 0.5mg/kg amphetamine (training dose). AMPH-appropriate responding simply plateaus. Similar to the proportion of saline responding in the MDMA dose response curve, the proportion of saline responding also decreases in a dose dependent manner with increasing doses of amphetamine (0.125-0.75mg/kg). The proportion of MDMA lever responding at all amphetamine doses remains consistently low with a slight nonsignificant decrease (0.25-0.75mg/kg).

Experiment 2A- D1 antagonist SCH23390: Comparison between Experiment 1 and Experiment 2A

Experiment 1 clearly shows the trend of an increase in the proportion of AMPH lever responding for 3.0mg/kg and 4.5mg/kg MDMA. Although the proportion of AMPH lever responding in Experiment 2A also significantly increases as the dose of MDMA goes up, this effect was significantly attenuated compared to Experiment 1. Similarly, while the decrease in MDMA-appropriate responding is smaller compared to Experiment 1, it still meets significance. Another interesting result of Experiment 2A was the rise in the proportion of saline lever responding, although this did not meet significance.

Overall, all of the changes between Experiment 1 and 2A on the proportion of MDMA, saline and amphetamine lever responding were less evident at 3.0 mg/kg, indicating that DA mediated influences in terms of MDMA's subjective effects at

this dose play a less dominant role compared to 4.5mg/kg MDMA. At the highest dose, the proportion of responding is almost equally divided between amphetamine, MDMA and saline. Therefore, it can be assumed that the discriminative cue properties of MDMA at 4.5mg/kg in conjunction with SCH23390 differ significantly from 1.5mg/kg MDMA in conjunction with SCH23390 but also from 4.5mg/kg MDMA by itself (Experiment 1). The fact that MDMA at 4.5mg/kg partially generalizes to MDMA, saline and amphetamine could also indicate confusion, as the subjects struggle to identify which substance has been administered. In drug discrimination literature 40-70% of drug-appropriate responding generally indicates partial antagonism of the stimulus. While less than 40% of responses made on the drug-appropriate lever points towards saline-appropriate responding (Young et al. 2004). In the current study 4.5mg/kg MDMA in conjunction with the D1 antagonist SCH23390 results in approximately 33% of MDMA, amphetamine and saline lever responding. It is argued that partial generalisation/antagonism findings are usually problematic to unravel (Young et al, 2001; Stolerman, 1993), in the current study the employment of a three-choice procedure has led to the possibility that neither drug stimuli nor the saline cue offer an appropriate association to match the cue experienced. Alternatively, it could be argued that some generalisation to both drug stimuli and vehicle has taken place, which could indicate that 4.5mg/kg MDMA in conjunction with SCH23390 appears to have some similarities with both drug stimuli and vehicle. However, it is important to note, that responding was also significantly impaired at this dose, which further clouds the results and subsequent interpretations.

Comparing patterns of responding of Experiment 2A and Experiment 2B

In Experiment 2B the D2 antagonist eticlopride was administered in conjunction with MDMA (1.5-4.5mg/kg). Similar to Experiment 2A with the D1 antagonist SCH23390, as the dose of MDMA increases, so does the proportion of saline and amphetamine lever responding. The increase in saline responding misses significance, but in contrast to Experiment 2A the increase in the proportion of amphetamine lever responding also misses significance. This is an important difference to Experiment 2A, as it indicates that there has been a significant reduction in the DA mediated influences at the higher MDMA doses. The fact that Experiment 2B does not show a dose dependent increase in amphetamine lever responding implies that eticlopride had a significant impact in changing MDMA's discriminative cue at 4.5mg/kg in comparison to Experiment 1. In line with this and in contrast to Experiment 2A, the proportion of amphetamine lever responding is also significantly less than in Experiment 1 at 3.0mg/kg and at 4.5mg/kg MDMA. However, MDMA-appropriate responding also decreased significantly as the dose of MDMA increased. Specifically, there was a notable reduction after 3.0mg/kg MDMA which is consistent with the findings in Experiment 1 and 2A.

