Assessment of the Reinforcing Properties of Orally Administered MDMA ('Ecstasy') in Rats

by

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ABSTRACT

The so-called “party drug” 3,4-Methylenedioxymethamphetamine (MDMA, or ecstasy) may share many of the addictive properties common to other CNS stimulants. In humans MDMA is primarily consumed orally in one more pills per session. However, animal research has mostly focused on examining the effects of MDMA as a function of other routes of administration. Route of administration can have profound effects on the subjective and reinforcing properties of drugs of abuse. This thesis assessed the locomotor-activating and reinforcing properties of MDMA when delivered orally. MDMA-induced hyperlocomotion was used to examine magnitude of response and onset of action as a function of *ip*, *sc* and oral administration. Significant route-dependant effects were found with *ip* producing higher locomotor activity than *sc* and oral respectively. Onset of action was slower for subcutaneous administration compared with both *ip* and oral administration. The reinforcing properties of MDMA were examined by use of the self-administration procedure. Oral MDMA self-administration was firstly examined using simple schedules of reinforcement as a function of two different vehicle substrates, water (under water deprivation) and saccharin. Oral MDMA maintained responding and reliable dose-response curves were obtained under both water and saccharin vehicle conditions. However, both saccharin and water vehicle conditions also acted as strong reinforcers in these studies. Further studies utilising a behavioural economic approach were conducted in order to delineate the reinforcing effects of MDMA from that of its parent vehicle. In addition, demand-curve analysis using both the Linear-Elasticity model (Hursh et al., 1988, 1989) and the Exponential Model of Demand (Hursh & Silberberg, 2008) were compared in order to evaluate each model and assess the relative reinforcing efficacy of oral MDMA. Demand curves for the oral self-administration of MDMA revealed that responding for MDMA was more elastic (lower P_{max}) than responding for saccharin-alone indicating that saccharin functioned as stronger reinforcer than did MDMA+saccharin. The results

of these studies provide evidence for the positive-reinforcing effects of MDMA when it is delivered via the oral route of administration, however, the relative reinforcing efficacy of orally delivered MDMA appears to be low.

ASSESSMENT OF THE REINFORCING PROPERTIES OF ORALLY ADMINISTERED MDMA ('ECSTASY') IN RATS

The popular club drug 3,4-methylenedioxymethamphetamine (MDMA) or 'ecstasy' is a prominent drug that continues to be abused around the world. The United Nations Office on Drugs and Crime (UNODC) estimated that in 2008 there were between 10.5 and 25.8 million users of ecstasy and related compounds (MDA, MDEA, MDA) accounting for approximately 0.2 to 0.6% worldwide prevalence in the 15-64 age bracket (World Drug Report 2010, UNODC). MDMA use in New Zealand appears to be of particular concern as the Oceania region accounts for the highest percentage prevalence rates of ecstasy-group drug use in the world with 3.6% to 4% of people aged 15-64 estimated to have used ecstasy in the past year (World Drug Report, 2010, UNODC). From 1998 to 2006 MDMA use in New Zealand increased with the percentage of people reporting previous use increasing from 3.1 to 8.0%. Users reporting MDMA use in the past year rose from 1.5 to 3.9% across the same time span (Wilkins & Sweetsur, 2008). The continued increase in popularity of MDMA as a drug of abuse has resulted in parallel increases in research into the addictive and long-term consequences of MDMA use (Green, 2004).

Never intended as a recreational drug, MDMA was first synthesized by pharmaceutical company Merck in 1912. MDMA was originally patented as part of a group of chemical intermediates in the syntheses for a novel clotting agent (Freudenmann, Öxler & Bernschneider-Reif, 2006). It was not till the early 1970's that MDMA re-emerged as a therapeutic agent (as an adjunct to psychotherapy), and also for it's non-therapeutic recreational effects (Grinspoon & Bakalar, 1986). MDMA is one of a number of 'club drugs' also including GHB, flunitrazepam

('roofies'), ketamine and LSD, so called due to their high frequency of use at night clubs and all night dance parties known as 'raves' (Teter & Guthrie, 2001). MDMA further rose to prominence in the 1980's and 1990's as a drug associated primarily with the rave scene (Parrott, 2001). MDMA was originally known as 'empathy' due its related psychoactive properties, though it later acquired the name the 'ecstasy', it's most common name (Parrott, 2001). In addition, MDMA has also been known by various other names including, E, X, XTC, adam and the 'love drug' (Freye, 2009; Smith, Larive & Romanelli, 2002).

In 1985 the US Drug Enforcement Agency used its emergency powers to classify MDMA as a Schedule I drug reserved for those drugs that have high abuse liability and no confirmed therapeutic actions (Green, Mechan, Elliott, O'Shea & Colado, 2003; Green, 2004; Parrott, 2001). In New Zealand MDMA is classified as a Class B drug with high risk of harm (Misuse of Drugs Act, 1975). Despite this, MDMA use continues to grow worldwide.

Ecstasy is primarily consumed in one or more oral doses most often in pill form, though it also available in capsules or as powder (Smith et al., 2002; Teter & Guthrie, 2001). The main active ingredient in ecstasy is MDMA and a typical pill contains approximately 80-150mg, though purity, doses and presence of other active ingredients can vary by pill type (Teter & Guthrie, 2001; Green et al., 2003).

Generally, MDMA has been considered a 'safe' drug not only by users but also the general public (Timár, Gyarmati, Szabó & Fürst, 2003). Part of this misconception may stem from the relatively low reports of MDMA-related death, for example, despite an estimated 500,000 people taking Ecstasy in the UK on any given weekend estimates for MDMA-related deaths are estimated to be just 12 per year (Green et al., 2003). Using a sample collected directly from a nightclub rave, Yacoubian and colleagues found that ecstasy non-users were more likely to

perceive risk associated both long-term and short-term use of MDMA than were past-year MDMA users (Yacoubian, Boyle, Harding & Loftus, 2003).

The perception of safety related to MDMA use is in stark contrast to the scientific evidence indicating that MDMA can cause long-term damage to serotonin neurons in a variety of experimental species including rats (Broening, Bowyer & Slikker, 1995; Scanzello, Hatzidimitriou, Martello, Katz & Ricaurte, 1993; Schenk, Hely, Lake, Daniela, Gittings & Mash, 2007), guinea pigs (Saadat, Elliott, Colado & Green, 2004) and non-human primates (Scheffel et al. 1998; Hatzidimitrou, McCann & Ricaurte, 1999). Studies conducted using rodents have indicated some recovery of function of the serotonin system over time with levels returning to near baseline levels after 52 weeks (Scanzello et al., 1993). However, deficits in non-human primates have been shown to persist for as long as seven years raising concerns about the severity of MDMA-induced serotonin neurotoxicity in humans (Hatzidimitriou et al., 1999). The evidence for MDMA-induced serotonin neurotoxicity in humans is less clear (Curran, 2000; Steele, McCann & Ricaurte, 1994), however studies conducted with positron emission tomography (PET) have shown decreased serotonin transporter binding in MDMA users compared with controls (McCann, Szabo, Scheffel, Dannals & Ricaurte, 1998).

Pharmacology of MDMA

MDMA is a ring-substituted amphetamine derivative that is structurally similar to CNS stimulants like methamphetamine, and hallucinogens such as mescaline (Schmidt, Leven & Lovenberg, 1987; Steel et al., 1994; Green et al., 2003, Farré et al., 2004). However, due to the unique properties of MDMA it cannot be classified as either “a true hallucinogen nor a potent stimulant” (Stone, Stahl, Hanson & Gibb, 1986, p.41). Instead the term ‘entactogen’ has been proposed as a new class of

drug to describe the effects of MDMA (Nichols, 1986; Oberlender & Nichols, 1988; Vollenweider, Gamma, Liechti & Huber, 1998; Morgan, 2000; Liechti & Vollenweider, 2001). Nichols reports that the term 'entactogen' relates to its ability to produce "a touching from within" (Nichols, 1986, p.308), a desirable trait initially reported to be of interest for therapeutic use.

Depending on drug doses and metabolism the acute effects of MDMA generally manifest between 30-60 minutes after ingestion and the peak effects are seen approximately 60-120 minutes after ingestion (Kolbrich et al., 2008b). The effects of a typical single oral dose of 80-150 mg of MDMA last for approximately 3-5 hours (Liechti & Vollenweider, 2001; Green et al., 2003).

Subjective effects of MDMA include a feeling of 'closeness' to others (Peroutka, Newman & Harris, 1988) and an increased state of well-being, happiness, extroversion and sociability (Liechti, Gamma & Vollenweider, 2001). MDMA also has euphoric properties producing a 'high', like many other CNS stimulants (Liechti & Vollenweider, 2001). MDMA does not produce classic hallucinations like other psychotropic drugs; rather users report an altered emotional consciousness. However, some individuals experience visual hallucinations, though these hallucinations are typically not well formed (as compared with other prominent psychotropic drugs). These hallucinations tend to manifest as flashes of light, colours and patterns serving to provide increased vividness and distortion for the user (Peroutka et al.; Liechti et al. 2001).

Physiological effects of MDMA include increases in heart rate, body temperature, and in both systolic and diastolic blood pressure. (Kolbrich et al., 2008b; Vollenweider et al. 1998). While modest effects on body temperature have been found in controlled human studies, the effects on temperature are almost certainly impacted by the conditions in which MDMA is generally taken; most usually hot

nightclubs or ‘raves’ with non-stop dancing, concurrent use of other drugs/alcohol and often-times poor access to hydration (Green, O’Shea & Colado, 2004). Tachycardia, jaw clenching and teeth grinding (bruxism), lack of appetite, difficulty concentrating, impaired balance, insomnia, forgetfulness and dry month/thirst have also been commonly reported symptoms of MDMA ingestion (Peroutka et al., 1988; Vollenweider et al. 1998).

Cellular mechanisms of MDMA action

MDMA is a potent indirect monoamine agonist and reuptake inhibitor that results primarily in the release of 5-HT and dopamine in the brain (Parrot, 2001). The primary acute effects of MDMA are thought to be mediated through MDMA’s affinity with the presynaptic serotonin transporter (SERT), resulting in reversal of the SERT and MDMA/5-HT exchange, thus causing a 5-HT efflux (Rudnick & Wall, 1992). Increased synaptic 5-HT has been shown to correlate with the mood altering and physiological effects of MDMA as shown by attenuation of those effects after blockade of the SERT with the 5-HT reuptake inhibitor citalopram (Liechti, Bauman, Gamma & Vollenweider, 2000; Liechti & Vollenweider, 2001). In addition, MDMA has moderate direct affinity for post-synaptic 5-HT2 receptors (Battaglia, Brooks, Kulsakdinun & De Souza, 1988) that have been implicated in the hallucinogenic properties of MDMA (Liechti & Vollenweider, 2001). Activation of the 5-HT2 receptors has also been implicated in dopamine efflux through a modulatory effect (Green et al., 2003).

Though to a lesser extent than serotonin, MDMA is also a potent indirect dopamine agonist both in vitro (Johnson, Hoffman & Nichols, 1986) and in vivo (Yamamoto & Spanos, 1988). Antagonism of the dopamine D2 receptor with haloperidol was shown to attenuate the euphoric effects of MDMA implicating dopamine’s role in the

'high' experienced after MDMA administration (Liechti & Vollenwieder, 2000; 2001). Dopamine release has also been linked with the stimulant-related effects of MDMA such as its locomotor activating (Spanos & Yamamoto, 1989) and reinforcing effects (Daniela, Brennan, Gittings, Hely & Schenk, 2004).

The second phase of MDMA's action results from an interaction between the depletion of vesicular 5-HT and deactivation of the enzyme tryptophan hydroxylase (TPH) (Schmidt & Kehne, 1990). Reversal of the SERT results in the massive acute release of vesicular 5-HT into the synapse, but also prevents deactivation via reuptake into the presynaptic terminal. Further depletion of 5-HT and its metabolites occurs due to deactivation of 5-HT's rate-limiting enzyme, TPH, therefore preventing further synthesis of 5-HT. The resulting depletion of 5-HT and its metabolites may be a factor in the subacute symptoms found after MDMA administration, such as drowsiness, muscle aches, difficulty concentrating and depression (Peroutka et al., 1988) as well as lack of appetite, lethargy, thirst and insomnia (Vollenweider et al., 1998).

Abuse Potential of MDMA

DSM-IV criteria for drug dependence contains features including the development of tolerance, manifestation of withdrawal symptoms, maintenance or escalations of drug use, increased drug-seeking behaviours and a lose of control of drug intake (Bickel, Madden & Petry, 1998). MDMA use (like that of other drugs of abuse) has been shown to meet the criteria for dependence and abuse according to DSM-IV criteria. For example, Cottler, Womack, Compton and Ben-Abdallah (2001) studied ecstasy use in adolescents and young adults and found that 30% of their sample had used ecstasy more than 5 times (the inclusion criteria for the study). Of those individuals 43% met the DSM-IV criteria for dependence and 34% met the criteria

for abuse. Crucially, all of the respondents reported either tolerance (35%) or withdrawal (59%) after MDMA use.

A key feature of a drug having abuse potential lies in its ability to act as a reinforcer (i.e. maintain behaviours that lead to its delivery). Primarily the subjective effects of drugs of abuse can act as positive reinforcers, but note that drug use may also be maintained by negative reinforcement contingencies such as removing unwanted withdrawal symptoms (Koob & Le Moal, 1997).

Tancer and Johanson (2003) measured the reinforcing subjective effects of MDMA by using a multiple choice procedure (MCP) in humans by asking participants to choose between the current dose of MDMA they had administered and a range of dollar values. MDMA produced dose-dependent increases in the dollar amount needed to switch from drug to money (the crossover point) compared with placebo. Participants in Tancer and Johanson's study also reported 'liking' MDMA more than both amphetamine and mCPP (a 5-HT agonist).

Greenwald (2008) re-evaluated the results Tancer and Johanson (2003) as part of a wider reanalysis of MCP data in terms of Behavioural Economic demand curves. Briefly, behavioural economics applies aspects of consumer demand theory to the experimental analysis of behaviour (Lea, 1978; Hursh, 1980, 1984; Greenwald, 2008). Demand for a good (reinforcer) reflects the price (effort required) in order to obtain that good. Demand curves plot *consumption* (reinforcers earned) as a function of *price* (effort required per unit of consumption) and typically produce a non-linear function that exhibits decreased consumption as a function of increased price. Demand curves (when plotted in logarithmic space) allow for the analysis of *elasticity of demand* or the sensitivity of consumption to changes in price. Elasticity of demand spans a continuum from *inelastic* demand, where prices rises are met with increased effort or expenditure in order to maintain access to the commodity, to

elastic demand where consumption decreases more than the proportional change in price. Importantly, different commodities (including drugs of abuse) differ in elasticity of demand. In essence those commodities that are defended more strongly represent greater inelasticity and stronger reinforcers relative to those with lower elasticity. For a more comprehensive discussion of Behavioural Economics see Chapter 4. Greenwald compared demand functions for several opiates and found that rank order inelasticity was highest for fentanyl followed by hydromorphone (in heroin users), methadone and hydromorphone (in heroin abstainers), respectively. When comparing psychostimulant drugs rank order inelasticity was *d*-amphetamine, MDMA, followed by MDMA + fluoxetine indicating that d-amphetamine required higher dollar amounts to be offered before switching preference from drug to money. The difference between MDMA and MDMA + fluoxetine conditions suggests that the addition of fluoxetine decreased the subjective value of the MDMA and subjects were likely to choose lesser dollar amounts before switching preference from drug to money.

Similarly, Sumnall and colleagues investigated hypothetical drug purchases in polysubstance drug abusers (Sumnall, Tyler, Graham & Cole, 2004; Goudie, Sumnall, Field, Clayton & Cole, 2007). They found that drug purchases for cocaine, amphetamine and ecstasy were elastic, while only demand for alcohol was inelastic. The authors suggest that alcohol was the preferred drug of choice of the polydrug user population they sampled as evidenced by inelastic demand. That is, demand for alcohol was defended by higher expenditure as price increased. Interestingly, when the price of ecstasy was increased, cocaine choices increased suggesting that cocaine acts as a substitute to MDMA when prices increase (and vice versa). The polydrug user population studied by Sumnall et al. indicated that MDMA and cocaine substitute for one another suggests that MDMA likely produces reinforcing effects similar to cocaine, a drug with well-known abuse potential. In a follow up

study Goudie et al. manipulated perceived quality of drugs and income levels (amount of money available to buy drugs for a hypothetical night out), while keeping prices fixed at or near prices for drugs on the street (in keeping with the real world higher quality drug commanded higher relative prices). Alcohol, cocaine, cannabis and ecstasy produced income elastic choices for poor quality drugs, that is despite restricted income participants continued to choose lower (and average for some drugs) quality drugs. Choices were income elastic for all high quality drugs except for alcohol. In effect higher quality drugs were chosen when income was high, but not when income was set to a low value representing the fact that high quality drugs are a luxury good.

While the reinforcing subjective effects of MDMA appear to mirror the similar effects of other prominent drugs of abuse, MDMA does not appear to produce drug craving or physical dependence often associated with the addictive potential of other more prominent drugs of abuse (Parrott, 2001). But MDMA, like other drugs of abuse such as cocaine and amphetamine does produce tolerance and dosage escalation (Parrott, 2001). Patterns of use of MDMA seem to change as a function of experience with the drug. While first time users typically start with a single or even half a tablet experienced users will often take multiple tablets in a binge session (Hammersley, Ditton, Smith & Short, 1999). Binging can be achieved either via 'stacking' (i.e. taking multiple doses at once) or 'boosting' (first taking a single pill, and another several hours after the first) or a combination of both (Hammersley et al.; Parrott, 2005). In a referral-type sample of ecstasy users, Hammersley et al. found significant variation in MDMA use allowed for subjects to be categorised as 'light', 'medium' or 'heavy' users. Light users reported MDMA use 'less than monthly', medium users reported 'more than monthly but less than weekly', while heavy users reported 'more than weekly but less than daily'. Heavy users were more likely to engage in binging than light users were with 76% of heavy users

reporting binging on MDMA compared with just 16% of light users. The incidence of increased binge dosing and escalation suggests that tolerance to the drug is occurring. Indeed, users self-reports have suggested than the positive effects of MDMA decline with increased usage, while the negative effects become more prominent (Petrouka et al., 1988). Irvine et al., (2006) found evidence for tolerance to MDMA's sympathomimetic and behavioural effects in a study that collected pharmacokinetic and physiological data before and after subjects attended a 'dance party'. Those who were experienced users showed blood concentrations of MDMA in the range of 'toxic to lethal', however, reported little to no adverse side effects.

While direct evidence for the abuse potential of MDMA in humans remains tentative, substantial evidence for its abuse potential comes from animal models, particularly drug self-administration (Schenk, 2009). The self-administration procedure provides drug reinforcers (typically delivered through intravenous catheters) contingent upon operant responding. Acquisition and maintenance of drug self-administration reflects the ability of the drug to act as a reinforcer and has been demonstrated across a wide variety of abused drugs (Spealman & Goldberg, 1978). The reinforcing effects of MDMA have been demonstrated by the self-administration procedure where it has been shown to initiate and maintain lever responding in a variety of species including, non-human primates, rats and mice (for details, see Chapter 3, Table 3.1).

In addition, MDMA has been shown to reliably produce conditioned place preference (CPP) (Cole, Sumnall, O'Shea & Marsden, 2003; Meyer, Mayerhofer, Kovar & Schmidt, 2002; Schechter, 1991; see Tzschentke, 1998, 2007 for a comprehensive review on CPP). MDMA administration also lowers the reward threshold for electrical brain stimulation; a model of the euphoria inducing effects of drugs of abuse (Hubner, Bird, Rassnick & Kornetsky, 1988).

The similarities between MDMA and other more prominent drugs of abuse suggest that MDMA has significant abuse potential. However few studies have attempted to quantify the relative abuse potential of MDMA using animal models. Animal models allow for assessment of abuse potential by comparing the strength of a drug reinforcer with that of other reinforcers, a measure known as relative reinforcer efficacy. Drugs can then be ranked as a function of their relative reinforcer efficacy; with those drugs that rank higher having more potential for abuse and thus more likely to lead to potential addiction.