Comparison of D1 and D2 receptor effects in MDMA's discriminative cue properties

Bubar et al. (2004) have previously shown that MDMA in conjunction with SCH23390 will result in a decrease in MDMA-appropriate responding. In a two-choice drug discrimination procedure, it was found that there was a significant increase in saline lever responding at lower MDMA doses (0.75 and 1.0 mg/kg) when MDMA was administered in conjunction with SCH23390. However, in the current study only the higher dose of SCH23390 (0.04mg/kg) demonstrated a small

but insignificant decrease of MDMA-appropriate responding at the training dose of 1.5 mg/kg. It is possible, that the lack of a significant finding in the current study with the training dose of MDMA is due to the three-choice procedure employed. This could have increased the subjects' sensitivity for the mediating influences of 5-HT and DA, which could have translated into an association of the MDMA discriminative cue with purely 5-HT mediated influences. Since MDMA's discriminative cue properties at the training dose are predominantly mediated by 5-HT release in the current study, the effect of the DA antagonist at this dose would have been nearly void.

Bubar et al. (2004) also investigated the effects of the D2 antagonist eticlopride on MDMA's discriminative cue properties. In contrast to the D1 antagonist SCH23390, it was demonstrated that eticlopride had no effect on MDMA-appropriate responding. However, both antagonists were reported to significantly reduce MDMA-induced hyperactivity at 3.0mg/kg. Furthermore, Goodwin et al. (2003) reported that the D2 antagonist haloperidol also had no effect in changing response proportions when administered with MDMA. In contrast to Bubar et al. (2004), Goodwin et al. (2003) utilised a three-choice drug discrimination procedure. Rats were trained to discriminate between MDMA, LSD and vehicle. It is possible that because DA-mediated influences are less salient at lower doses, only the D1 receptor plays a role at lower doses. In line with this, Experiment 2A found a small, but significant reduction in the proportion of amphetamine lever responding at 1.5mg/kg MDMA (from 4% to 0%) with the lower dose of 0.02 mg/kg SCH23390.

However, as the dose of MDMA increases, the D2 antagonist eticlopride also elicits significant changes in response proportions, which could indicate that as DA mediated influences increase, influences mediated by D2 receptors also become more salient. Additionally, in the current study with the higher doses of MDMA (3.0-4.5mg/kg) some noteworthy differences between the D1 and the D2 antagonists become apparent. While an important effect of D1 receptor blockage was to increase saline responding (lower dose SCH23390), the most salient effect of D2 receptor blockage was the significant decrease in the proportion of amphetamine lever responding.

The role of D1 vs. D2 receptors in MDMA induced behaviour and cognitive effects

In terms of locomotor effects, research has shown that both D1 and D2 antagonists will reduce MDMA induced hyperactivity (Bubar et al., 2004). However, the specific changes in locomotor behaviour differ depending on which DA antagonist was administered. Ball et al. (2003) found that MDMA (5.0mg/kg) in conjunction with eticlopride completely blocked MDMA-induced locomotion for the entire time frame (100min). However, the D1 antagonist would still result in MDMA-induced locomotion but there would be a delayed onset (peak at 65 min after MDMA injection). Since, the antagonist tests in Experiment 2A and B both were performed 30minutes after the antagonist injection and 15 minutes after MDMA injection, it is possible that this may have distorted the current data. Correspondingly, the effect that the D1 antagonist SCH 23390 had in terms of

proportion of responses may have been more salient if the antagonist test had taken place shortly after SCH23390 injection.

Ball et al. (2003) also reported that MDMA in conjunction with SCH23390 had less effect in decreasing excitatory neuronal responses in the striatum, compared to eticlopride, which blocked almost all MDMA-induced excitation. While most studies have found significant results with eticlopride in terms of having an effect on MDMA induced locomotion (Bubar et al. 2004; Ball et al. 2003) it is important to note that the MDMA doses were also markedly higher compared to Bubar's (2004) drug discrimination study (3.0-5.0mg/kg vs. 0.375-1.0mg/kg). This could be taken as further prove that DA-mediated effects at higher MDMA doses (>3.0mg/kg) become more relevant. Additionally, it appears that at least some of these effects are primarily mediated by D2 receptors. However, to draw a definite conclusion, the sample size and most importantly the amount of probe sessions were not sufficient in the current study.

Contrary to the notion that MDMA-mediated effects are predominantly mediated by D2 receptors (>3.0mg/kg), Harper (2011) found that MDMA-induced memory impairments in a radial arms maze were significantly decreased when MDMA (3.0mg/kg) was given in conjunction with SCH23390 but not in conjunction with eticlopride. However, although the current drug discrimination task and the DMTS task are both conditional discrimination tasks, an important difference between them is that in the current procedure the drug acts as a stimulus, whereas in the DMTS task, drugs interact with the perception of stimuli that signal reinforcement (i.e. lever position). It was suggested by Harper (2011) that MDMA-

induced impairments in the DMTS task were the result of perseveration (i.e. a response bias towards returning to a lever that was chosen on the preceding trial). Therefore, it may be that D1 vs. D2-like receptors play different roles with respect to the subjective experience of MDMA as a stimulus (as in the current study) versus their role in response perseveration or behavioural flexibility (as revealed by an analysis of response patterns in the DMTS procedure).

Differential Effects of DA antagonists between high and low MDMA doses

While higher doses of MDMA generally begin to feel more and more amphetamine-like through increased DA agonist action, high doses of MDMA in conjunction with a DA antagonist only partially generalize to vehicle and only partially antagonize MDMA and amphetamine. Especially at the highest MDMA dose (4.5mg/kg) the proportion of responses is almost equally divided between MDMA, saline and amphetamine with both DA antagonist. As discussed above, this could be an indication that the rats were unable to associate 4.5mg/kg in conjunction with a DA antagonist with the previously experienced training drug stimuli (1.5mg/kg MDMA vs. 0.5mg/kg amphetamine), while the concurrent increase in the proportion of saline lever responding may simply a by-product of this. However, assuming that this is the case, than the role that DA release plays at 4.5mg/kg is essentially more important than 5-HT release in terms of mediating discriminative cue properties. The apparent confusion in responding at 4.5mg/kg in conjunction with the DA antagonist could indicate that a decrease of DA release at these doses has a significant effect in changing the discriminative cue of MDMA. However, if DA release only played a minor role in mediating MDMA's discriminative cue

properties than it can be postulated that MDMA-appropriate responding would be significantly enhanced when 4.5mg/kg MDMA is given in conjunction with a DA antagonist. Specifically, as 5-HT release is considered to surpass DA release at all MDMA doses (Baumann et al. 2008). In line with this, Experiment 1 also illustrated that amphetamine lever responding surpasses MDMA-appropriate responding at 4.5mg/kg, which further supports the notion of the salient role that DA release plays at this dose.

Future research should consider administering both DA antagonists in conjunction with amphetamine, as this could also help to clarify to what extent amphetamine's discriminative cue properties are mediated by D1 and D2 receptors. This in turn would provide some indication whether and to what extent amphetamine's discriminative cue properties are mediated by 5-HT release. It has been widely reported, that amphetamine also elicits 5-HT release (e.g., Schenk, 2011). While amphetamine's 5-HT release is minor compared to MDMA's, the extent to which amphetamine's discriminative cue properties are mediated by 5-HT release remain largely unexplored. Overall, having an understanding of how D1 and D2 receptor antagonists act on amphetamine's discriminative cue properties will allow for another level of comparison between MDMA's and amphetamine's subjective effects.

The effects of 5-HT2c antagonists on MDMA induced DA release

A previous study has suggested that some of the neurochemical and behavioural changes that occurred after MDMA was administered in conjunction

with SCH23390 are due to SCH23390 affinity for 5HT2c rather than its ability to block D1 receptors. Ramos et al. (2005) suggested that MDMA-induced behavioural sensitization was facilitated by SCH23390 ability to agonize 5-HT2c receptors, rather than antagonizing the D1 receptors. In line with this, Ross et al (2005) showed that 5-HT2c receptor antagonists ameliorate DA release. More specifically, it has been shown that MDMA in combination with 5-HT2c agonists reduce MDMA-induced hyperactivity which in turn is revealed when MDMA is administered in conjunction with a 5-HT2c antagonist (Bankson & Cunningham, 2002). Fletcher et al. (2006) argued that the effect of increased hyperactivity is a result of a general rise in DA activity. Secondly, blocking of the 5-HT2c receptor will amplify activity at other 5-HT receptors which have a more stimulatory profile. Overall, it appears that 5-HT2c receptor antagonist will lead to increased extracellular DA release, which in turn amplifies MDMA and amphetamine induced hyperactivity.