This thesis addressed several gaps in the current literature concerning the study of MDMA. To date the reinforcing effects of MDMA as an oral drug have often been overlooked in the literature. Animal models used for assessing the reinforcing effects of MDMA have almost exclusively been studied using parenteral routes of administration such as intravenous (*iv*) (in self-administration studies), intraperitoneal (*ip*) or subcutaneous (*sc*) injection (in conditioned place preference). In particular, the differences between *iv* and oral administration have the potential to produce markedly different profiles of the reinforcing effects of MDMA (see Chapter 2). As MDMA is consumed almost exclusively in pill form in humans, studies of the effects of oral doses are prudent in order to better understand the human condition with regard to MDMA's reinforcing and addictive properties. The first goal of this thesis was to establish a paradigm with which the oral effects of MDMA can be studied. Methods generally used for the study of alcohol reinforcement in rats were adapted in order to accomplish this goal. The second objective of this thesis was to examine the abuse liability of MDMA through quantitative methods. Relative reinforcing efficacy of a drug can be considered synonymous with its potential for abuse. Relative reinforcing efficacy can be studied by utilizing an economic framework and examining changes in consumption (reinforcers consumed) as a function of changes in price (response requirement). The field of behavioural

economics provides two theoretical models with which relative reinforcing efficacy can be quantified.

Chapter 2 ROUTE OF ADMINISTRATION

While all psychoactive compounds have their primary effects in the Central Nervous System (CNS) the route with which those compounds reach the CNS can produce profound differences in the effects of those compounds (Farré & Camí, 1991). Route of administration affects the speed and the efficacy of a drug during its passage across the blood brain barrier into the brain. The most common routes of administration human drug use are intravenous (*iv),* inhalation, intranasal (e.g. snorting of cocaine) and ingestion (oral or *po*). It is common for most drugs to be confined to a single route of administration but several drugs are known to be used via multiple routes leading to very different abuse and efficacy profiles (e.g. methamphetamine can be taken orally, injected intravenously, snorted or smoked all producing distinct pharmacological and pharmacokinetic effects (de la Torré et al., 2004).

A drug's effectiveness is a function of the combination of its pharmacodynamic and pharmacokinetic parameters, however, route of administration can also serve to modulate the pharmacokinetic parameters for a given drug (Farré & Camí, 1991; Smith, Jones & Walker, 1996). The route of administration is an important determinant of a drug's effect as it will change not only the rate of absorption, but also the metabolism of that drug based on the site of absorption (Strang et al., 1998). This in turn will affect the drug's bioavailability or the proportion of the active drug circulating in the systemic system. For example, first pass metabolism in the liver and gut after oral ingestion will decrease bioavailability relative to *iv* administration that results in almost instantaneous absorption and a fast onset of action (Farré & Camí, 1991). Route of administration can also have dramatic effects on the onset of action of a drug and will affect the time taken to reach peak

plasma concentration (T_{max}) and peak plasma concentration (C_{max}) (e.g. Baumann et al., 2009).

MDMA Pharmacokinetics

MDMA is metabolised through two major pathways. Firstly, MDMA is metabolised to HHMA by the enzyme CYP2D6 in humans or the similar enzyme CYP2D1 in rats (Baumann et al., 2009). HHMA is then subsequently metabolised to HMMA by the enzyme COMT. The minor secondary pathway involves metabolism of MDMA to its active metabolite MDA and subsequent metabolism to HHA and HMA by CYP2D6 and COMT respectively (Baumann et al., 2009; de la Torré et al., 2000; Lim & Foltz, 1988). The major metabolism pathways for MDMA in rats and humans are shown in Figure 2.1 (Baumann et al., 2009). Oral administration of MDMA in human participants results in detectable plasma levels 15-30 minutes after ingestion (Kolbrich et al., 2008a). Studies have consistently found increased area under the concentration time/curve (AUC) more than the proportional increase in dose suggesting that MDMA exhibits non-linear pharmacokinetics in humans (de la Torré et al., 2000; Farré et al., 2004; Kolbrich et al., 2008b), non-human primates (Mechan et al., 2006) and rats (Baumann et al., 2009). For example, de la Torre et al. (2000) tested a range of MDMA doses from 50 to 150mg in human volunteers and found that maximum concentration (C_{max}) and AUC for MDMA increased as a function of dose. Despite the three fold difference between the 50 and 150mg doses the authors report an increase in AUC greater than 10 fold. In addition, Kolbrich and colleagues (2008b) found that 82% of participants had detectable levels of MDMA 47 hours after receiving a high dose (1.6mg/kg) of MDMA; in those same subjects only 23.5% showed detectable levels of MDMA after a low dose (1.0mg/kg) after the same duration. Time to maximum concentration (T_{max}) for MDMA was 2.4 hours and was similar across both high and low doses. However, C_{max} was significantly higher for the high dose than it was for the low

dose. It has been suggested that the non-linear pharmacokinetics of MDMA are a result of auto-inhibition of the enzyme CYP2D6 by MDMA at high doses; which in turn results in decreased metabolism of MDMA and MDA (Baumann et al, 2009; de la Torré et al, 2000; Kolbrich et al, 2008b). The auto-inhibition of CYP2D6 by MDMA is thought to occur via formation of a metabolic inhibitory complex that starts within an hour of ingestion. The deficit in levels of the enzyme CYP2D6 may be long lasting with up to 10 days required before a return to basal levels of CYP2D6 (Yang et al., 2006). The inhibition of the metabolism of MDMA may have direct consequences for those who use MDMA regularly, particularly those who are frequent users or those who 'stack' or 'boost' multiple doses of MDMA. Farré et al. (2004) examined the effects of repeated doses of MDMA on the pharmacokinetics of MDMA in human participants. Subjects were given two 100mg doses of MDMA separated by 24 hours. Results showed that plasma concentrations were increased by 77% and C_{max} was increased by 29% compared to the first dose, suggesting inhibition of MDMA metabolism, rather than just simple accumulation of drug in the system. Similarly, Mechan et al. (2006) studied the effects of multiple oral doses of MDMA in squirrel monkeys and found evidence for non-linear pharmacokinetics of MDMA after both single doses as well as multiple doses of MDMA delivered three hours apart.

Baumann and colleagues have recently systematically characterised the effect of route of administration on the pharmacokinetics of MDMA in rats. Baumann et al. (2009) administered either a low (2 mg/kg) or high (10 mg/kg) dose of MDMA to rats via either the *ip*, *sc* or *po* routes of administration. Results indicated non-linear pharmacokinetics for all three routes of administration due to larger than proportional increases in C_{max} for the high dose compared with the low dose. C_{max} values varied as a function of route of administration with *ip* producing greater C_{max} values than *sc* and *po* respectively. In addition, both low- and high-dose oral

administration showed significantly decreased AUC compared with the *ip* and *sc* routes of administration. T_{max} varied as a function of route of administration with *sc* administration producing the slowest time to maximum concentration compared with the *ip* and *po* routes. The authors suggest that oral MDMA is likely subject to decreased absorption as well as first pass metabolism in the liver or gut which results in decreased blood levels of MDMA. The decrease in circulating MDMA will result in less centrally active MDMA and thus produce marked differences in its action when delivered via the oral route. It has been suggested that in rats MDMA survives mostly untouched after enzymatic degradation in the liver, such that the majority reaches the bloodstream (Finnegan et al., 1988).

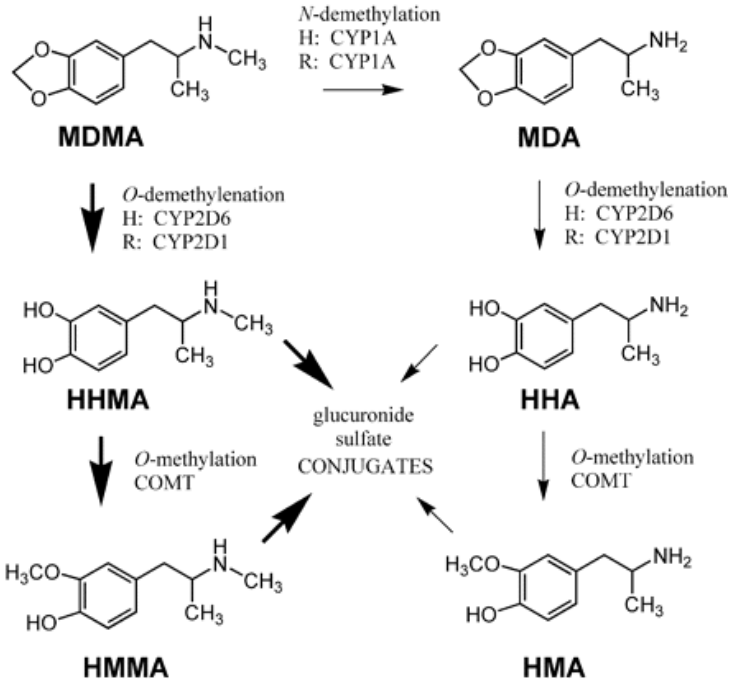

Figure 2.1: Metabolism of 3,4-methylenedioxymethamphetamine in humans and rats (Baumann et al., 2009).

Despite delays in onset of action and decreased absorption of MDMA evident in pharmacokinetic analysis of blood metabolites there still appears to be significant behavioural and pharmacological effects resulting from administration of oral

MDMA. For example, Finnegan et al. (1988) found that MDMA produced equivalent depletions of 5-HT in the hippocampus regardless of whether either the oral or subcutaneous route of administration was used. However, using primates Ricaurte and colleagues showed that the oral route of administration resulted in decreased 5-HT depletion relative to *sc* administration (Ricaurte, DeLanney, Irwin & Langston, 1988). Baumann et al. (2009) found no effects on either cortical or striatal 5-HT levels two weeks after either a high (10 mg/kg) or low (2 mg/kg) doses of MDMA given to rats, irrespective of the route of administration used (either *ip*, *sc*, or *po*).

A limited number of studies have used behavioural assays in order to examine effects across a range of routes of administration. Measurement of locomotor activity permits the analysis of MDMA's efficacy as function of route of administration as well as the timecourse of MDMA's locomotor activating effects. Many studies have shown that administration of MDMA results in dose-dependent increases in locomotor activity (for examples see, Gold & Koob, 1988; Spanos & Yamamoto, 1989; McNamara, Kelly, & Leonard, 1995; Daniela, Brennan, Gittings, Hely & Schenk, 2004). However, relatively few studies have tested MDMA-induced hyperactivity as a function of route of administration. De Souza and colleagues found that oral MDMA produced dose dependant increases in locomotor activity after oral administration (De Souza, Kelly, Harkin & Leonard, 1997). The highest locomotor activity scores were noted for 20 mg/kg MDMA with subsequent decreases in locomotor activity after 40 and 80 mg/kg doses. This result may reflect increased occurrences of serotonin syndrome symptoms apparent after high MDMA dose administration (Spanos & Yamamoto, 1989); most notably low body posture and head weaving that may be mutually exclusive with the forward locomotion associated with stimulant-type induction of hyperactivity. In addition, De Souza and colleagues showed significant increases in temperature and lethality as a function of increasing oral doses of MDMA indicating that MDMA has significant

pharmacological actions even when delivered orally. However, the dose range used is their study was extremely high (20 – 320 mg/kg MDMA *po*) and no comparisons were made between the oral route and other common routes of administration typically used in animal models. Crean, Davis and Taffe (2007) measured the effect of high (5 mg/kg) and low doses (1.78 mg/kg) of MDMA delivered via either the *im* or *po* routes of administration on homecage activity in rhesus monkeys. They found homecage activity was decreased in the high dose *im* condition. Activity was decreased after high dose oral administration, but to a lesser extent than that of the intramuscular route suggesting that there are route dependent effects of MDMA on locomotion in non-human primates. However, the results from Crean et al.'s study showed a decrease in locomotor activity in contrast with increased locomotion seen in rat studies; although it must be noted that the procedure for measuring locomotion in rats and primate studies vary substantially. The rhesus monkeys studied by Crean et al. were confined to their homecages during testing and had transmitters surgically implanted in order to measure gross motor movements. In contrast, rats are typically tested in novel open field chambers and locomotion is measured via the use of infrared beam breaks. Despite differences in the behavioural assay across species Crean et al. showed that oral MDMA was less effective than intramuscular administration across the same dose range indicating that despite the contrasting behavioural effects across species the MDMA was less efficacious when delivered via the oral route.

The pharmacokinetic effects of route of administration on MDMA and its metabolites have recently been systemically studied (Baumann et al., 2009), however locomotor activity has not as yet been examined in this context. The following study was designed to test the effects of route of administration on MDMA-induced hyper-locomotion. The effects of MDMA administered via either the intraperitoneal, subcutaneous or oral route were examined in order to measure total activity, time to

peak activity and the time course for each route of administration. Although this initial study did not assess the reinforcer effects of MDMA, the open field locomotor paradigm provided a rapid method which to quantify the magnitude and time course of the effects produced by MDMA as a function of route of administration.

Experiment 2.1: Effect of Oral, Subcutaneous and Intraperitoneal Administration of MDMA on Locomotor Activity

Method

Subjects:

Subjects were 91 naïve male Sprague-Dawley rats weighing between 250-300 grams bred in the Victoria University Animal Facility. Animals were housed in polycarbonate cages with cage tops made of wire mesh with free access to food and water, except during experimental sessions. Animals were maintained in a temperature controlled environment with ambient temperature 21°C and 70% humidity and a 12:12 hour light/dark cycle, lights on at 7am. Research was approved and animals were treated in accordance with ethical guidelines set forth by the Victoria University of Wellington Animals Ethics Committee.

Apparatus/materials:

Equipment:

Experiments were run in eight ENV-515 Open Field Test chambers (Med-Associates Inc, Vermont, USA) used to measure rat locomotor activity. Chambers were 45 cm x 45 cm with white acrylic floors and clear Perspex walls 30 cm high. Each chamber was housed in a sound and light attenuating cubicle. Infrared light beams equally spaced and positioned 1.5 cm above the floor formed a 16 by 16 lattice. Experiments were controlled by Activity Monitor 5 software using the following parameters, box size 3; resolution 100ms; resting delay 1000ms (Med Associates, Vermont, USA). During behavioural tests a white-noise generator external to the cubicles was used to mask any ambient noise.

Solutions:

+/-3,4-methylenedioxymethamphetamine hydrochloride (ESR, Porirua, New Zealand) for injections were made at doses of 0, 10, and 20mg/ml in a vehicle of sterile 0.9% physiological saline. Injections were administered either *sc* or *ip* at 1ml/kg of bodyweight. MDMA HCl for oral gavage was prepared at doses of 0, 10 and 20mg/ml in a vehicle of dH_2O. Gavage was administered *po* at 1ml/kg of bodyweight. All drug doses refer to the salt.

Procedure:

In order to decrease distress related to administration method animals' were habituated to oral gavage or injection in the home cage with vehicle administration five days prior to commencing behavioural testing. Vehicle administration continued for three days.

On the test day, animals were transported to the testing room and placed inside the locomotor activity chambers. Animals were left in the chambers for a habituation period of 30-minutes during which time the activity monitoring software was active and white-noise generator was on. At the end of the habituation period each animal was given either an injection (*ip* or *sc*) or oral gavage before being placed back into the chamber. Following injections/gavage behaviour was recorded for a further 60 minutes.

Animals were randomly assigned to one of nine different drug-administration groups. Groups and subject numbers are detailed in Table 2.1.

Table 2.1: Experiment 2.1 conditions

Route of Administration	Dose (mg/kg)	Subjects numbers (*n*)
Oral (*po*)	20.0	12
	10.0	6
	0.0	6
Intraperitoneal (*ip*)	20.0	10
	10.0	9
	0.0	12
Subcutaneous (*sc*)	20.0	12
	10.0	12
	0.0	12

Data Analysis:

Locomotor activity data were collected and analysed in 5-minute time bins. Briefly, animals were considered ambulatory if they moved at least three beam breaks away from their current position in less than specified resting delay (1000 ms). An ambulatory episode continued until such time as the animal failed to meet the above criteria. Ambulatory counts represent the sum of all X and Y beam breaks during ambulatory episodes. Binned activity data was calculated as ambulatory counts for that time bin averaged across all animals. Total activity scores represent the group average of the summed ambulatory counts across all time bins. Time to peak activity for each condition was determined by averaging the time at which peak ambulatory counts occurred for each subject. Obtained values represent the average time bin from which peak activity was recorded. The habituation period that consisted of the first 30 minutes of each session prior to drug treatment was discarded and not used in the subsequent analysis.

Results and Discussion

Figure 2.2 shows locomotor activity data for all conditions broken down as a function of route of administration. Figure 2.2 indicates that both 20 and 10mg/kg MDMA and all three routes of administration produced MDMA-induced hyper-locomotion. As expected the highest rates of locomotor activity were found for 20mg/kg MDMA for each of the three routes of administration tested (Figure 2.2 top panel). In general, total activity counts were dose dependent with higher doses producing higher total activity than lower doses, with the exception of the *ip* route of administration which produced similar behaviour for both the 20 and 10mg/kg doses of MDMA. Figure 2.2 (bottom panel) shows that 20mg/kg MDMA produced a similar increase in activity for both the *ip* and *sc* routes of administration, which were in turn higher than activity for the same dose when delivered via the *po* route. Overall the intraperitoneal route was the most effective at producing MDMA-induced hyper-locomotion at the doses tested, followed by the subcutaneous and oral routes of administration. A two-way ANOVA was conducted using factors of dose (3 levels: 0, 10 and 20mg/kg) and route of administration (3 levels: *ip*, *sc* and *po*) and found a significant interaction (F (4, 82) = 3.494, p = 0.011). However, the interaction was no longer significant when the each of the vehicle conditions was removed from the analysis, F (2, 55) = 0.636, p = 0.533). With the vehicle conditions removed the main effect of dose failed to reach significance (F (2, 55) = 3.393, p = 0.71), but there was however a significant main effect of route of administration (F (2, 55) = 12.310, $p < 0.01$). Tukey post-hoc analysis revealed that the oral route of administration produced significantly less locomotor activity than did either the subcutaneous or intraperitoneal routes of administration ($p < 0.01$). There was no significant difference between the *ip* and *sc* routes of administration.

Figure 2.3 shows the timecourse analysis as a function of MDMA dose for the *ip* (top panel), *sc* (middle panel) and *po* (bottom panel) routes of administration.

Figure 2.4 shows the timecourse of MDMA-induced locomotion as a function of dose. Figure 2.4 indicates that both the 20mg/kg *ip* (top panel) and 10mg/kg *ip* doses (middle panel) produced the highest peak activity and the highest time to peak activity (T_{max}) (see Table 2.2). The *sc* route of administration produced lower peak activity relative to the *ip* route but higher than the *po* route of administration; however, though overall activity was lower for *po* than it was for *sc*, the *po* route showed a lower T_{max} indicating a faster onset of action. Time to peak activity (T_{max}) data is shown in Table 2.2. A two-way ANOVA revealed a significant main effect of route of administration (F, (2, 55) = 3.766, p = 0.029) but not of dose (F (2, 55) = 0.385, p = 0.538) on T_{max}. The interaction term was not significant (F (2, 55) = 1.371, p = 0.262). Post-hoc analysis indicated that the subcutaneous route produced significantly slower time to peak activity than did the oral or intraperitoneal routes.

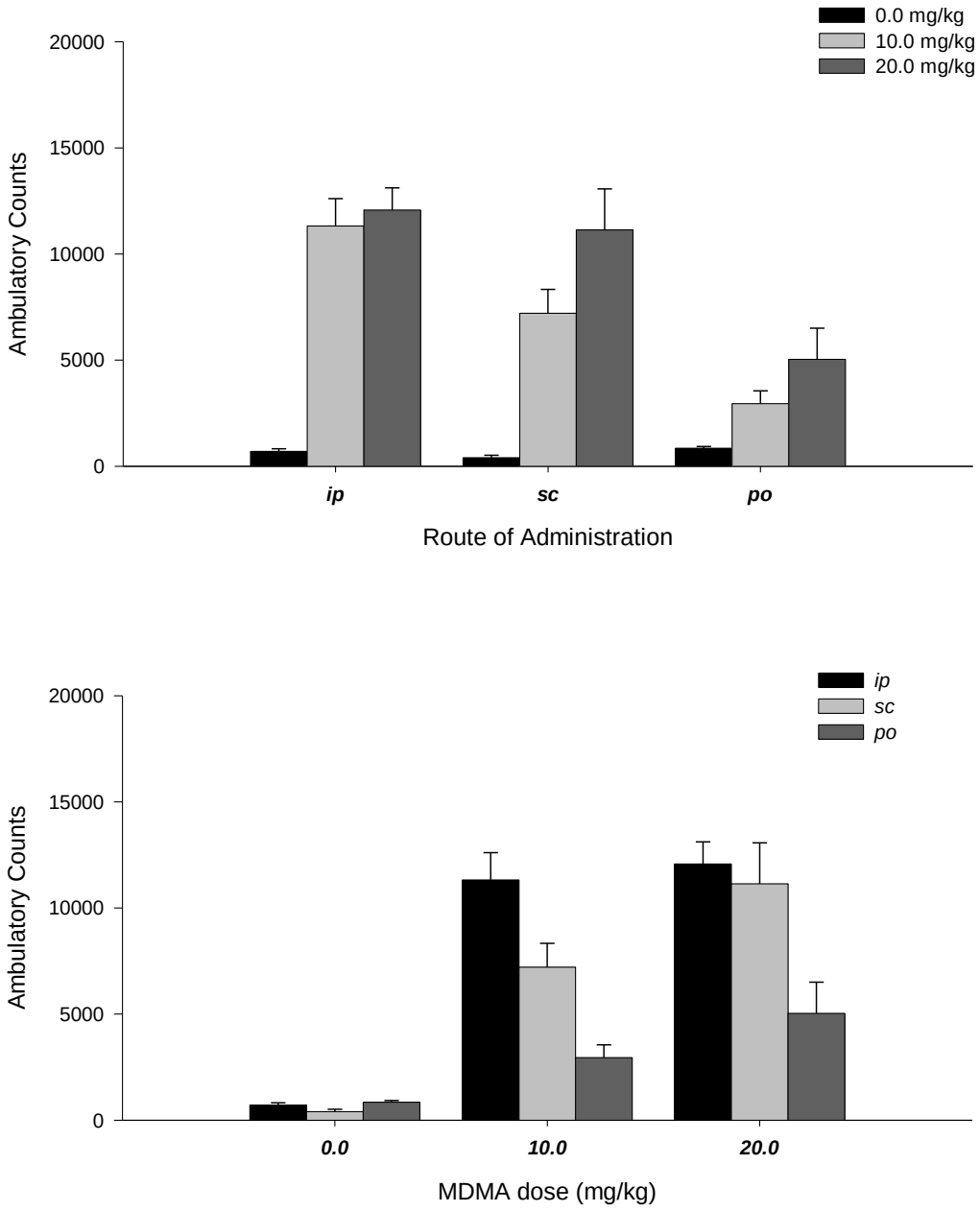

Figure 2.2: *(top panel)* Effect of route of administration on MDMA-induced hyper-locomotion in rats. Bars represent average total ambulatory counts (+SEM) as a function of dose for *ip*, *sc* and *po* routes of administration respectively. *(bottom panel)* Comparison of route of administration on MDMA-induced hyper-locomotion for vehicle, 10 and 20mg/kg MDMA doses. Error bars represent standard error of the mean.

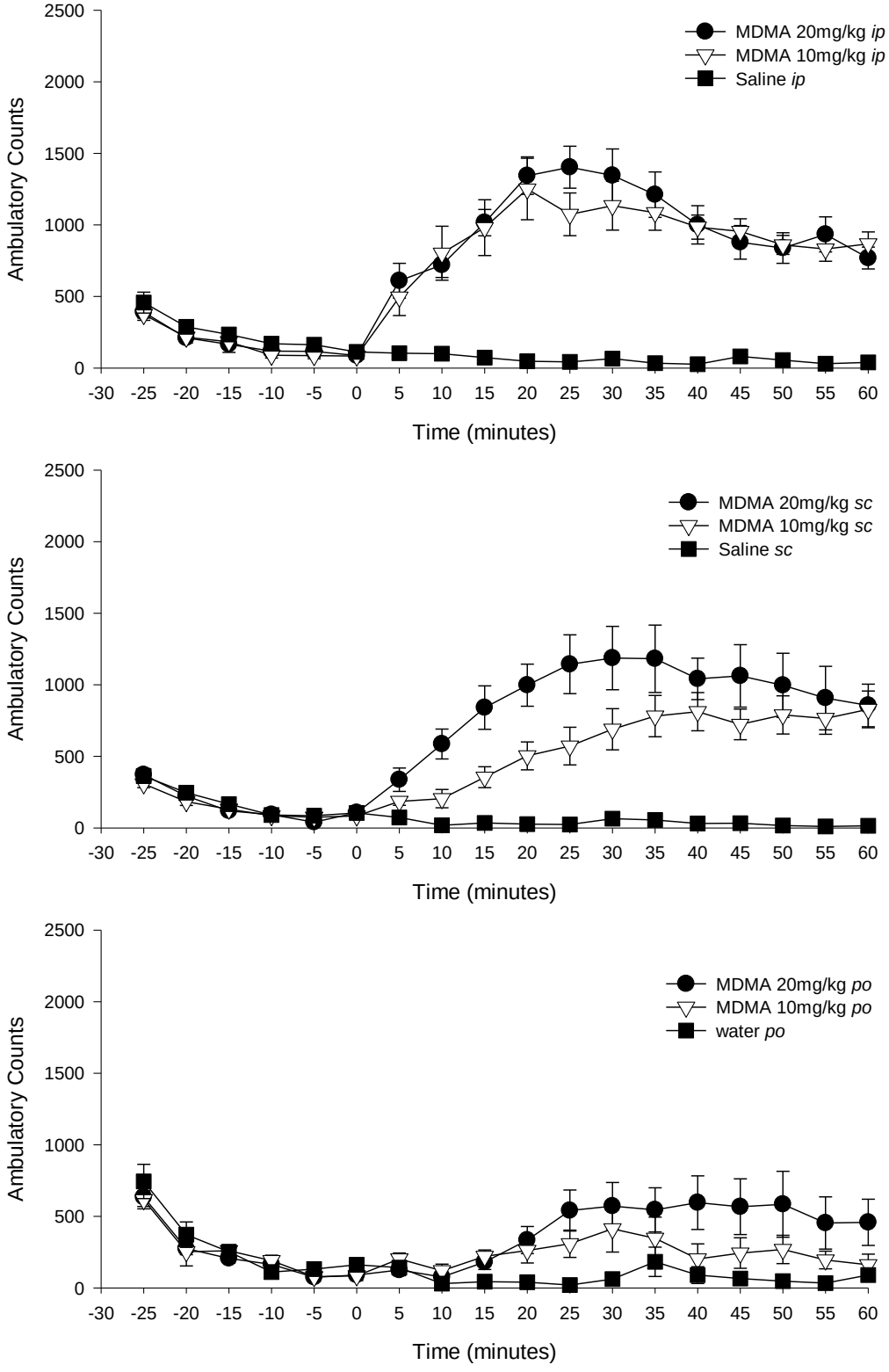

Figure 2.3: Timecourse for MDMA-induced locomotor activity for intraperitoneal (*ip) (top panel)*, subcutaneous (*sc) (middle panel)* and oral (*po) (bottom panel)* as a function of MDMA dose. Drug injections were delivered at timepoint zero. Error bars represent standard error of the mean.

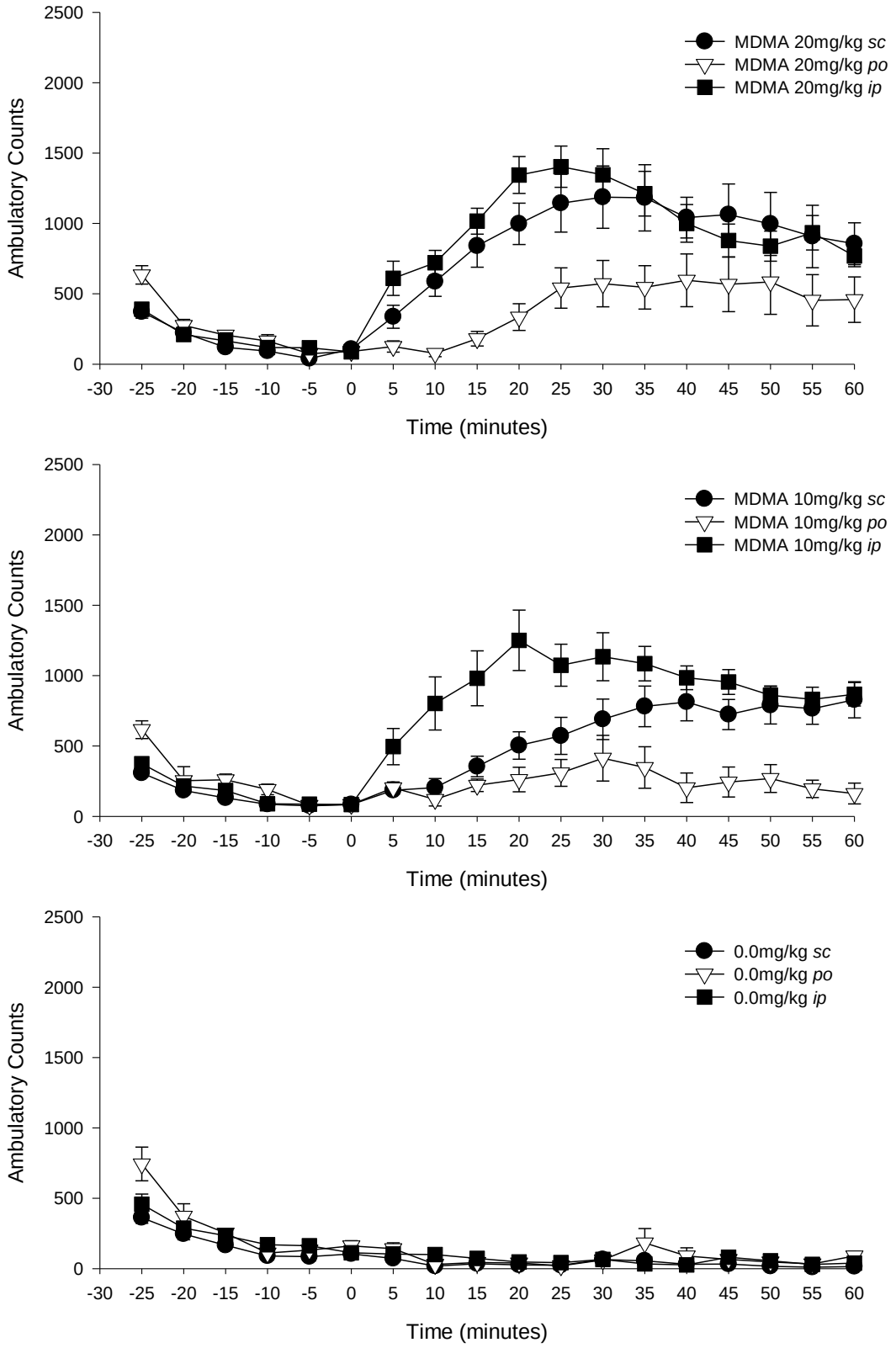

Figure 2.4: Timecourse for MDMA-induced locomotor activity for 20mg/kg *(top panel)*, 10mg/kg *(middle panel,)* and vehicle administration *(bottom panel)* as a function of route of administration. Drug injections were delivered at timepoint zero. Error bars represent standard error of the mean.

Table 2.2: Time to maximum activity (T_{max}) as a function of three routes of administration.

Route of Administration	Dose (mg/kg)	T_{max}	*SE*
Oral (*po*)	20.0	36.25	4.97
	10.0	31.67	8.43
Intraperitoneal (*ip*)	20.0	28.00	2.26
	10.0	29.44	4.03
Subcutaneous (*sc*)	20.0	35.42	2.98
	10.0	45.42	4.37

As expected both the route of administration and dose of MDMA had prominent effects on the locomotor activating effects of MDMA. The intraperitoneal route of administration produced both the highest overall locomotor activity counts as well as the highest peak locomotor activity. In addition, the *ip* route exhibited the fastest onset of action as measured by T_{max}. The subcutaneous route produced moderate locomotor activating effects, typified by a slower onset of action (T_{max}) that gradually increased over the timecourse measured (relative to the to the *ip* route). Though slower to reach peak activity the 20mg/kg *sc* dose of MDMA produced similar levels of total activity compared with the 20mg/kg *ip* dose of MDMA. This was not the case for the 10mg/kg dose of MDMA where the *sc* dose produced not only a slower onset of action but also lower total locomotor activity counts. The decreased locomotor activity for the 10mg/kg *sc* dose is consistent with a decrease in central bioavailability due to slower absorption and increased systemic metabolism. Notably this was not case for the 20mg/kg MDMA condition where both *ip* and *sc* routes produced similar activity levels suggesting a ceiling effect for the MDMA-induced hyperlocomotion at that dose.

The oral administration of MDMA resulted in dose dependent increases in locomotor activity when administered orally, however MDMA-induced hyperlocomotion was low when delivered orally compared with the *ip* and *sc* routes of administration.

High dose MDMA treatment (20mg/kg) produced modest increases in locomotor activity approximately 40-45 percent lower than either the *ip* or *sc* routes of administration. The 10mg/kg MDMA dose produced significant route-dependent effects with *ip* producing more total activity counts than the *sc* route and the oral routes respectively. The lower activity counts after oral administration are consistent with decreased bioavailability due to increased metabolism and absorption found in oral administration relative to the subcutaneous and intraperitoneal routes of administration. This decrease in central bioavailability results in less dopamine release resulting in lower overall MDMA-induced hyperlocomotion.

It was expected that the oral route of administration would result in the slowest onset of action due to slow passage from the stomach to the large intestine (the primary absorption site). Thus it was expected that the oral route would show the highest time to peak activity (T_{max}). However, this was not the case and in fact the subcutaneous route showed the highest T_{max} value, followed by the oral and *ip* routes respectively. Though it produced an attenuated effect on total activity counts compared with the subcutaneous route, the oral route was faster to reach its peak effects. This result corresponds with the research of Baumann et al. (2009) who found that the T_{max} value for MDMA plasma concentration was higher for the subcutaneous route than it was for *po* and *ip* routes for both high (10mg/kg) and low (2 mg/kg) doses. In addition, they found that C_{max} and *AUC* values for both high and low doses were highest for *ip*, followed by sc with *po* producing the lowest values for both parameters. Though the current research did not attempt to correlate the behavioural and neurochemical parameters, together with the results of Baumann et al.'s study, these data suggest that the behavioural data closely matches the pharmacokinetic profiles for the different routes of administration.

The results from the current study are also consistent with findings from others stimulant drugs, such as cocaine and methylphenidate. Dow-Edwards and colleagues (Dow-Edwards, Fico, Osman, Gamagaris & Hutchings, 1989) found that subcutaneous cocaine produced a two-fold increase in locomotor activity compared with the same 40mg/kg dose delivered orally, though both *po* and *sc* administration resulted in sensitization to the cocaine locomotor activating effects over the course of 15 days of treatment. Plasma levels of cocaine were tested 15 or 45-minutes post-cocaine administration and the authors found that cocaine concentration remained similar or increased at each time point for subcutaneous administration, however oral cocaine showed decreased plasma concentration when tested after 45-minutes. The increasing plasma concentration for the *sc* administration suggests that peak effects had not yet occurred, while the decreasing plasma concentration for the oral route suggests that peak effects have already occurred prior to testing at 45-minutes. Similarly, Gerasimov et al. (2000) found that *po* administration of methlyphenidate (MP) to rats showed both slower onset and lower total locomotor activity than the same dose delivered *ip*. In addition the authors showed that the increased locomotor activity corresponded closely to measured dopamine release in the nucleus accumbens.

In the current study some dose and route combinations (most notably the 20mg/kg dose delivered via the *sc* or *ip* routes) used in the current experiment approximated equal locomotor effects despite the differences in the route with which it was administered. These results were not anticipated or planned, thus the current results do not enable dose equivalence to be determined, at least not across all routes used. For oral administration of MDMA doses higher than 20mg/kg higher may have resulted in a larger MDMA-induced locomotor response. However, De Souza et al. (1997) found that a single oral dose of 20mg/kg MDMA actually produced overall higher locomotor activity than did 40mg/kg MDMA and that the

latter dose produced lethality in 1 out of the 12 rats tested suggesting that other factors may prevent further dose-dependent increases in hyperactivity. De Souza and colleagues also showed that additional increases in dose further decreased locomotor activity (80mg/kg *po*) and lethality (80-320mg/kg *po*). However, future studies might serve to test a greater range of doses in order to calculate equivalent doses for MDMA as a function of differing routes of administration using locomotor activity. Though even with the use of equivalent doses in future experiments care must be given to conclusions generated. For example, Porrino (1993) tested equivalent doses of cocaine on locomotor activity across intravenous (1mg/kg) and intraperitoneal (10mg/kg) administration. Despite producing similar effects on locomotor activity scores, the differing routes of administration produced markedly different patterns of local cerebral glucose utilization across brain regions suggesting that cocaine produced differential activation of neuronal circuits based on alternative routes of administration. Local cerebral glucose utilization has also been mapped after exposure to MDMA and the results suggest that MDMA produces a similar pattern of glucose utilization to that of other drugs of abuse such as cocaine, *d*-amphetamine and phencyclidine (Wilkerson & London, 1989; Quate, McBean, Ritchie, Olverman & Kelly, 2004). Though never directly tested, it remains a distinct possibility that route of administration may promote changes in neuronal circuit activation for equivalent doses of MDMA in much the same way as the results reported by Porrino for cocaine.

The results of this study show a clear decrease in the efficacy of MDMA when delivered orally with regard to its locomotor activating effects. However, it must be noted that the current study used gavage for assessing the effects of oral MDMA while using injection methods for both subcutaneous and intraperitoneal routes of administration. Though unlikely, it remains possible that the differing methods of administration were the cause or at least contributed to the differences noted in the

experiment above. Oral gavage has been shown to induce stress and is associated with a number of other consequences such as breathing interference as a consequence of the intubation tube (often metal), stomach distension and accidental tracheal placement instead of the stomach (Balcombe, Barnard & Sandusky, 2004). Brown, Dinger and Levine (2000) conducted a study into the effect of gavage of various compounds with rats. They found that gavage with corn oil, but not 1% methylcelluose/0.2% tween 80 or water produced volume dependent increases in plasma corticosterone one hour after gavage. Sham gavage for each of the compounds produced plasma corticosterone within normal values suggesting that the gavage itself did not produce large scale changes in stress-induced corticosterone levels. However elevated corticosterone levels have been shown to modulate dopamine release and increase the reinforcing efficacy and locomotor activating effects of drugs of abuse (Piazza & Le Moal, 1996). However it seems unlikely that gavage would have produced a significant change in corticosterone levels in the current study as the volumes were small (1ml/kg) and the animals were habituated to gavage prior to the experiment. Additionally, examination of the control (0.0mg/kg) conditions revealed no effects of the gavage procedure, and locomotor activity was ostensibly identical to that of both *sc* and *ip* injection.

It remains unclear to what extent the dose and route dependency of the locomotor activating effects of MDMA correspond with changes in the reinforcing efficacy of the drug itself. While dose has been established as a major factor in the self-administration of MDMA (see Chapter 3 for details), route of administration has mostly been neglected. It seems likely however that route of administration will modulate the reinforcing properties of MDMA primarily through differences in metabolism (decrease in potency) and onset of action (delay between response and reinforcer). Faster onset of action, but not duration of action appears to be a better predictor of relative reinforcer efficacy (Winger, Hursh, Casey and Woods, 2002; Ko,

Terner, Hursh, Woods & Winger, 2002; Lile et al., 2003). Specifically if rapid onset of effects (neurologically or behaviourally) corresponds to reinforcing efficacy of a drug then it may be that *po* MDMA may still act as a reinforcer, albeit a relatively weak one. The following chapter explores the issue of the reinforcing effects of MDMA when tested via the oral route of administration.

Chapter 3 Oral Administration of Drugs of Abuse

Much of the behavioural evidence for the reinforcing properties of drugs of abuse comes from the literature concerning self-administration of drugs of abuse in animal subjects. In the self-administration model animals are trained to make operant responses in order to obtain access to various pharmacologically active compounds. By allowing the animal access to the drug compound contingent upon operant responses (i.e., lever presses) the consumption of that compound is wholly dependent on the animals' behaviour. Those compounds that support operant responding are considered to have some 'hedonic' or reinforcing properties, while those that do not maintain responding are either not reinforcing, or provide some other effect that may instead suppress responding.

Weeks' (1962) pioneer procedure for drug self-administration involved the implantation of chronic indwelling intravenous (*iv*) catheters through which drug solutions could be infused. Adapted to study drugs of abuse across a wide range of drug classes including psychomotor stimulants, opiates, sedatives and hypnotics (Spealman & Goldberg, 1978) this method of drug delivery is still the primary method used today in self-administration studies in non-human primates, rats and mice.