5-HT antagonist or DA agonist ?

In general, the trends in response proportions that were observed in Experiment 2C are more easily explained through the antagonist action on the 5-HT system, rather than agonist effects on the DA system. Specifically, it appears that MDMA-appropriate responding is reduced at 1.5mg/kg, which could be explained through the blocking of 5-HT2c receptor. Correspondingly, there is also a nonsignificant increase in the proportion of amphetamine lever responding. These effects are also present but far smaller for 0.5mg/kg RS 102221. However, as the MDMA dose goes up, it becomes increasingly difficult to explain the results through

an increase in DA release. Specifically, MDMA-appropriate responding (at 4.5mg/kg) is higher with 1.0mg/kg RS102221 than it is with any other antagonist (Experiment 2A and B) or when MDMA is given by itself (Experiment 1), while the small increase in amphetamine lever responding is comparatively low (Experiment 2A and B) and significantly less than in Experiment 1. Again, a similar trend but on a smaller scale was found with the lower dose of RS 102221 at 3.0mg/kg. Generally, however, the proportion of amphetamine lever responding when MDMA was given with RS 102221 is comparable to the proportion of amphetamine lever responding when MDMA was administered with the two DA antagonists. However, compared to Experiment 2A and B, MDMA in conjunction with the 5-HT2c antagonist only led to a very minimal increase in saline lever responding.

Interestingly, the only noteworthy variation in MDMA-appropriate responding at 1.5mg/kg occurred in Experiment 2C when MDMA was administered with the 5-HT2c antagonist. This lends further support to the notion that the behavioural effects of relatively low doses of MDMA (1-1.5mg/kg) appear to be primarily mediated through its influence on the 5-HT system.

The effects of RS 102221

McCreary et al. (2003) showed in a two-lever drug discrimination study that trained rats to discriminate between a 5-HT agonist (fenfluramine) that administering RS 102221 in combination with fenfluramine did not lead to any changes in proportion of responding. They argued that this was due to RS 102221's inability to cross the blood-brain barrier. In line with this, SB206553 a 5-HT2c antagonist that

more easily crosses the blood-brain barrier has been found to completely block fenfluramine-appropriate responding and consequently led to a significant increase in the proportion of saline lever responding (McCreary et al. 2003).

While it has been shown that MDMA completely substitutes for fenfluramine (Schechter, 1997), the results of the current study can only partially be explained by the notion that RS 102221 may not cross the blood brain barrier. While 5-HT2c antagonist tests did not result in any significant response proportion changes from 1.5-4.5mg/kg MDMA lends support to the notion that the antagonist did not have an effect, the response proportions in Experiment 2C differ substantially from those in Experiment 1. For example, MDMA-appropriate responding decreased in a dose dependent manner in Experiment 1, yet this was not the case in Experiment 2C, which supports the notion that 5-HT release was inhibited due to successful 5-HT2c blocking. Additionally, there is a significant difference in the proportion of amphetamine lever responding, which was significantly less in Experiment 2C compared to Experiment 1. Alternatively, however, this could be interpreted as another indication that RS 102221 was not successful in blocking 5-HT2c receptors, as it is postulated that 5-HT2c antagonist should lead to an increase in DA release due to the receptors typical role in inhibiting DA release. The inconsistency of the effects of Experiment 2C coupled with the small sample size and the small number of probe sessions prevents from drawing definitive conclusions.