The face validity of self-administration of drugs of abuse is evidenced by the fact that almost all pharmacological compounds abused by humans are also self-administered by animals, with the general exception of the hallucinogens (Griffiths Bigelow & Henningfield, 1980; Self & Nestler, 1995). Many of the properties of the drug used are also observed in both humans and animals and thus animal models can serve as a viable alternative to the study of drugs in humans where polydrug use is prevalent and doses consumed vary widely.

The self-administration procedure allows for the direct reinforcing effects of drugs to be measured and also for the relative reinforcing efficacy of drugs to be compared. In addition, pharmacological manipulations such as administration of agonists or antagonists can help us to better understand the neurological mechanisms through which drugs of abuse produce their effects.

Self-administration of MDMA

MDMA has generally been considered a low-efficacy reinforcer (Schenk, 2009). This is backed up by research using the self-administration paradigm that shows that MDMA is a weaker reinforcer compared to other typical stimulant drugs like cocaine (Lamb & Griffiths, 1987; Lile, Ross & Nader, 2005; Ratzenboeck, Saria, Kriechbaum & Zernig, 2001), methamphetamine (Wang & Woolverton, 2007) or amphetamine (Dalley, et al., 2007). Early reports on the self-administration of MDMA showed that both rhesus monkeys (Beardsley, Balster & Harris, 1986) and baboons (Lamb & Griffiths, 1987) would continue to respond when MDMA was substituted in the place of cocaine suggesting that it functioned as a reinforcer. Later attempts have shown that in addition to non-human primates both mice (Trigo et al., 2006) and rats (see Schenk (2009) for a review) will readily self-administer MDMA even without prior training or experience with other drugs of abuse. Table 3.1 summarises current literature published on MDMA self-administration over the course of the last 25 years; in the case of studies that have tested multiple self-administered drugs the results from those conditions have been omitted from the table unless a direct comparison was merited. MDMA self-administration has most commonly been shown using fixed ratio (FR) schedules of reinforcement. In addition, progressive ratio (Lile et al., 2005; Trigo et al., 2006; Wang & Woolverton, 2007; Schenk et al., 2007), concurrent choice (Banks et al., 2008a,b,c) and runway (Wakonigg et al., 2003) procedures have also provided evidence for the reinforcing

effects of MDMA. Drug seeking has been measured using reinstatement of previously extinguished MDMA reinforced responding with non-contingent drug priming injections or with non-drug stimuli previously paired contingently with MDMA infusions (Ball, Walsh & Rebec, 2007; Banks et al., 2008a; Schenk, Hely, Gittings, Lake & Daniela, 2008). MDMA has also been shown to be self-administered in the home cage when available as a drinking solution (Reinhard & Wolffgramm, 2005, 2006) and is self-administered directly into the brain when delivered by the *icv* route of administration (Braida & Sala, 2002). The pharmacology of the reinforcing effects of MDMA and its individual stereoisomer's (Fantegrossi et al. , 2002, 2004; Wang & Woolverton, 2007) has been examined as well as the effects of various antagonists on MDMA maintained self-administration (Fantegrossi et al., 2002; Braida & Sala, 2002; Daniela et al., 2004; Brennan, Carati, Lea, Fitzmaurice & Schenk, 2009). The effects of ambient temperature have been shown to modulate the reinforcing strength of MDMA such that high ambient temperature will increase responding for MDMA (Cornish et al., 2003) and low ambient temperature can attenuate the reinforcing strength of MDMA (Banks et al., 2008a).

In a recent review on rodent self-administration studies De La Garza and colleagues (2007) note that different laboratories have produced vastly different results with regard to MDMA intake despite it producing reinforcing effects across a range of laboratories and paradigms. Table 3.1 includes estimates of the maximum MDMA intake found in each study across the range of MDMA self-administration papers published to date. In some studies, such as those employing a progressive ratio, it was impossible to calculate intake levels from the reported parameters so intake levels for those studies have been omitted. In other cases, such as the runway procedure employed by Wakonigg et al. (2003), the intake of MDMA was constrained such that levels of intake did not functionally vary making comparisons to other studies invalid. Values in the table are by necessity estimates as often

times authors fail to report MDMA intake levels consumed during studies, instead opting to plot reinforcers obtained or responses as a function dose. The values in the table represent maximum estimates based on the highest total dose of MDMA earned in a session irrespective of the dose it was obtained from. For simplicity's sake, I have opted to use the maximum intake value rather than an average across all doses as in many cases dose-response figures were inverted U-shaped functions that also include some doses that no not support MDMA self-administration to high levels, thus including those values in averages would provide underestimates of the maximum intake and inhibit direct comparison.

A brief examination of Table 3.1 reveals different results across species and paradigms. When exclusively examining rat studies the results can be broadly divided into three categories; those studies that show low levels of intake (i.e. less than 4 mg/kg) (e.g. Ratzenboeck et al., 2001; Ball et al., 2007; De La Garza et al., 2007); those studies that show mid level intakes (i.e. 6-8mg/kg) (e.g. Cornish et al, 2003; Reveron, Maier & Duvauchelle, 2006, 2009; Feduccia, Kongovi & Duvauchelle, 2010); and finally those that maintain high levels of intake (i.e. more than 20mg/kg) (e.g. Schenk, Gittings, Johnstone & Daniela, 2003; Schenk et al., 2008; Daniela et al., 2004; Daniela, Gittings & Schenk, 2006; Brennan et al., 2009). It is clear that there are vastly different results across laboratories that have reported MDMA self-administration. It is unclear why such large differences exist, though different strains of rats and training protocols likely contribute to these differences (Schenk, 2009). Individual variability between subjects may also contribute heavily to these differences. For example, Banks et al. (2008b) reported a range of individual subject intake values that ranged from 4 to 22 mg/kg in a study of four Rhesus monkeys. It is possible that the non-linear pharmacokinetics of MDMA has a substantial effect on the reinforcing properties of MDMA. Increased exposure to high levels of MDMA may lead to sensitisation to the reinforcing effects

of MDMA or alternatively to the development of tolerance, particularly to the 5-HT mediated effects. Schenk (2009) suggests that 5-HT neurotoxicity may be a significant factor in MDMA's reinforcing effects, by reducing serotonin release relative to dopamine release. Thus MDMA may become a more efficacious reinforcer contingent upon high levels of exposure to MDMA.

Of interest for the present study, Reinhard and Wolffgramm (2005, 2006) found that rats consumed an extremely low amount of MDMA when the drug was made available orally with cumulative doses reaching an average of 4.433mg/kg (*SE* = 1.212) after 49 weeks of access to the drug. Over half of that cumulative dose was said to be consumed during the first 12 weeks of the study. Of particular note is the fact that rats failed to consume high levels of MDMA when it was freely available to them; indeed, the authors report that consumption decreased almost to the point of complete cessation by the end of the 49 week study. It is unclear from their study whether the decrease in consumption was related to taste of the unadulterated MDMA solution or to a lack of reinforcing effects. However, the use of MDMA in humans when consumed orally suggests that it retains reinforcing properties under those conditions. Ergo, it is logical to conclude that MDMA should also function as a reinforcer in animal subjects when delivered orally. There is substantial support for the reinforcing effects of pharmacologically active compounds when delivered orally (Meisch, 2001; Ator & Griffiths, 2003). While the majority of this research has focussed on alcohol, it has also been established with other drugs of abuse such as cocaine (Falk,ma & Lau, 1991; Miles, Everitt & Dickenson, 2003), amphetamine, ketamine (Carroll & Stotz,1983), pentobarbital (Meisch & Lemaire, 1988) and PCP (Carroll & Meisch, 1980; Carroll, 1982). The majority of research utilising oral administration has been tested using rhesus monkeys, though rats and mice have been used to a lesser extent (Meisch, 2001). The following section will examine

research and paradigms used for testing the reinforcing qualities of orally administered drugs.

Table 3.1: A summary of research related to MDMA self-administration in animals.

Authors	Species	Methods	Dose of MDMA	Estimated M.D.C[1]	R.O.A.[2]	Findings
Beardsley *et al.* (1986)	Rhesus monkey (n=4)	FR10 MDMA/saline substitution from cocaine baseline	3-300 *µ*g/kg/inj	7.8 mg/kg	IV	MDMA substituted for cocaine and was self-administered by at least 3 of the 4 subjects for at least one tested dose. In most cases MDMA was obtained at a rate much lower than cocaine.
Lamb & Griffiths (1987)	Baboons (n=3)	FR160 – 3hr TO MDMA substitution from cocaine baseline.	0.1-3.2 mg/kg/inj	9.6 mg/kg	IV	MDMA substituted for cocaine and maintained dose dependent consumption and response rates. All except the lowest dose produced responding higher than vehicle responses. MDMA self-administration was maintained at levels lower than that of cocaine.
Ratzenboeck *et al.* (2001)	Long Evans rats (n=19)	FR1, 150 TO	0.032-10 mg/kg/inj	3.5 mg/kg	IV	No difference in MDMA S.A. between animals who experienced cocaine S.A. before MDMA. MDMA produced lower rates of responding than cocaine. MDMA S.A. higher during a second dose response determination.
Fantegrossi *et al.* (2002)	Rhesus monkeys (n=5)	FR10:60 sec TO FR30:45 sec TO Substitution from cocaine	0.001-0.3 mg/kg/inj SR(+/-) MDMA S(+) MDMA R(-) MDMA	4.8 mg/kg Racemate only	IV	Racemic MDMA and both of its isomers maintained similar biphasic dose response functions though peak responding was shown at different doses. Response rates were lower than both Methamphetamine and cocaine. 5-HT_2 Antagonist ketanserin and 5-HT_{2A} antagonist MDL 100907 attenuated responding for (+) MDMA while both abolished responding for (-) MDMA.
Braida & Sala (2002)	Wistar rats (n=18)	FR1	0.01-2.0 *µ*g/inj	80 *µ*g/kg	ICV	Subjects responded for ICV MDMA dose-dependently with a biphasic function with 1.0 *µ*g/inj producing the highest response rate and 2.0 *µ*g/inj the lowest.

[1] Maximum Daily Consumption: This value is an estimate of the maximum intake of MDMA consumed during self-administration sessions. Values have been estimated using number of responses/infusions and doses and corresponds to the peak amount consumed irrespective of dose (in cases of multiple doses tested only the highest figure is presented. Estimated values have been reconstructed from presented tables and figures and are approximate values only. Where possible actual values from published results have been reported and are noted as such above.
[2] Route of administration.

Authors	Species	Methods	Dose of MDMA	Estimated M.D.C[1]	R.O.A.[2]	Findings
Schenk *et al.* (2003)	Sprague-Dawley rats (n=11)	FR1 2,6 or 24 hr session length	0.25-2.0 mg/kg/inj	21 mg/kg	IV	Demonstrated acquisition of MDMA in naïve rats that subsequently showed increased responding when drug dose was decreased (from 1.0 to 0.5 mg/kg/inj) and extinction when MDMA was substituted with saline. Additionally rats produced dose dependent responding across the range of doses tested and subjects continued to respond throughout a 24-hr test session though most responding occurred primarily at the beginning of the session.
Cornish *et al.* (2003)	Hooded Wistar rats (n=40)	FR1, 20s TO Measured at normal 21°C and High 30°C ambient temperature	0.1-1.0 mg/kg/inj	7.5 mg/kg normal temp only	IV	Rats freely administered MDMA in a dose dependent manner and to a lesser extent than cocaine controls. Reponses for MDMA (and cocaine) were significantly increased in the high temperature condition. There was a contingent decrease in hyperactivity at the high temperature indicating that increased locomotion was not responsible for the increased responding for MDMA.
Wakonigg *et al.* (2003)	Long Evans rats (n=5) Sprague-Dawley rats (n=6)	Runway procedure	1.0 mg/kg/inj	--	IV	MDMA produced shorter average runtime than saline controls for both strains suggesting positive reinforcing effects.

Authors	Species	Methods	Dose of MDMA	Estimated M.D.C[1]	R.O.A.[2]	Findings
Daniela *et al.* (2004)	Sprague-Dawley rats (n=6)	FR1 plus DA D_1 receptor antagonism	0.25-2.0 mg/kg/inj	22 mg/kg	IV	Antagonism with the D_1 receptor antagonist shifted the dose response curve for MDMA self-administration to the right implicating dopaminergic mechanisms in the self-administration of MDMA.
Fantegrossi *et al.* (2004)	Rhesus monkey (n=4)	FR10:60 sec TO Cocaine/saline substitution	0.03-0.3 mg/kg/inj SR(+/-) MDMA S(+) MDMA R(-) MDMA	1.26 mg/kg Racemate only[3]	IV	Dose response curves were determined several times over the course of 18 months of cocaine and MDMA administration. Results showed that responding for racemic and (-) MDMA were attenuated between the first and last dose effect determinations. Results were less mixed for (+) MDMA with at least one animal showing attenuation and another increased responding between determinations. The effect was specific to MDMA and suggests long term changes in reinforcing effects may be apparent after long term dosing.
Lile *et al.* (2005)	Rhesus Monkey (n=4)	Progressive Ratio MDMA substitution from cocaine baseline	0.01-0.56 mg/kg/inj	--	IV	Subjects initially trained on cocaine, various doses of MDMA substituted for cocaine self-administration under PR schedules. MDMA maintained lower peak BP's than cocaine over a lesser dose range . Authors suggest MDMA is a lower efficacy reinforcer than cocaine
Reinhard & Wolffgramm (2005, 2006)	Wistar rats (n=16)	Free-choice two bottle test	50mg/L	4.433 mg/kg[4]	PO	The only study to have examined oral administration of MDMA in respect to its reinforcing abilities. Subjects strongly preferred water to the MDMA containing solution. Stable pattern of low level consumption of MDMA over a 49 week period with subjects decreasing intake over the course of the study. Consumption resumed after a 12 week abstinence period, but rats failed to defend access strongly when MDMA was adulterated with aversive quinine.

[3] Data reported, averaged across all doses tested

[4] Data reported, represents cumulative intake over 49 weeks of testing.

Authors	Species	Methods	Dose of MDMA	Estimated M.D.C[1]	R.O.A.[2]	Findings
Trigo *et al.* (2006)	Mice	FR1 nosepoke Progressive Ratio	0.06-1.0 mg/kg/inj	4.25 mg/kg	IV	Established MDMA self-administration in naïve mice making the procedure suitable for the study of K.O. strains. Dose-dependant effects found for both acquisition and responding. Progressive ratio found dose dependent decreases in B.P with increased dose.
Daniela et al. (2006)	Sprague-Dawley rats (n=30)	FR1, FR2, FR5	0.5-1.0 mg/kg/inj	22 mg/kg	IV	Subjects compensated to increases in response requirement and maintained similar rates of drug intake despite increased economic strain. Responding ceased when put into extinction and resumed after reinstatement of the drug reinforcer. Additionally subjects required more sessions to extinguish when a light stimulus previously paired with MDMA was present during extinction. Removal of both the light and the drug however resulted in rapid extinction. Responding also decreased in the drug present/light absent condition suggesting that the conditioned reinforcing properties of the light may be important for the self-administration of MDMA.
Reveron *et al.* (2006)	Sprague-Dawley rats (n=12)	FR1 Acquisition phase, days 1-10 Maintenance phase days 11-20	0.5-1.0 mg/kg/inj	6.5 mg/kg	IV	During the acquisition phase rats received 1.0 mg/kg/inj MDMA. Responding and drug intake increased during the maintenance phase. Subjects showed experience dependent changes in temperature (hypothermic-normal) and locomotor activity (normal to potentiated) as a function of increased exposure to MDMA.

Authors	Species	Methods	Dose of MDMA	Estimated M.D.C[1]	R.O.A.[2]	Findings
Schenk *et al.* (2007)	Sprague-Dawley rats (n=23)	FR1 Progressive Ratio	0.25-1.0 mg/kg/inj	--	IV	Acquisition was tested with 0.25 and 1.0 mg/kg/inj MDMA over 6-hour daily sessions and approximately 60% of the animals acquired MDMA self-administration over the 15 day test period compared with 100% of the cocaine comparison group. There were no real differences in the latency to acquire MDMA self-administration as a function of dose. MDMA produced dose-dependant increases in BP as a function of increased dose.
Ball *et al.* (2007)	Sprague-Dawley rats (n=14)	FR5:6 sec TO-extinction – reinstatement	0.3 mg/kg/inj	2.5 mg/kg[5]	IV	Tested acquisition across 14 daily sessions followed by extinction by removal of drug plus light/tone stimuli. After extinction (average 5 days) reintroduction to the light/tone stimulus resulted in reinstatement of the previously extinguished responding showing that stimuli paired with self-administered MDMA can lead to reinstatement/relapse. However, a 5.0mg/kg injection failed to reinstate responding.
Dalley *et al.* (2007)	Lister Hooded rats (n=6)	FR1	0.5 μg/kg/inj (as the free base)	23.3 μg/kg		Rats acquired MDMA self-administration at a slower rate than both Methamphetamine and *d*-amphetamine though responding was approximately equal across drugs after the 21-day testing period. Enduring deficits in attention were found after a 6 weeks withdrawal period in response to challenges as measured with a 5 choice serial reaction time task.
Wang & Woolverton (2007)	Rhesus monkey (n=6)	Progessive ratio Substitution from cocaine/saline BL	0.025-0.8 mg/kg/inj of MDMA, (+)-MDMA or (-)-MDMA	6 mg/kg Racemate only		Progressive ratios were used to test relative reinforcing efficacy of MDMA or its individual isomers. Both (+/-), and (+)-MDMA functioned as a reinforcer with the (+) isomer producing more responding (albeit not significantly). The (-) isomer (primarily 5-HT release) did not function as a reinforcer in 3 of the 5 animals tested. Cocaine and Methamphetamine both produced higher numbers of drug infusions suggesting that MDMA is a weaker reinforcer than those two more prominent drugs.
Trigo *et al.* (2007)	Wild type mice SERT K.O. mice	FR1 nosepoke	0.03-0.25 mg/kg/inj	--	IV	WT mice steadily acquired MDMA self-administration but SERT K.O. mice did not acquire self-administration at any dose.

[5] Average reported data for the last 5 days of self-administration testing.

Authors	Species	Methods	Dose of MDMA	Estimated M.D.C[1]	R.O.A.[2]	Findings
De La Garza *et al.* (2007)	Wistar rats (n=20)	FR2 or FR1	0.185-1.5 mg/kg/inj	2.7 mg/kg[6]	IV	Experiment 1 showed low rates of responding though one rat (of 5) did show dose dependent responding. In a second experiment subjects showed greater responding for MDMA during the dark cycle (active phase) than during the light cycle. However, when doses were changed to a lower dose or saline responding for MDMA did not recover.
Schenk *et al.* (2008)	Sprague-Dawley rats (n=9)	FR5-extinction-reinstatement	0.5 mg/kg/inj	24 mg/kg	IV	Subjects maintained high rates of responding for MDMA prior to extinction. After extinction both priming injections of MDMA or cocaine dose-dependently reinstated previously extinguished responding despite only being given infusions of vehicle. Reinstatement was also observed after the return of the light stimulus, though to a much lesser extent.
Banks *et al.* (2008a)	Rhesus monkeys (n=5)	Concurrent FR30 (MDMA) : FR30 (Food) Both 30 sec TO	0.03-0.3 mg/kg/inj	2.25 mg/kg[5] ±0.43	IV	Examined the effects of ambient temperature using a concurrent choice procedure between MDMA and food. At room temperature subjects preferred MDMA over food for all doses except the lowest. At the high ambient temperature subjects also showed a preference for the lowest dose over food, suggesting an increase in relative reinforcing efficacy. At low ambient temperatures only the high dose of MDMA showed a clear preference indicating the relative reinforcing efficacy may have decreased. MDMA given non-contingently dose dependently increased responding when saline was substituted for MDMA however this reinstatement was not effected by changes in ambient temperature.
Banks *et al.* (2008b)	Rhesus monkeys (n=4)	Concurrent FR30 (MDMA) : FR30 (Food) Both 30 sec TO	0.03-0.3 mg/kg/inj	4-22 mg/kg[7]	IV	Subjects self-administered MDMA for a period of at least 6 months. After which PET scans were taken and to examine SERT availability. It was found that subjects in the MDMA group showed no differences in SERT availability than control subjects. In comparison a cocaine control group found up regulation of the SERT. It is unclear why do deficits were found though the possible remains that either the process of self-administering the drug or more likely the total amount of drug consumed was not sufficient to produce detectable deficits.

[6] Data reported.

[7] Range of the weekly average reported.

Authors	Species	Methods	Dose of MDMA	Estimated M.D.C[1]	R.O.A.[2]	Findings
Banks *et al.* (2008c)	Rhesus monkeys (n=4)* reanalysis of data obtained from (Banks *et al.,* 2008a)	Concurrent FR30 (MDMA) : FR30 (Food) Both 30 sec TO	0.03-0.3 mg/kg/inj	--		MDMA produced a dose dependent decrease in overall response rate. In addition MDMA caused a dose dependent decrease in RR for both drug and food. In comparison cocaine did not produce a decrease in RR for food. Running RR for individual drug levers did not vary as a function of dose suggesting that RR is independent of reinforcing strength.
Reveron *et al.* (2010)	Sprague-Dawley rats (n=9)	FR1-20 sec TO	0.5-1.0mg/kg/inj	6.6 mg/kg	IV	Responding for MDMA increased gradually during an initial 10-day acquisition phase at 1.0mg/kg/inj. Responding and MDMA intake were significantly higher during a second 10-day maintenance phase when the dose was decreased to 0.5mg/kg/inj. MDMA subjects showed decreased core body temperature during the acquisition phase and higher locomotor activity during part of the acquisition and all of the maintenance phase compared with saline controls.
Brennan *et al.* (2009)	Sprague-Dawley rats (n=7)	FR1 plus DA D_2 receptor antagonism	0.5-2.0 mg/kg/inj	22 mg/kg	IV	Measured the effects of the D_2 receptor antagonist eticlopride. Eticlopride increased responding fro MDMA partially implicating the D_2 receptor in the rewarding effects of MDMA.
Feduccia *et al.* 2010	Sprague-Dawley rats (n = 16)	FR1-20 sec TO High ambient temp 32°C Normal room temp 23°C	0.5-1.0mg/kg/inj	5.75 mg/kg total intake days 1-10 ≈ 35 mg/kg total intake days 11-20 ≈ 45mg/kg	IV	No effect of ambient temperature on intake of MDMA. Subjects showed increased intake during the maintenance phase (MDMA 0.5mg/kg/nj, days 11-20) compared with the acquisition phase (MDMA 1.0mg/kg/inj days, 1-10). Subjects showed increased core temp in the high ambient temp condition, but only during the maintenance phase. Locomotor activity was enhanced for both high and normal temp conditions during maintenance phase. High ambient temp significantly increased extracellular levels of 5-HT in the NAcc compared with room temperature but temperature had no effect on extracellular DA in NAcc.

Oral self-administration of MDMA

The benefits of an oral model of MDMA self-administration include the ability to more closely replicate the human condition in which MDMA is almost exclusively taken orally. Also an oral method would bypass issues regarding catheter patency and allow for long-term parametric studies to be conducted (Meisch, 2001). In addition, it would be possible to examine the long-term effects of continued MDMA administration over longer time spans than is currently possible with *iv* self-administration in rodents. Long-term studies may also help to elucidate the effects of tolerance upon MDMA self-administration and enable the study of the occurrence of other deleterious health sequelae such as MDMA–induced serotonin deficits.

Typically an oral drug is perceived only to be reinforcing if the intake of a particular compound exceeds the consumption of that compound's vehicle solution (Meisch, 2001). In general this means that the animal indicates a preference for the drug over vehicle solutions. Much like drugs tested using the *iv* administration procedure, drugs that are delivered orally will commonly produce inverted u-shaped dose-response functions. The inverted u-shaped dose-response curve consists of both an ascending and a descending limb. In the former responding increases as a function of increases in dose, where as in the latter the inverse is true. Under generally unrestricted drug access, subjects consume approximately the same total amount of drug per session regardless of the dose of drug, this is known as self-regulation or titration (Griffiths et al., 1980). Regulation of drug intake is evidenced in the descending portion of the dose-response curve where subjects increase or decrease responding in order to maintain relative similar levels of drug intake across doses, for example as doses increases the subject can regulate drug intake by decreasing responding

during a sessions and thus obtaining less reinforcers, but approximately the same level of drug intoxication.

Meisch (2001) outlines several major factors that can hinder studies of oral self-administration and must be overcome for successful oral self-administration. Firstly, consumption of oral compounds is often low due to factors such as the aversive taste, even in experienced animals. Secondly, if consumption of a drug is too low then subjects may not experience the pharmacological effects of the drug in question. Finally, a delay in onset of action of up to 5 minutes caused by oral administration is thought to decrease the likelihood that operant conditioning will occur (Meisch, 2001). Conditioned stimuli have been shown to have an important role in the development and maintenance of self-administration. For example, in *iv* self-administration removal of a conditioned stimulus (e.g. a light) paired with drug infusions will result in a decrease in responding (Daniela et al., 2006; Schenk & Partridge, 2001). In addition, presence of a conditioned stimulus is integral to studies of second-order schedules that can maintain self-administration over large response requirements by response contingent presentation of conditioned reinforcers to maintain responding in the absence of frequent drug reinforcement (Schindler, Panlilio & Goldberg, 2002). In spite of the delay in onset of action for oral drugs the taste of the solution can come to serve as a conditioned stimulus for the presence of the drug despite a lengthy delay between responding its central effects (Meisch, 2001). So while the drug taste itself may start as potentially aversive it may actually come to signal the presence of up coming drug effects.

Strategies have been developed in order to overcome the initial reluctance to consume oral drugs including substitution and fading procedures. Substitution procedures rely on animals that have already developed self-administration of another drug (often alcohol). Different drugs can then either be substituted

wholly for the previous solution or they can be mixed initially and the original solution faded by decreasing its concentration over time. In a fading procedure (e.g. Samson, 1986) high rates of drinking are maintained by another fluid (usually sucrose or saccharin). Gradually, doses of the drug are added to the solution in increasing concentrations over several, sometimes many, sessions. When intake is relatively stable the concentration of the adulterant solution is gradually decreased until only the drug remains. This has proven to be a reliable way of demonstrating self-administration of oral drugs.

Another common method used to establish oral self-administration is to employ a procedure that produces schedule-induced polydipsia (SIP) (Falk, 1961). SIP is the excessive intake of water that can be induced when animals are exposed to intermittent schedules of food reinforcement. Typically with this method animals are placed in a chamber that is programmed to deliver food pellets at a Fixed Time (FT) duration (for example FT60 seconds). In addition to the presentation of food; animals are also given access to two water bottles; one containing a drug solution and the other its vehicle (usually water). As animals increase their consumption of liquid (above levels normally seen) exposure to the drug solution increases. Over time a long-lasting preference can develop for the drug solution (Falk & Lau, 1995).

Critically important in SIP is the learning history of the animal. In a study by Falk and colleagues it was shown that prior history with an adulterated drug solution that was then subsequently faded was necessary for the animals to reliably choose a cocaine solution to water. After training almost all animals showed a clear preference for the cocaine solution over water. The presumed mechanism for this effect is that the preferred vehicle becomes paired with the gustatory response to the drug resulting in the development of a strong conditioned

reinforcing effect even after fading of the vehicle solution (Falk, Neal & Lau, 1997).

The following experiments were designed to assess the reinforcing effects of MDMA when consumed orally. In Experiment 3.1 a method similar to that of Reinhard and Wolffgramm (2005, 2006) was adopted whereby animals were given free access to both water and a solution containing MDMA in the home cage to see whether animals would readily consume MDMA when it was freely available. Experiment's 3.2 and 3.3 used operant methods to measure the reinforcing efficacy of MDMA when the drug was delivered orally rather than intravenously, as has been the case in rodent studies up until now. Finally, Experiment 3.4 systematically replicated the procedure of Daniela et al. (2004) to determine whether oral MDMA self-administration is mediated through dopaminergic mechanisms through concurrent administration of the DA D_1-like antagonist SCH 23390.

Experiment 3.1A: Two choice free access in the home cage: Preference between MDMA and water

Preference between drug and non-drug containing solutions has been previously used to determine whether a drug compound acts as a reinforcer. Experiment 3.1 was designed to test preference between water and MDMA-containing solutions. This was done by monitoring daily MDMA intake in the home cage when subjects had free access to both MDMA and water simultaneously. Of additional interest was the amount of MDMA consumed when animals have free access to the drug in the home cage as a comparison with rates of intake in the operant paradigm used in Experiments 3.2, 3.3 and 3.4.

Experiment 3.1 was a partial replication of Reinhard and Wolffgramm (2005, 2006), such that this study focused solely on the choice between water and MDMA in the home cage (in the original study the authors were interested in concurrent availability of MDMA and THC). The dose used in the current study differed from that used by Reinhard & Wolffgramm but was chosen based on pilot studies of an operant self-administration task in which MDMA solutions served as the reinforcer (see Experiment 3.2 for further details on this task). The dose used for the free-access choice procedure was selected on the basis that it was the dose that promoted the highest response rate during the oral self-administration operant pilot studies.

To date the studies conducted by Reinhard and Wolffgramm are the only studies to have tested MDMA's effects as a reinforcer when delivered orally. Reinhard and Wolffgramm (2006) found that consumption of MDMA was highest through the first several weeks of the study but decreased to almost nothing by the end

of the 49 weeks of access. They concluded that subjects held a strong preference for water over the drug containing solution when both solutions were concurrently available. As noted in Table 3.1, the subjects consumed an average of 4.433 mg/kg of MDMA over 49 weeks. However, the drug was available in a 50mg/L solution suggesting that the subjects very rarely consumed much fluid from the drug-containing bottle. Wolffgramm and colleagues have previously shown that some drugs, including alcohol, the opiate etonitazene and *d*-amphetamine, will produce addiction-like profiles using this paradigm (Heyne & Wolffgramm, 1998). Initially drinking behaviour is said to be "controlled" where consumption can be modulated by external circumstances such as changes in housing conditions, etc. These changes will have effects on the level of drug consumption, for example, increasing or decreasing intake. Whereas an "addicted" profile is indicative of a loss of control over drug consumption indicated by marked increases in intake and inflexibility of drug consumption (Wolffgramm, Galli, Thimm & Heyne, 2000). For example, Galli and Wolffgramm (2004) showed that when rats were given access to water or *d*-amphetamine (100, 200, 400mg/L) in the home cage subjects initially showed controlled drug intake that was modulated by housing conditions, i.e. subjects consumed more *d*-amphetamine when housed in isolation than when they were group housed. After 40 weeks of access, six of the animals tested showed a large increase in drug consumption, while the remaining six animals did not. After a 10-week forced abstinence those animals showed an increase and subsequently maintained high rates of drug consumption even when the drug solutions were adulterated with bitter tasting quinine. In contrast, those animals that did not show increased drug consumption remained flexible in their drug intake, i.e. adulteration with quinine reduced intake. In a comparison group with only 16 weeks access to *d*-amphetamine none of the animals showed increased drug consumption, suggesting that length of access to the drug has an impact

on the change from “controlled” drug intake to “addicted”. This result also suggests that not all subjects will progress to the point of addiction and some will remain in the controlled intake state in perpetuity. In an earlier study, Heyne and Wolffgramm (1998) showed that the ability to choose was important to development of the addiction-type profile as rats that were forced to drink *d*-amphetamine as the only available solution remained controlled in their drug intake and did not show perseverance in the face of quinine challenge as did subjects who instead had free choice.

The following experiment tested MDMA consumption in rats given free access to MDMA in the home cage with the intention of comparing intake rates between home cage access and future experiments examining the operant delivery of reinforcers. Previous research with *iv* self-administration indicates that MDMA acts as a reinforcer in animal models (see Table 3.1). Furthermore, ethanol intake rates in home cage free-choice drinking paradigms by a range of ethanol-preferring mice and rats are positively correlated with oral ethanol self-administration in the same strains, but negatively correlated with conditioned taste aversion (Green & Grahame, 2008). The relationship between home-cage free-choice drinking and self-administration would imply that MDMA will provide reinforcement in both free-choice and operant paradigms. The results of Reinhard and Wolffgramm (2005, 2006) indicate only low rates of consumption of MDMA, however only a single dose of MDMA was tested. It remains a possibility that the single dose used in that study was not sufficient to induce substantial central effects of MDMA. The current study seeks to expand upon that research by using a higher dose of MDMA, one that that has been shown to maintain high rates of oral self-administration in pilot studies conducted previously. The use of a higher dose may help to promote the MDMA-induced reinforcing effects. In addition, the high rates of responding observed during the

pilot study indicate that the dose used in this study is not aversive as response rates were roughly equivalent with those seen for vehicle alone (See Experiment 3.2 for more details).

Method

Subjects:

Subjects were six naïve male Sprague-Dawley rats bred in the Psychology Animal Facility at Victoria University of Wellington, housed individually in polycarbonate cages with cage tops made of metal grating in the testing room. The testing room was maintained on a reversed 12:12 hour light/dark cycle, with lights on at 6pm, and maintained at a constant temperature between 19-21°C. Animals were maintained at 85% of their free-feeding weights throughout the duration of the experiment. Mean weight at the beginning of the experiment was 380 grams (*SD* = 32.66). Animals were treated in accordance with ethical guidelines set forth by the Victoria University of Wellington Animals Ethics Committee.

Apparatus/materials:

Equipment:

Home cages were modified to allow the attachment two 120ml graduated drinking bottles (Habitrail Safari, Living World, Rolf C. Hagen Inc.) with a single ball drinking mechanism to the front of each cage. Brackets for water bottles were positioned on the front of the cage 20mm from the side and 30mm from the top of the cage. The brackets were positioned 80mm apart from one another.

When bottles were fitted into the brackets the spout protruded 20mm into the cage and was positioned 45mm above the cage floor.

Solutions:

+/-3,4-methylenedioxymethamphetamine hydrochloride (ESR, Porirua, New Zealand) was mixed with tap water at a concentration of 81.25mg/L. Drug weights refer to the salt.

Procedure:

Animals were placed on a restricted diet and subsequently reduced to 85% of their free feeding weights. Once baseline weights had stabilised, the standard water bottles were removed and replaced with a single graduated sipper bottle filled with tap water in either the left of right hand position on the front of the cage (alternated across rats). Animals were acclimatised to the new drinking bottles over a 3-day period. Subsequently a second bottle was filled with MDMA solution and placed in the free bracket of each cage.

Daily measurements of fluid drinking were conducted between 10am and 12pm every day. Briefly, both bottles were removed and the contents measured and recorded. If any bottles needed refilling they were refilled at this time. Additionally, the animals were weighed before being placed back into their home cages and replacing the sipper bottles. Animals were fed a restricted diet (85%) consisting of Diet 86 pellets (Sharpes, New Zealand).

The location of the drug and water bottles was alternated after 21 days and data collection was continued for a total of 45 days.

Results and Discussion

Daily intake measures for MDMA and water were averaged over successive 3-day periods for analysis. Figure 3.1 (top panel) shows the total average liquid intake in mls over 3-day time bins for MDMA and water. Subjects showed a clear preference for water over the MDMA-containing solution drinking on average 22.75mls (*SE* = 1.31) of plain water in comparison to 3.70mls (*SE* = 0.47) of MDMA. A two-way repeated measures ANOVA between type of solution (water vs. MDMA) and time was performed. There was a significant interaction between type of solution and time (F (14, 70) = 4.827, $p < 0.01$). Paired sample *t* tests revealed that water intake was significantly greater than MDMA (all tests significant, $p < 0.05$). In order to examine how intake changed over time, paired sample *t* tests were conducted between only the first and last time blocks for both MDMA and water. MDMA intake was significantly lower (*M* = 3.17, *SD* = 2.49) during the last time block than it was during the first (*M* = 7.72, *SD* = 2.86). This difference was significant, t (5) = 4.526, $p = 0.008$. In contrast, water intake significantly increased (t (5) = -4.235, $p = 0.008$) from the first time block (*M* = 17.44, *SD* = 4.66) to the last time block (*M* = 27.28, *SD* = 4.11). Figure 3.1 (bottom panel) shows the median percentage of liquid intake from the drug-containing solution. During the first 3-day block, subjects consumed approximately a quarter of their fluid intake from the MDMA solution (*Mdn* = 23.24, *SD* = 4.32) though individual subject variation was high with the minimum percentage MDMA intake being 7.27% and a maximum percentage MDMA intake of 39.09%. Subsequently percentage intake from the drug containing solution decreased to less than 12% of the total fluid intake for the remainder of the study. A one-way ANOVA revealed a significant main effect of time on the percentage of intake from the drug-containing solution (F (14, 70) = 2.611, $p = 0.04$).

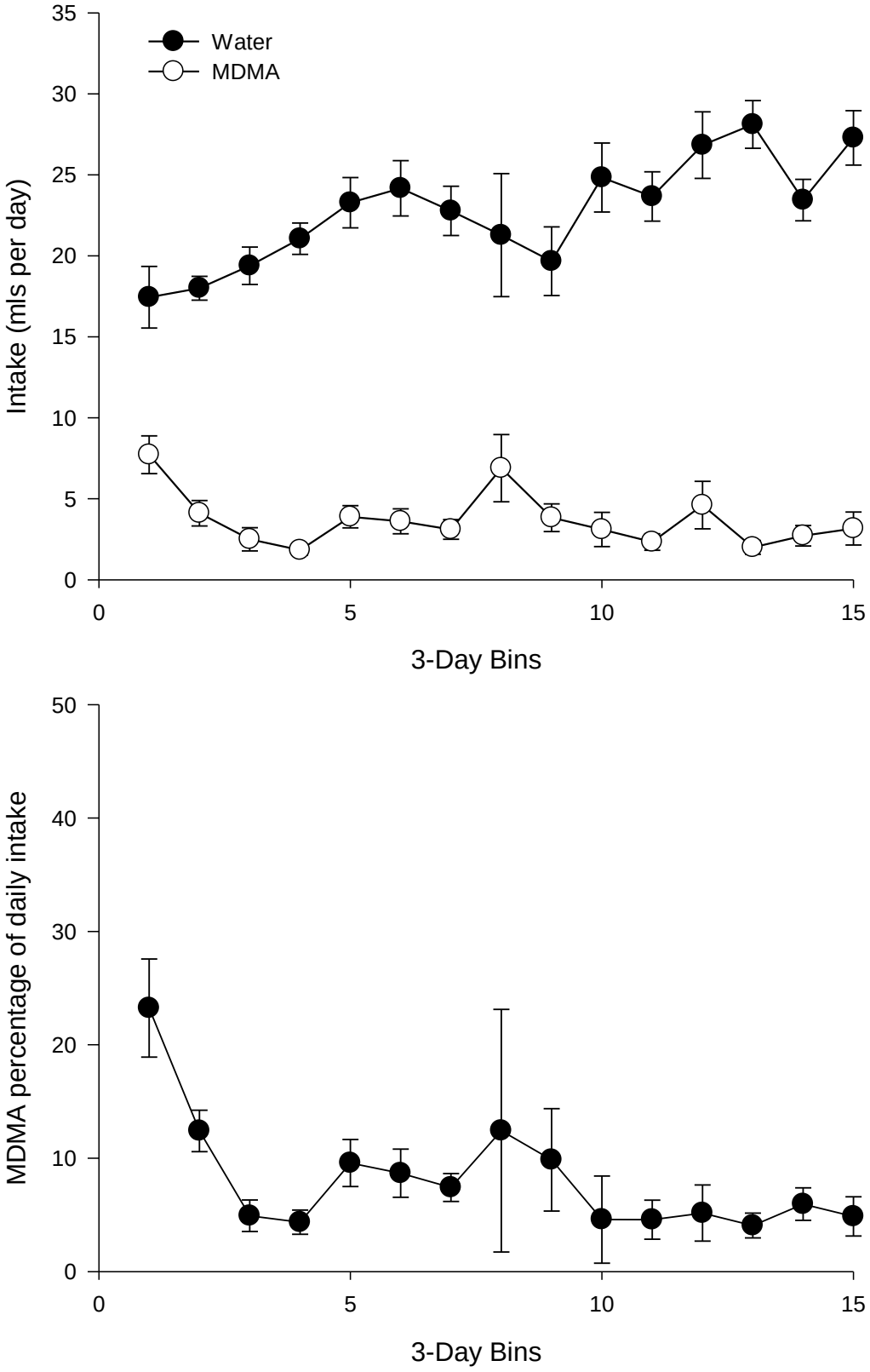

Figure 3.1: (*Top panel)* Average liquid intake (mls) for MDMA (open circles) and water (closed circles) as a function of 3-day time bins. (*bottom panel)* Percentage of daily intake consumed from the MDMA bottle averaged across 3-day time bins. On day 22 the bottle of position of drug and water containing solutions was rotated. Error bars represent standard error of the mean.