However, taking a closer look at previous research helps to clarify some of the issues encountered in Experiment 2C. Thus, it has shown that the selective 5-HT2 antagonist pirenpirone or the 5-HT2a/c antagonist ritanserin while leading to a

significant reduction only partially blocked MDMA-appropriate responding (Goodwin & Baker, 2000; Smithies & Broadbear, 2011). In Smithies & Broadbear's (2011) three-way drug discrimination study, MDMA in conjunction with ritanserin led to 62% from 100% of MDMA-appropriate responding, and importantly there was an increase from 0- 37% in the proportion of amphetamine lever responding. Additionally, MDMA was only administered at 1.5mg/kg. The decrease in MDMA-appropriate responding is, in fact comparable to the results in the current study. Accordingly, MDMA-appropriate responding decreased from 90% to 65% when 1.5mg/kg MDMA was administered in conjunction with RS 102221. However, unlike in Smithies and Broadbear's (2011) study, the proportion of amphetamine lever responding did not increase significantly from Experiment 1 to 2C in the current study.

In line with this, Schechter (1991) showed that a neurotoxic regimen of MDMA (20mg/kg twice daily, 4 days) only led to a significant decrease in MDMA-appropriate responding at 1.0mg/kg MDMA. However, while MDMA-appropriate responding decreased substantially at 1.5mg/kg (by 20%) this difference was not significant. It appears that only para-chlorophenylalanine (p-CPA), which inhibits 5-HT synthesis, appears to almost completely block MDMA-appropriate responding (33% with 1.5mg/kg) in a two choice drug discrimination procedure (Schechter, 1991). Overall, it appears that RS102221 in the current study was somewhat successful in decreasing 5-HT release, but this did not appear to have an effect in increased DA action. Future research should utilise a 5-HT2c antagonist that more easily crosses the blood-brain barrier, to avoid inconsistencies and uncertainties encountered in Experiment 2C.

<u>Future Research</u>

In line with this, while 5-HT2c antagonist tests are interesting because of their ability to decrease 5-HT release and increase DA release, future research should look at completely blocking 5-HT release. As mentioned above, past research (Schechter 1991) has indicated that the most reliable way to suppress MDMA-appropriate responding is through administering a regime of p-CPA. Therefore administering a treatment of p-CPA for rats that have been trained to discriminate MDMA from amphetamine and saline and subsequently running generalization tests with 3.0 and 4.5mg/kg could be of interest in order to clarify exactly the role that the DA and the 5-HT system have in terms of MDMA's discriminative cue properties. Utilising DA antagonist tests could further specify a dissociation between D1 and D2 receptors. Specifically, while the current research and previous research (Bubar et al. 2004) have not been able to implicate a role for D2 receptors in MDMA's discriminative cue properties at low doses, a possible role for D2 receptors may be unmasked after 5-HT release has been suppressed.

Furthermore, it would be of interest to collect neurochemical data which could then be linked to the behavioural data. To this end, 5-HT and 5-HIAA could be measured in different parts of the brain in vitro. This neurochemical data could then be linked to behavioural data. Rats could undergo daily discrimination probe sessions with 4.5mg/kg of MDMA. It would be expected that initial responding after p-CPA regimen would primarily result in increased amphetamine lever responding, which should be mirrored by 5-HT depletion in the brain. However, after approximately 5 days, it would be expected that MDMA appropriate responding

would begin to gradually increase, indicating the recovery of the 5-HT system which should in turn be mirrored by an increase in 5-HT in the brain.

Incidentally, drug discrimination research, especially when it involves three-way procedures, are potentially affected by very long training periods. This in turn could result in tolerance or sensitization to some effects, which may not be accounted for and bias the data in an unidentifiable manner. Similarly, some effects may only arise after repeated or chronic exposure, although drug discrimination literature is primarily concerned with acute effects. This problem is difficult to avoid in this type of research, but may be alleviated if neurochemical data is collected simultaneously.

The current study and past research have to a large extent neglected to show a role for MDMA-induced norepinephrine (NE) release in MDMA's behavioural effects. In vitro studies have indicated that MDMA induced NE release almost matches that of 5-HT release (Rothman et al. 2001). Additionally, NE has also been implicated to play an important role in MDMA induced hyperactivity (Selken & Nichols, 2007). Therefore, future research should perform NE antagonist tests. Moreover, in order to illuminate DA and 5-HT roles in MDMA's discriminative cue properties NE and 5-HT or DA antagonists could be combined at different doses of MDMA.