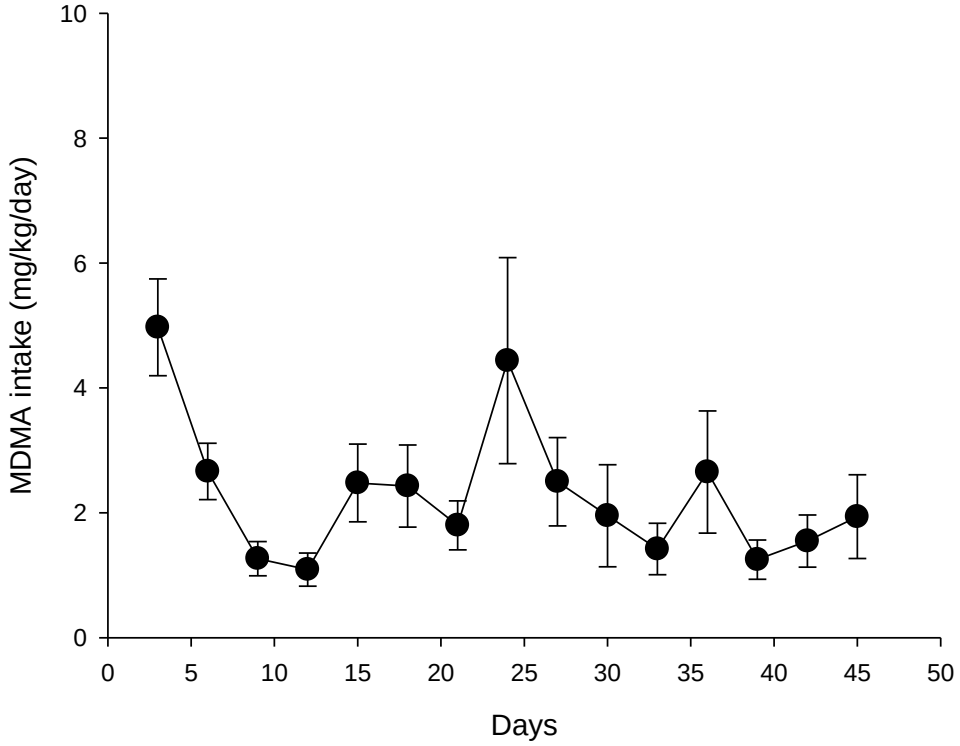

Figure 3.2: Average daily intake of MDMA plotted as a function of 3-day average consumption. On day 22 bottle position for MDMA and water-containing solutions was rotated. Errors bars represent standard error of the mean.

Over the course of the 45 days of study subjects had a mean total intake of 34.40 mg/kg of MDMA (*SE* = 5.55, range, 17.82 – 53. 07mg/kg). Figure 3.2 shows total MDMA intake summed across successive 3 day periods. MDMA intake was erratic with largest MDMA intake during the first 3-day block, and during the 8th block that corresponded with the alternation of the bottle position.

To examine changes in the pattern of MDMA consumption for individual rats the cumulative intake of MDMA (mg/kg) for each individual was plotted as a function of the cumulative total of each 3-day time bin. Figure 3.3 shows that total intake varied considerably across rats. In general, rates of intake of MDMA were stable within individuals, with the exception of rat 6 for whom intake increased markedly when the bottle position was alternated. Rat 6 showed a persistent

increase in rate of drinking from the drug bottle after the change in position.

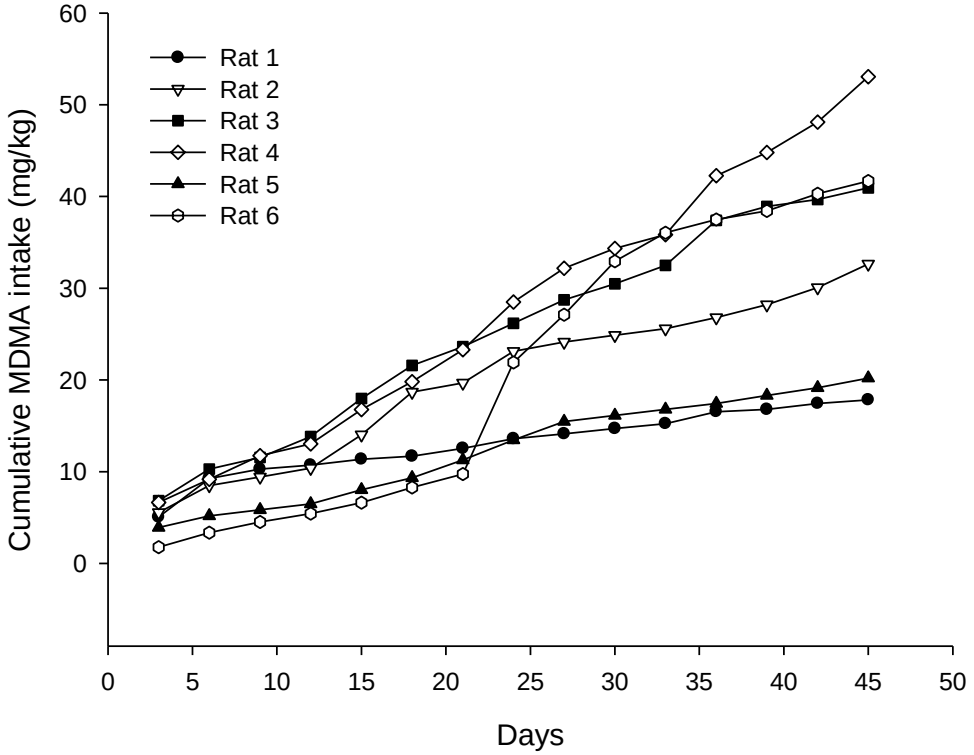

Figure 3.3: Cumulative MDMA intake in mg/kg plotted as a function of consecutive 3-day averages for 6 rats. NB. On day 22 bottle position was rotated for all subjects.

Overall this study showed that subjects had a clear preference for the water-only containing bottle. However, all subjects continued to sample from the drug-containing solution though subjects consumed less than 20% of their daily fluid intake from the drug-containing solution. Visual inspection of the data revealed relatively few instances of an animal consuming no MDMA on a given day. Across all six animals no MDMA intake was only recorded in 14 out of a total of 270 data points, representing approximately 5% of all recorded observances.

Average MDMA intake was 0.83mg/kg per day (*SE* = 0.07); considerably less than that consumed in operant studies of *iv* self-administration (see Table 3.1). However, this study yielded higher rates of MDMA intake in comparison with the

results of Reinhard and Wolffgramm (2005). In their study subjects consumed an average of 4.433 mg/kg of MDMA across the entire course of the 49 weeks of study. In a comparatively shorter period of time (45 days or 6.4 weeks) subjects in this study consumed on average 34.40 mg/kg of MDMA (*range* = 17.82 - 53.07). It remains unclear why there is such a large disparity between intake across both studies, however the dose used in the current study was approximately 1.6 times stronger than the dose used by Reinhard and Wolffgramm. It is not clear whether the amount of MDMA consumed in the current study produced any pharmacological effects because no tests were administered during the course of the study. However, low doses of MDMA in rats have been shown to disrupt performance on a number of tasks, for example, delayed matching to sample (Harper, Wisnewski, Hunt & Schenk, 2005; LeSage, Clark & Poling, 1993) and the radial arm maze (Braida, Pozzi, Cavallini, & Sala, 2002; Kay, Harper & Hunt, 2010). In addition low doses of MDMA have been shown to produce robust drug discrimination (Oberlender & Nichols, 1988; Schechter, 1987, 1991), conditioned place preference (CPP) (Schechter, 1991) and reduce the reward threshold for electrical brain stimulation (Hubner, Bird, Rassnick & Kornetsky, 1988). However all of these paradigms use bolus injections (*ip* or *sc*) that produce relatively rapid effects; this method differs markedly from the slow accumulation of MDMA that would occur during the free access paradigm. It is also unclear when (light vs. dark cycle) and how frequent visits to the drug bottle were. It would be beneficial for future studies to use a method that would allow for the analysis of drinking bout time as well as bout length.

One factor that may contribute to the drug preference is experience with the drug in question. The following experiment represents a partial replication of Experiment 3.1A using MDMA-experienced animals. Subjects were tested

using the free-access choice procedure described in Experiment 3.1A in order to test whether previous exposure to oral MDMA consumption would have any effect on the concurrent choice between MDMA and water in the home cage.

Experiments 3.1B: Effect of prior drug history on drug preference using a two-bottle free access choice procedure in the home cage

Experienced animals for the current experiment were selected from those animals that had previously participated in an operant-based oral self-administration procedure and had significant exposure to oral doses of MDMA. As subjects in the current study were already experienced with oral consumption of MDMA it was expected that there should be some transfer of overall intake rates from the operant task to the free-access paradigm. Thus it is hypothesised that rats in the current study will consume MDMA via the free-access method equivalent to the operant method and that subjects should show higher levels of drinking and MDMA consumption than seen in the naïve rats studied in Experiment 3.1A.

Method

Subjects:

The subjects used in this experiment were 12 male Sprague-Dawley rats previously used in Experiment 3.2 who had significant experience with oral administration of MDMA via the operant method (for a detailed description of the subjects' experience see Experiment 3.2). Subjects were housed individually in polycarbonate cages with cage tops made of metal grating in the testing room. The testing room was maintained on a reversed 12:12 hour light/dark cycle, with lights on at 6pm, and maintained at a constant temperature between 19-21°C. Prior to the initiation of the current experiment, animals had been tested on a 21-hour water deprivation schedule. This water deprivation scheme was discontinued and animals were reduced to 85% of their free feeding weights.

Subjects' weights ranged between 350 and 530 grams prior to being placed on the restricted diet. Housing conditions remained the same as used previously. Research was approved and all animals were treated in accordance with ethical guidelines set forth by the Victoria University of Wellington Animals Ethics Committee.

Apparatus/materials:

Equipment:

See Experiment 3.1A.

Procedure:

A single bottle filled with tap water was placed in either the left or right position of each cage and rats were allowed to acclimatise themselves to the new water bottles for 3-days. The water bottles were then removed and refilled with water, and an identical set of bottles was filled with 81.25mg/L MDMA solution and placed in the free bracket on each cage. Assignment of water/drug bottles to the left and right positions was alternated across rats. Animals had free access and choice to both bottles at all times.

Daily measurements of the volume in each bottle were taken between approximately 10:00am and 11:00am each morning. At this time the rats were also weighed and fed based on their 85% free feeding weight and any bottles that required filling were refilled.

Consumption of both drug and water was recorded 7-days a week for a period of 14 days. On the eighth day the position of the water and drug bottles was reversed in order to take into account any bias effects

Results and Discussion

Total MDMA intake per day was collected and the results are shown in Figure 3.4. On the first day of MDMA availability intake was the highest with intake decreasing rapidly on the second and subsequent days to a relatively stable daily intake of MDMA solution. The range of intake varied across rats with the highest average intake across the 14-day period being 1.2mg/kg/day while the lowest intake among all of the subjects being 0.3mg/kg/day (*SD* = 0.29). Examination of the raw data revealed that on occasion some animals would not consume any fluid from the MDMA bottle, though these days only happened on occasion and only once for more than a single day.

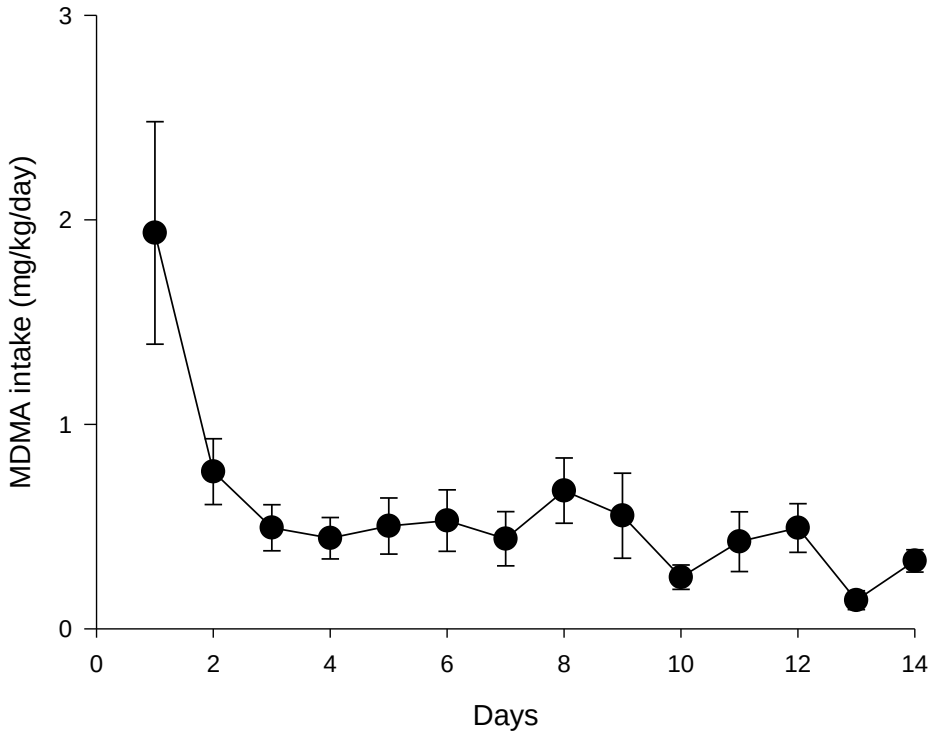

Figure 3.4: Average MDMA intake (mg/kg) per day during using a two-bottle (MDMA vs. water) free access paradigm for 12 MDMA-experienced rats. Error bars represent standard error of the mean.

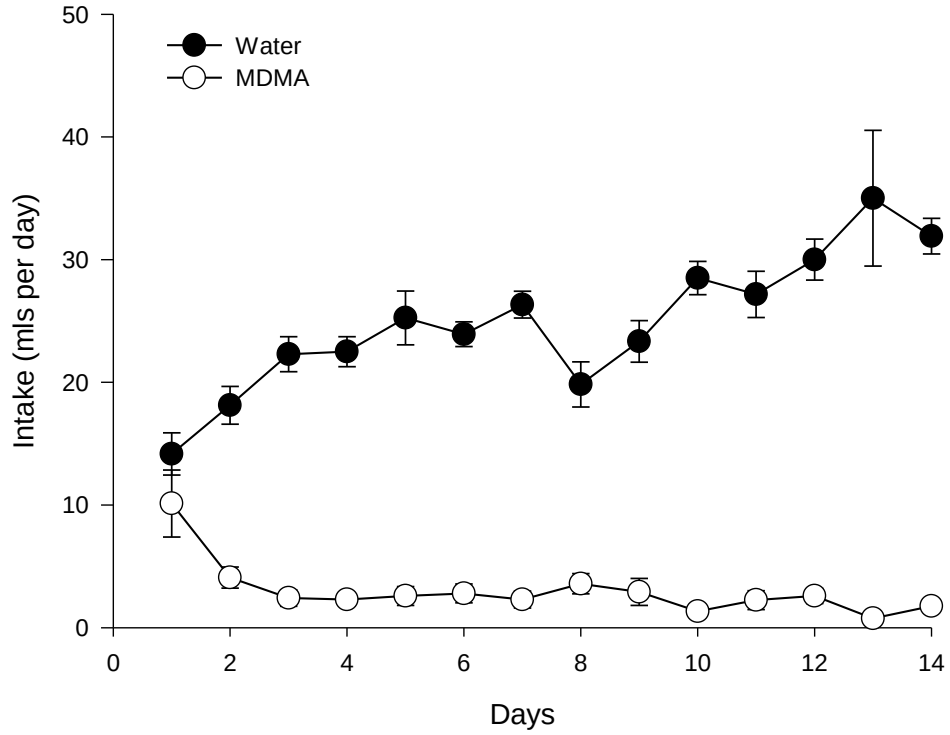

Figure 3.5: Average liquid intake in mls from two bottles containing either MDMA (81.25 g/L) (filled circles) or water (open circles) for 12 MDMA-experienced rats. Error bars represent standard error of the mean.

While MDMA intake appeared to be low, consumption of plain water during the experiment was high. As can be seen in Figure 3.5, MDMA and water intake (millilitres) were approximately even during the first day of testing, however, from day two onwards water intake began to increase while MDMA intake remained low. Animals' could clearly differentiate between the bottles that contained water from those that contained the solution including MDMA. Despite the high rates of responding for this dose of MDMA seen in Experiment 3.2A, this did not carry over to the free-access situation when plain water was available as an alternative. Clearly animals were able to discriminate between solutions and showed a clear preference for the plain solution. The ability to discriminate the solutions was likely the result of taste, though despite the differences in taste animals did continue to drink from the MDMA bottle and produced little evidence of exclusivity for the plain water bottle.

After eight days the position of the drug-containing bottles was switched which resulted in a noticeable decrease in water intake and a small increase in MDMA consumption, though this difference was transitory suggesting that subjects rapidly acquired the new location of each of the bottles after an initial period of confusion.

Despite their previous exposure to MDMA solutions the animals in this study reduced their intake of MDMA rapidly to a very low level and maintained high levels of water intake throughout the experiment. When an MDMA solution was simultaneously available with plain water the water solution was preferred despite previous experience with oral reinforcement with MDMA. The results found in this study are comparable with those found by Reinhard and Wolffgramm (2006), though animals in the current study showed higher rates of intake. However, this study only lasted for a fraction of the time (2 weeks compared with 49 weeks) than that of Reinhard and Wolffgramm's study.

Despite the fact that rats in this study consumed on average more MDMA per day, it is possible that rats in the current study may have completely extinguished their MDMA drinking much like those in Reinhard and Wolffgramm's study did after long-term exposure to the drug. Additionally, the fact that the subjects in the current study were drug experienced may have contributed to the higher rates of MDMA intake seen in this study in comparison to those of Reinhard and Wolffgramm.

In contrast to expectations the rats in this study did not show a preference for the MDMA containing solution. In addition, the MDMA-experienced animals did not show a significantly greater consumption of MDMA despite extensive experience compared with that of the naïve animals tested in Experiment 3.1A

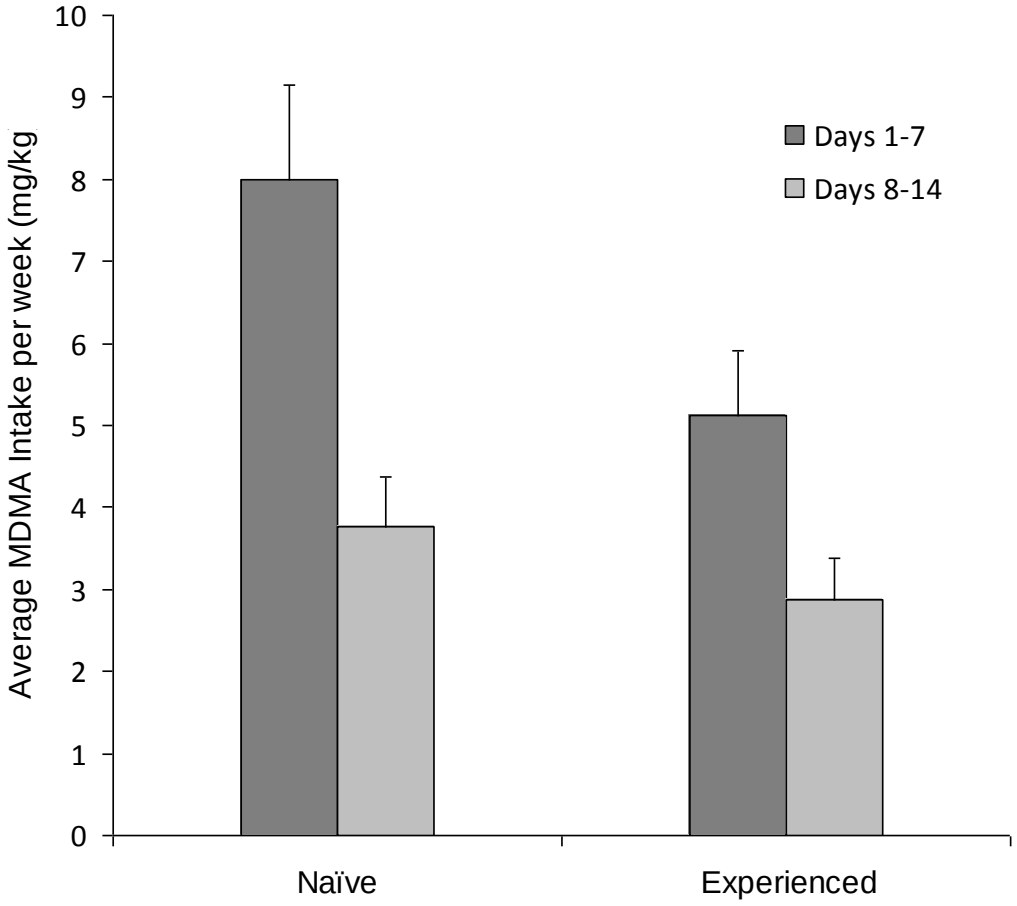

Figure 3.6: Comparison of weekly MDMA intake (mg/kg) across naïve (n = 6) or MDMA-experienced animals (n = 12). Error bars represent standard error of the mean.

Figure 3.6 shows a comparison between the results from Experiment 3.1A using naïve animals and Experiment 3.1B using MDMA-experienced animals. Due to differing lengths of each experiment only the first two weeks of Experiment 3.1A was including in order to facilitate comparisons. Surprisingly, rats who were naïve to MDMA prior to the beginning of the experiment actually showed greater levels of intake for both the first and second weeks of the study. The rats that had prior exposure to MDMA (while participating in Experiment 3.2) showed lower levels of MDMA intake than those of the naïve group. The MDMA-experienced group showed a smaller decrease in MDMA consumption from

week 1 to week 2 relative to the naïve group, but the overall amount of MDMA intake was on average lower for the experienced compared with the naïve animals. It is unclear why the experienced animals consumed less MDMA than their naïve counterparts, though the experienced animals had ample time for tastes preferences or tolerance to develop that may have had an impact on the results of this study.

Further research is necessary in order to further examine the effects of free-access to oral MDMA solutions. For example, the bitter taste of MDMA may promote preference for the plain solution over the drug-solution irrespective of the drug effect associated with it. In addition, only a single dose of MDMA was used throughout the course of this study, which prevents any analysis of dose-dependent effects on intake. In order to more fully examine the oral reinforcing effects of MDMA, further experiments utilised an operant paradigm in order to examine the effects of dose on oral self-administration of MDMA.

Experiment 3.2: Operant self-administration of a MDMA/water solution

The literature reviewed previously has focused primarily on measuring MDMA self-administration using the *iv* route of administration. Those studies indicate that MDMA functions as a reinforcer when delivered via the *iv* route of administration in animal subjects. However, MDMA is primarily consumed as an oral drug in humans and thus the *iv* route of administration may not fully replicate the human experience with MDMA use. It is likely that differences in metabolism and absorption will have profound effects on the pharmacological profile of orally administered MDMA and these effects may alter the effectiveness of MDMA to serve as a reinforcer. The results of Experiment 2.1 showed that onset of action for MDMA was delayed when delivered orally as measured by locomotor activity. In addition, the maximal effect of MDMA was substantially lower when the drug was tested via the *po* route than it was when delivered *ip* or *sc*. The attenuated response of orally delivered MDMA on locomotor activity suggests that there may also be decreases in MDMA's reinforcing effects when delivered via that route. This notion is supported by the results of Reinhard and Wolffgramm (2005, 2006) that showed that MDMA was consumed only at low levels and in a controlled pattern when it was made available in drinking water. The results of Experiment 3.1 also showed that low levels of MDMA were consumed using a similar method.

In the majority of studies MDMA self-administration has been tested using instrumental responding under continuous or ratio schedules. To date, operant methods have not been used to test the reinforcing properties of oral administration of MDMA. The absence of this research remains an intriguing omission since it is not yet understood if MDMA will produce reinforcing effects in animal models that use oral rather than intravenous delivery. The following

experiment presents an initial study using an operant paradigm to test the effects of orally delivered MDMA. The experiment was modelled after those studies conducted in our laboratory using *iv* self-administration and simple schedules of reinforcement (e.g. Schenk et al., 2003, 2008).

Method

Subjects:

Subjects were 12 experimentally naïve male Sprague-Dawley rats bred in the School of Psychology Animal Facility at Victoria University of Wellington. Subjects were aged approximately eight weeks old at the start of training and weighed between 240-285 grams at the beginning of testing. Subjects were housed individually in polycarbonate cages cage tops made of metal grating situated in the testing room. The testing room was maintained on a reversed 12:12 hour light/dark cycle with lights on at 6pm and maintained at a constant temperature between 19-21°C. Animals were fed ad libitum with food pellets (Diet 86, Sharpes, New Zealand) but water access was restricted and only available for a 90-minute period subsequent to daily test sessions. Experimental sessions were conducted five days a week, however on days when subjects did not have experimental sessions they were given free access to water in the home cage. Restricted access to water was reinstituted approximately 20-hours prior to the next week's scheduled experimental sessions. Animals were treated in accordance with ethical guidelines set forth by the Victoria University of Wellington Animals Ethics Committee.

Apparatus/materials:

Equipment:

Experimental sessions were conducted in six ENV-008 modular test chambers (Med Associates Inc.) equipped with two retractable levers (ENV-112CM, Med Associates Inc.) situated at the front of the chamber to either side. Chambers were enclosed in light and sound attenuating cubicles.

Liquid reinforcers were delivered by a liquid dipper (ENV-202M-UP, Med-Associates Inc.) in the volume of 0.1 cc per reinforcer. The liquid dipper for presentation of reinforcers was situated in the centre at the front of the chamber 2 cm from the chamber floor. The two retractable levers were positioned to the left and right 8 cm from the liquid dipper and 3 cm from the sides of the chamber. At the rear of the chamber a house light was positioned 22 cm directly above the chamber floor.

Solutions:

+/-3,4-methylenedioxymethamphetamine hydrochloride (ESR, Porirua, New Zealand) was mixed with tap water at doses ranging between 0.040625mg/ml and 0.825mg/ml. All drug weights refer to the salt.

Procedure:

Acquisition Phase:

Water-deprived rats were initially auto-shaped during daily 90-minute sessions to lever press and were reinforced with a single dip (0.06cc) of tap water. Auto-shaping lasted for approximately five days. During the auto-shaping procedure the right lever would insert into the chamber and the house light would illuminate

signalling the availability of reinforcement. If no response was recorded within 30-s the lever would retract, the house light would extinguish and the liquid dipper would activate providing the subjects with a free reinforcer. After a 30-s timeout a new trial would commence with insertion of the right lever and illumination of the house light. Responses to the lever within 30-s of the lever insertion resulted in presentation of the dipper followed by a 5-s ITI after which the next trial began. At the conclusion of each days testing animals were given access to water bottles for 90 min resulting in a 21-hour water deprivation regime across all conditions.

Experimental testing began when all rats had successfully acquired lever pressing. At the beginning of each test session the right retractable lever would insert into the chamber and the house light would illuminate. Animals were reinforced for responding on the right hand lever on an FR1 schedule of reinforcement with a single dip of MDMA solution (dose range: 0.01mg/kg/reinf – 0.2 mg/kg/reinforcer). The left-hand retractable lever was never inserted into the chamber during test sessions thus had no scheduled consequence. After completion of the response requirement (FR1) the lever was retracted, the house light extinguished and the liquid dipper was activated, resulting in presentation of the reinforcer. The liquid dipper was set in the normally up position resulting in reinforcers being available for collection until another successful response requirement was met (resulting in the loss of any previously available, but not collected, reinforcer). A 10-s ITI preceded the start of the next trial.

After establishing responding at FR1 the response requirement was increased in order to promote increased responding during daily test sessions. Initially the fixed ratio was increased to FR2 and later FR4.

Testing Phase

After extensive experience with MDMA-reinforced responding a dose-response curve determination was conducted. Beginning with the largest dose, animals were tested on each dose of drug for four consecutive days on an FR4 schedule of reinforcement. The drug doses tested during this experiment in chronological order were: 0.2, 0.08, 0.04, 0.02, 0.01mg/kg/reinforcer and vehicle solution (H_2O).

Results and Discussion

For each rat the number of responses made on the FR4 schedule was averaged across the four daily sessions of each dose. Figure 3.7 shows the mean total responses across rats at each dose. This figure shows that animals responded in a dose dependent manner, as dose decreased the subjects' responding increased thus maintaining intake of MDMA at a relatively stable level. This group function was fairly representative of the dose response curves for the individual rats (see Figure 3.9). With the exception of Rat 41 the rats were sensitive to changes in dose of MDMA; however, idiosyncratic differences in the total rate of responding were apparent. Subjects 33, 36 and 46 produced more step-like dose-response functions where responding decreased sharply with increases in dose.

A one-way ANOVA with repeated measures found a significant main effect of dose, F (4, 44) = 30.361, $p < 0.001$. Post-hoc contrasts found significant differences in responding between doses (all $p < 0.05$) with the exception of that between the 0.08mg/kg and 0.2mg/kg/reinforcer doses (F (1, 11) = 2.745, p = 0.126).

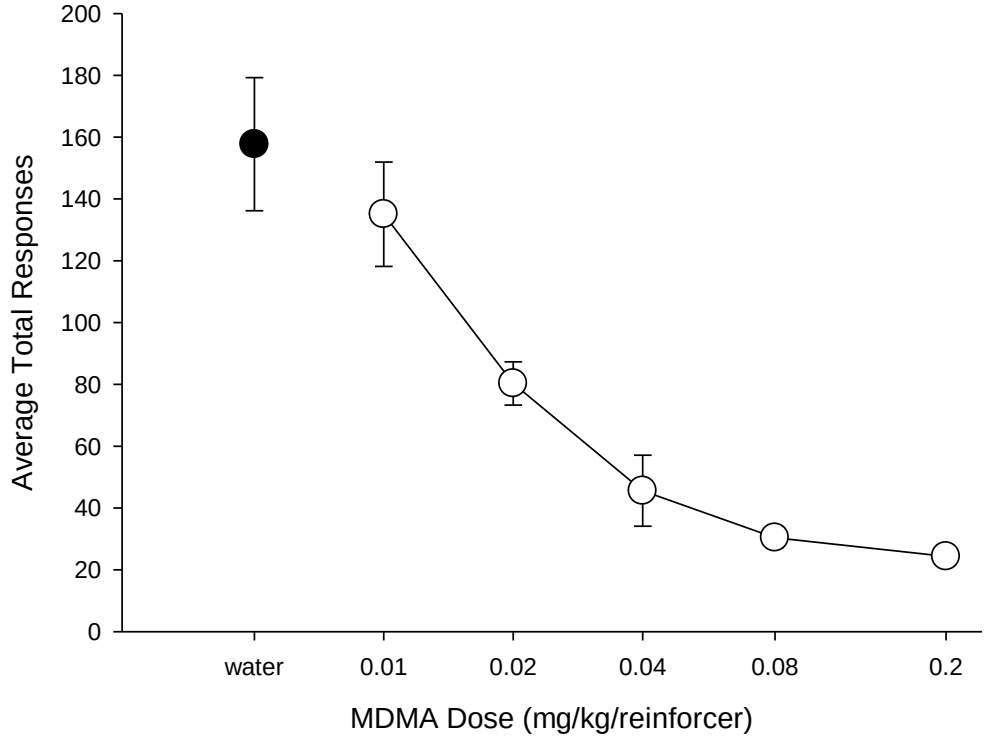

Figure 3.7: Dose-response curve for the oral self-administration of MDMA in a water vehicle. Subjects were reinforced on an FR4 schedule. Error bars represent standard error of the mean.

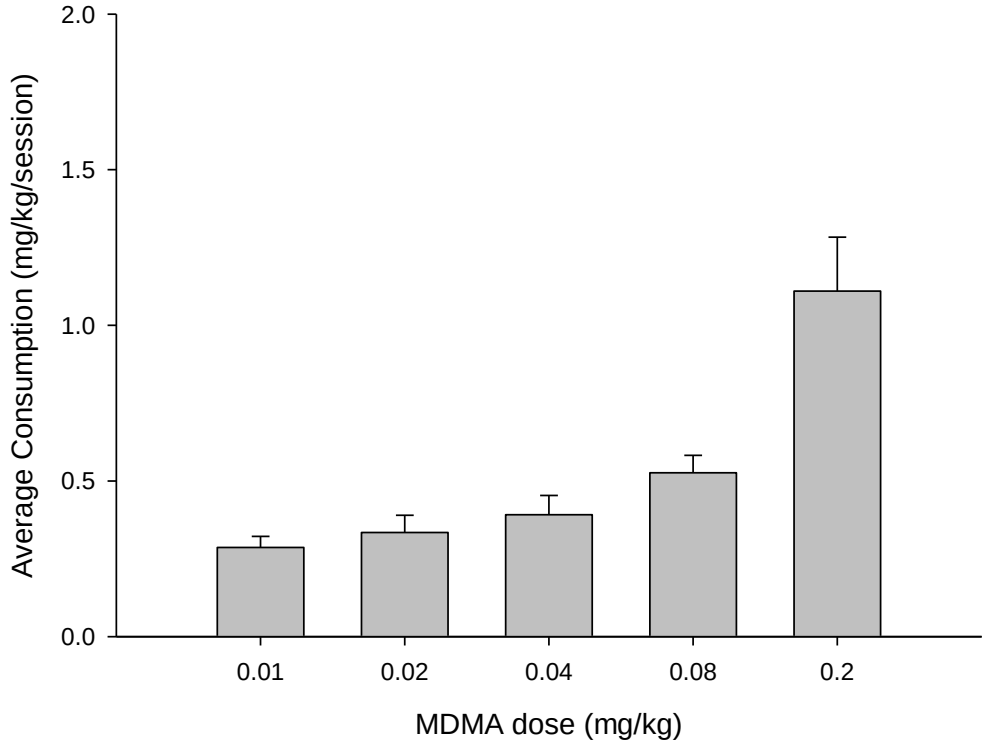

Figure 3.8: Average daily MDMA consumption in mg/kg plotted as a function of MDMA dose. Error bars represent standard error of the mean.

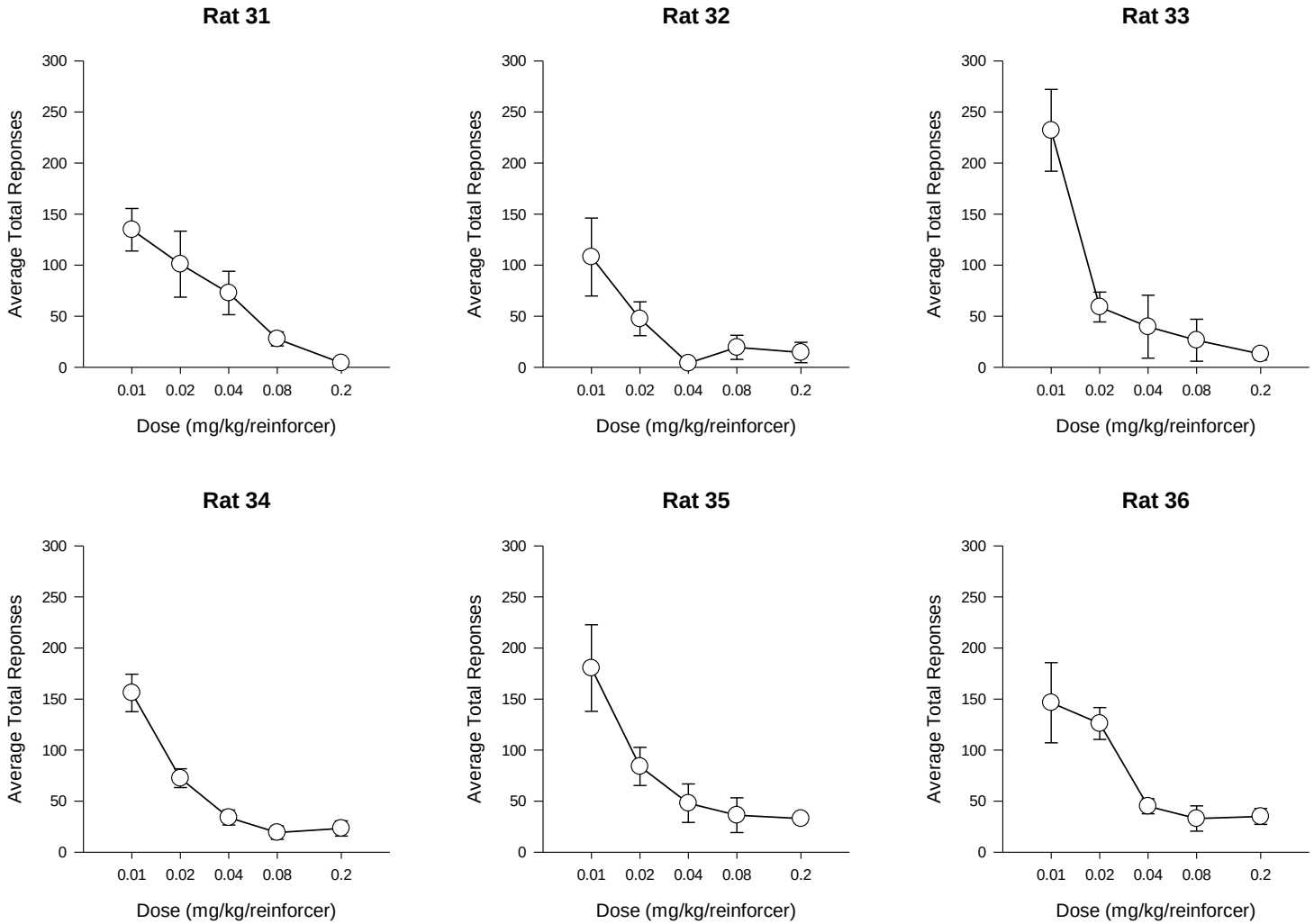

Figure 3.9: Dose-response curves for the oral self-administration for MDMA for 12 rats. Subjects were reinforced according to an FR4 schedule of reinforcement. Error bars represent standard error of the mean.

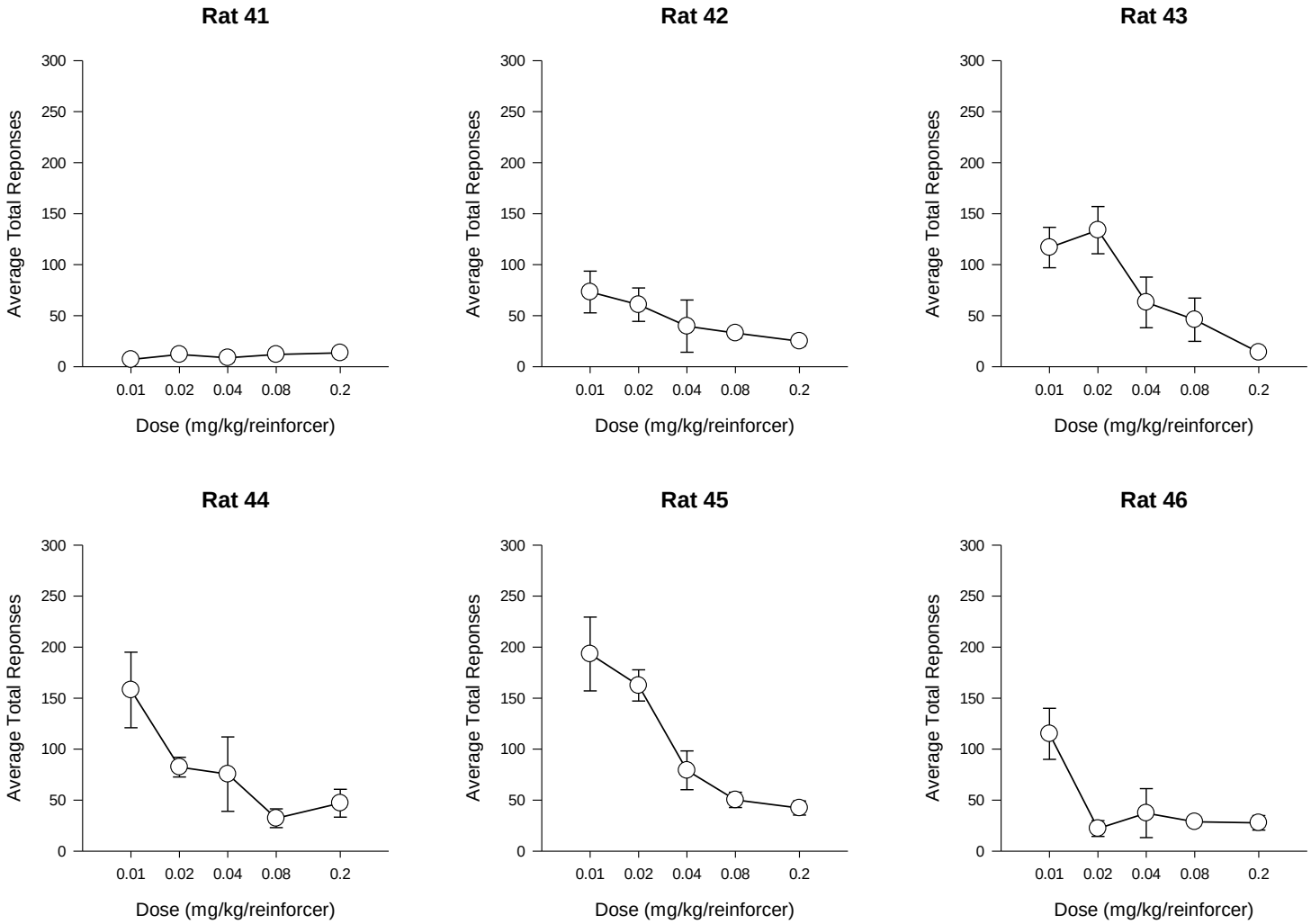

Figure 3.9: (cont) Dose-response curves for the oral self-administration for MDMA for 12 rats. Subjects were reinforced according to an FR4 schedule of reinforcement. Error bars represent standard error of the mean.