Response Rate

In the current study, it was found that response rates decreased significantly with increasing MDMA doses, until it was substantially impaired at 4.5mg/kg (Experiment 1). However, if the reason for this decline in response rates was due to MDMA induced stimulant effects (i.e. ambulation) it would have been expected that response rates were less impaired when MDMA was administered in conjunction with a DA antagonist, but this was not the case. In Experiment 2A and B, response rates were comparable to response rates in Experiment 1. Thus, neither the D1 antagonist SCH23390, nor the D2 antagonist eticlopride had a significant impact on response rates. Generally, it is suggested that higher doses of MDMA will elicit the 5-HT syndrome (Schenk, 2011). In line with this, it appeared that while the 5-HT2c antagonist RS102221 did not have an overall effect in increasing response rates at high doses, almost all of the rats finished 10 responses in the allocated time frame. However, there is a significant difference in response rates when 1.0mg/kg RS102221was given in conjunction with 1.5mg/kg MDMA. At this instance, responding is significantly decreased compared to response rates in Experiment 1, 2A and B. Responding was most severely disrupted with the D1 antagonist SCH23390 (1.0mg/kg) with only two rats finishing ten responses at the highest MDMA dose. Thus, the fact that less rats were impaired at 4.5mg/kg MDMA when MDMA was administered in conjunction with RS102221 could indicate that the antagonist had some impact in attenuating 5-HT release and some of the behavioural effects associated with the 5-HT syndrome.

Overall, there were no significant differences in responding between male and female rats at any MDMA dose. However, there were some differences in terms of response and response rates at the amphetamine training dose (0.5mg/kg) between

male and female rats. In the current study it was decided to combine the data of the male and female rats, because the focal point was the analysis of MDMA's discriminative cue properties. However, Zhou, Cunningham & Thomas (2003) have shown that augmented hyperactivity in female rats after cocaine administration occurred 15 minutes after injection, whereas an increase in hyperactivity in female rats after MDMA injection occurred 30 minutes after injection. It is possible that amphetamine administration results in comparable hyperactivity onset times than cocaine, which could perhaps explain the significant increase at 0.5mg/kg in amphetamine-appropriate responding for female rats. In the current study test session would finish after 30 minutes. Therefore the issue of gender inconsistency was avoided in terms of MDMA, and appeared to only play a minor role after amphetamine administration.

However, future research should consider utilizing only male or female rats, as it is possible that the fact that female rats showed significantly enhanced amphetamine-appropriate responding could have led to significant gender differences in terms of experiencing amphetamine's discriminative cue properties. This in turn could easily lead to gender differences in amphetamine lever responding at increased MDMA doses, although in the current study there were no significant differences between male and female response choices at 3.0 and 4.5mg/kg MDMA-appropriate responding. Yet, responding at 4.5mg/kg MDMA was significantly impaired which could have masked this effect. Accordingly, future research should consider using 4.0mg/kg as the highest MDMA dose, in order to attenuate response rate impairments.

Implications

        The fact that relatively high doses of MDMA become increasingly treated like amphetamine, due to a rise in DA-mediated influences suggests that at these doses MDMA could produce effects that are more typically associated with DA-releasing agents. The most important implication of this finding is a rise in MDMA's addictive potential. While past research has shown that MDMA has some abuse potential and is self-administered by rats (e.g., Schenk, 2011), anecdotal reports have implied that most MDMA users remain casual consumers and deny dependency issues (Morgan, 2000). The findings of the current research go some way in explaining these inconsistencies. Generally, laboratory studies tend to utilise doses above 1.5mg/kg (which is the equivalent of a normal dose for humans) or subjects are exposed to the drug for longer and more regularly (Marston et al. 1999). In line with this Tancer & Johanson (2001) have shown that more experienced human drug users were more likely to identify MDMA as amphetamine, indicating increased sensitivity to MDMA-induced DA release, which perhaps occurs after chronic exposure. MDMA-induced DA release may initially act as a secondary agent in mediating MDMA's subjective effects, DA release will become more relevant with prolonged exposure or increased doses, potentially leading to addiction.

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