In order to calculate consumption of MDMA by the animals the total number of reinforcers was multiplied by the dose and the volume of each reinforcer before being divided by each rat's body weight on each session. Consumption of MDMA was analysed by examining the average amount of drug consumed as a function of dose and is displayed in Figure 3.8. Data were averaged across each of the four sessions tested for each dose and the group function represents the average of all 12 rats. Figure 3.8 shows consumption increased with increasing dose with the highest consumption of MDMA occurring for the highest dose tested. A one-way ANOVA was conducted and showed a significant main effect of dose, F (4, 44) = 20.613, $p < 0.001$. Post-hoc paired samples t tests were conducted and showed a significant difference between 0.08 and both the 0.04 (t (11) = -3.302, $p = 0.007$) and the 0.2mg/kg/reinforcer doses (t (11) = -3.893, $p = 0.003$). Consumption of MDMA was highest for the largest dose 0.2 mg/kg/reinforcer, despite subjects having the lowest response rate at that dose. Note that some responding would be likely due to the water-deprivation conditions; hence it is unclear whether the consumption at the highest dose was the result of the drug's effects or alternatively a result of fluid deprivation. Fluid deprivation seems unlikely as an explanation as the animals were exhibiting extremely low rates of reinforcement during the 0.2mg/kg/reinforcer condition; gaining on average 4 reinforcers per session, or the equivalent of only 0.4ml.

Responses throughout the session were analysed to look at the time-course of drug-reinforced responding throughout self-administration sessions. Figure 3.10 (top panel) shows the average number of responses throughout the 90-minute sessions in 10-minute bins as function of drug dose. For all conditions the highest rate of responding occurred within the first 10-minutes of the session. Animals continued to respond at a slower rate during minutes 11-20 but

responding had virtually ceased by minutes 21-30. A two-way repeated measures ANOVA showed a significant interaction between dose and bin, F (40, 440) = 22.725, $p < 0.001$. There was an inverse relationship between dose and rate of responding suggesting that water deprivation was not the sole reason for responding. Lower doses of drug had higher response rates than did higher doses of MDMA. Figure 3.10 (bottom panel) plots the percentage of total responding plotted as a function of 10-minute time bins and indicates that the majority of responding for each dose (range 58%-64%) occurs during the first 10 minutes of each session, after which responding sharply decreases during the next two time bins before dropping to virtually nothing.

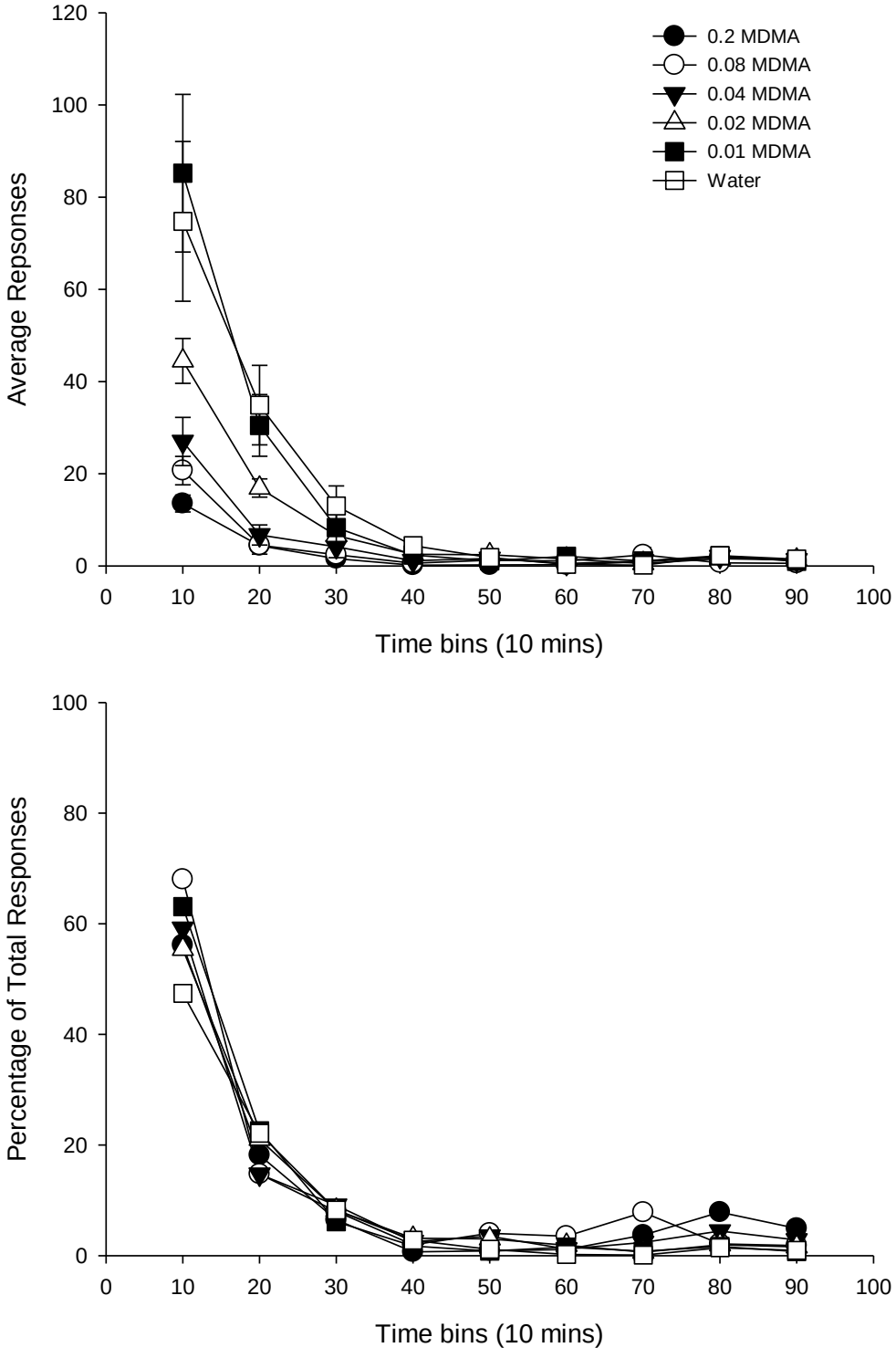

Figure 3.10: (Top panel) Timecourse of responding for the oral self-administration of MDMA in rats. Total responses for each rat were binned into 10 min blocks and averaged across each day of training for a particular dose. Data represent averages across rats. Error bars represent standard error of the mean. (Bottom panel) Percentage of total responding for each dose plotted as a function of time bin.

In addition to testing different doses of MDMA subjects were also tested with water (vehicle) to determine any effects on behaviour potentially caused by thirst-activated responding due to the water deprivation. The results are shown in Figure 3.7 and show that total responses in a session for water were higher than it was for any of the drug conditions. A paired sample *t* test was conducted between vehicle and the dose that produced the highest total responding; 0.01mg/kg/reinforcer. Responding for vehicle was significantly higher than responding for the 0.01mg/kg/reinforcer dose, t (11) = 2.224, p = 0.048. It was hypothesised that rats would show a preference for MDMA-containing solutions, but in contrast the highest response rate was for the drug-free vehicle. Several factors may have contributed to this finding. Firstly, the animals in this study were maintained on a rather strict water deprivation regime. Animals only had access to water during the 90-minute sessions followed by a further 90-minute period of free access to water in the home cage. Thus deprivation may have contributed to high levels of responding for water. Animals were clearly able to discriminate water from the drug-containing solutions since water produced significantly higher responding than each of the five drug doses tested. However, it is possible that the discrimination evidenced here is caused by differences in the taste of the solutions as opposed to the effect of the drug itself.

Unfortunately taste of the drug-containing solutions causes a potential confound that may be a factor in the dose-effect curves produced. With each reduction in dose there is also a reduction in the taste of the solution. Stronger doses of the drug have a stronger taste due to a higher drug concentration and are potentially more aversive, thereby suppressing responding at higher concentrations. However, with no adulterant to mask the taste of the drug it is unclear whether the increases in responding were the result of the drug dose

manipulations or merely a response to a reduction in the potentially aversive taste of the drug-containing solutions. This may explain why responding was highest for the lowest of the drug doses tested, as that solution may have tasted more akin to water resulting in a less aversive solution. Of note here is the absence of a typical inverted U-shaped dose-response function consisting of both an ascending and descending limb. It, however, remains possible that the ascending limb of the dose response curve is being masked by the high rates of responding for the vehicle solution.

Due to the water deprivation regime used in this study the drug solutions may be acting as a compound stimulus consisting of the reinforcing drug properties, in addition to the reinforcing properties of the vehicle solution. Of note here is that water deprivation (leading to thirst) may be acting as an establishing operation (Michael, 1993) that increases the value of the vehicle component. Establishing operations are environmental events, conditions or states that alter the reinforcing effectiveness of a reinforcer either by increasing reinforcing value (motivating operations) or decreasing value (abolishing operations) (Tapper, 2005). For example, hunger is a motivating operation for food reinforcers; food has greater reinforcing value for someone who has not eaten than to someone who has. Similarly, satiety can act as an abolishing operation in that food has less reinforcing value when someone has recently eaten. For example, water has more value to a thirsty man in the in middle of the Sahara desert than to someone who is visiting a mall. In addition establishing operations can act to increase or decrease goal-directed behaviours related to a given reinforcer (Michael, 1993; Tapper, 1995). For example the thirsty man in the desert will expend more of his time searching for water than would the man visiting the mall. In the context of the current experiment thirst would increase the value of water as a reinforcer and may contribute greatly to the reinforcing strength of the

combined drug-vehicle compound solution. Generally speaking water is a weak reinforcer under conditions of free access, but much stronger under restricted conditions (for example see Case, Nichols & Fantino, Experiment 3, 1995). Because thirst may enhance the reinforcing properties of the vehicle, in this case water, making firm conclusions about the reinforcing properties of the actual drug solution independent of the reinforcing value of the vehicle is difficult.

Further testing may have determined the contributions of the reinforcing properties of the drug versus the vehicle solution. If lower doses of MDMA were tested and it produced compensatory responding above levels seen for the vehicle solution then it would be likely that the drug had at least some reinforcing properties. Alternatively, if as dose decreased, responding decreased then it would be evidence for the ascending limb of the dose-response curve.

As both taste and water-deprivation have confounding effects preventing clear interpretation of the current results a further study was designed in which the effects of water-deprivation were removed and palatability of the solution was increased by adulterating the solution with sweetened saccharin solution in order to ameliorate taste-related factors in the oral self-administration of MDMA.

Experiment 3.3: Operant self-administration of an MDMA/saccharin solution

The results of Experiment 3.2 showed that MDMA produced results consistent with the descending limb of the dose-response curve. The results are also consistent (at least partially) with dose response curves generated for *iv* administration of MDMA and other drugs of abuse; however, the results of Experiment 3.2 are potentially confounded with both tastes and/or with the water deprivation conditions under which the animals were tested. In order to overcome these confounding variables a further experiment was conducted to reassess the dose-response function in an MDMA solution containing saccharin as the vehicle. Rats respond readily for saccharin solution so it was no longer necessary for subjects to be water-deprived prior to experimental sessions. Saccharin was chosen over sucrose for its non-caloric nutritional value in order to eliminate any confounds produced by the addition of calories to the solution. Another concern arising from Experiment 3.2 was that food intake was not controlled for in that study. Though food was not available during testing sessions, there was no control over stomach content prior to daily sessions. The presence of food in the stomach may alter the efficacy of the drug by slowing absorption by delaying the gastric emptying allowing for increased metabolism due to a delay in reaching the gastrointestinal tract wherein the majority of absorption occurs. Thus under such circumstances testing the drug under conditions that control for stomach content is highly desirable. To this end subjects in the current experiment were tested at 85% of their free feeding weights and were fed their normal diet of rat chow post-session in order to directly control stomach content during experimental sessions.

Much like water in the previous experiment, it is expected that saccharin-alone will produce relatively high rates of responding. Of particular interest is whether

the MDMA/saccharin solution will produce a typical dose-response function and replicate the findings of Experiment 3.2 in non water-deprived animals.

Method

Subjects:

This study used the same 12 subjects that had been previously used for Experiments 3.2 and 3.1B. Subjects were 12 male Sprague-Dawley rats who had extensive experience with orally delivered MDMA solutions. Subjects were approximately ten months old at the beginning of this experiment and weights ranged from 395 – 523 grams (*M* = 452, *SD* = 34.8) Animals were maintained at 85% of their free feeding weights with post-session feeding (Diet 86, Sharpes, New Zealand). Water was freely available in the home cage. Subjects were housed individually in polycarbonate cages cage tops made of metal grating situated in the testing room. The testing room was maintained on a reversed 12:12 hour light/dark cycle with lights on at 6pm and maintained at a constant temperature between 19-21°C. Experimental sessions were conducted five days a week.

Apparatus/materials:

Equipment:

Sessions were run in same chambers as those used during Experiment 3.2. However, during this study the dipper cup used to deliver the reinforcers was decreased to 0.02cc per reinforcer.

Solutions:

MDMA hydrochloride (ESR, Porirua, New Zealand) was mixed in a vehicle of 0.2% (w/v) Sodium Saccharin (Sigma-Aldrich, New Zealand) dissolved in tap water. Due to the reduction in reinforcer size caused by the change in dipper cup, each drug dose was increased by a factor of five in order to produce an equivalent amount of drug in each reinforcer across Experiments 3.2 and 3.3 in order to accommodate for direct comparisons between these two studies. The following doses were tested in this study; 0.02; 0.04; 0.08; 0.16mg/kg/reinforcer and vehicle-alone (0.2% saccharin solution). All drug weights refer to the salt.

Procedure:

Subjects were run five days a week in daily 60-minute sessions. It was noted during Experiment 3.2 that few responses were made in the last third of the 90-session so session times in this study were decreased accordingly.

At the beginning of each experimental session the right hand lever was inserted into the chamber and the house light illuminated. Subjects were reinforced on an FR4 schedule with a single dip of liquid reinforcer (drug or vehicle). Following reinforcement there was a 10-s ITI before starting the next trial that was signalled by the insertion of the right-hand retractable lever and illumination of the house light.

Initially subjects were tested with 0.2% saccharin vehicle solution as the reinforcer. Testing for saccharin was continued for 14 days at FR4, with only the last five days testing used for subsequent analysis. Following saccharin testing each drug dose was tested for five consecutive sessions (Monday to Friday) before examination of the next dose the following Monday. The order of

testing was run in an ascending dose sequence beginning with 0.02, followed by 0.04, 0.08 and 0.16mg/kg/reinforcer respectively. Due to data loss caused by a computer error during testing of the 0.8mg/kg/reinforcer dose a further five days testing was conducted; however only the last five sessions were subsequently used in the data analysis.

Results and Discussion

The mean number of responses per session was calculated across each of the five sessions of each dose. The results can be seen in Figure 3.11. Results show that the mean total numbers of responses was highest for saccharin and for the 0.02 mg/kg/reinforcer dose of MDMA. Lower total responding was shown for the 0.04, 0.08, and 0.16mg/kg/reinforcer MDMA doses respectively. A one-way repeated measures ANOVA found a significant main effect of Dose, F (4, 44) = 14.76, $p < 0.001$. Pair-wise comparisons revealed significant differences between all MDMA doses ($p < 0.01$) but no significant difference between the lowest dose of MDMA (0.02 mg/kg/ reinforcer) and the saccharin vehicle.

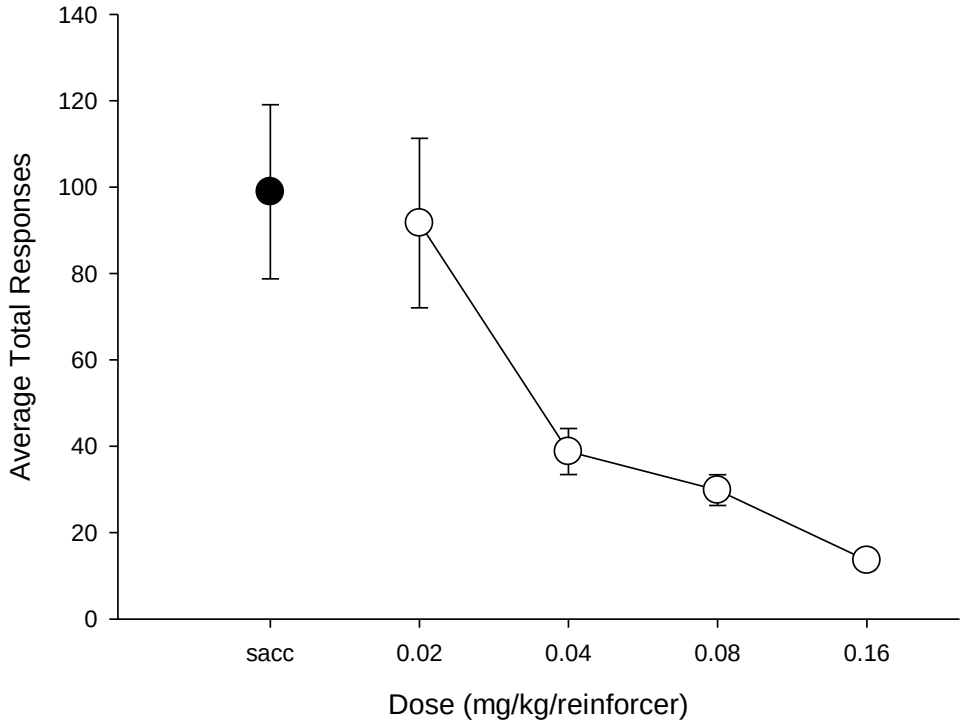

Figure 3.11: Dose-response curve for the self-administration of oral MDMA in 0.2% saccharin vehicle. Subjects were reinforced on an FR4 schedule of reinforcement. Open circles represent drug-containing solution. Closed circles represent the vehicle-alone condition. Error bars represent standard error of the mean.

Overall oral self-administration produced a typical dose-response curve with greater responding found for lower doses of drug. As expected responding for the saccharin vehicle remained high, though the lowest dose of MDMA tested produced comparable responding. The dose of MDMA appears to have strong control over responding and animals continue to respond for MDMA in a dose-dependent pattern.

Data collected from individual subjects are shown in Figure 3.12. Rats 33, 35, 36, 42, 44, 45 and 46 all produce typical dose-response curves, while rats 31 and 34 produce functions that exhibit dose-dependant responding, though to a lesser degree. Rats 32, 41 and 43 did not produce dose-appropriate responding, however, these animals also exhibit low response rates during the

vehicle-only condition indicating an overall deficit in responding (data not shown).

Further analysis was conducted to examine the total consumption of MDMA across each dose of drug. The average total consumption of MDMA (mg/kg) is shown in Figure 3.13 and did not vary as a function of dose, F (3, 33) = 2.011, p = 0.131. There were no significant differences found between levels of intake across doses indicating that animals adjusted their responding according to the dose of the drug. Thus, lower doses of orally administered MDMA exhibited higher rates of responding as can be seen in Figure 3.11, while the total amount of drug consumed stayed the same across different doses tested.

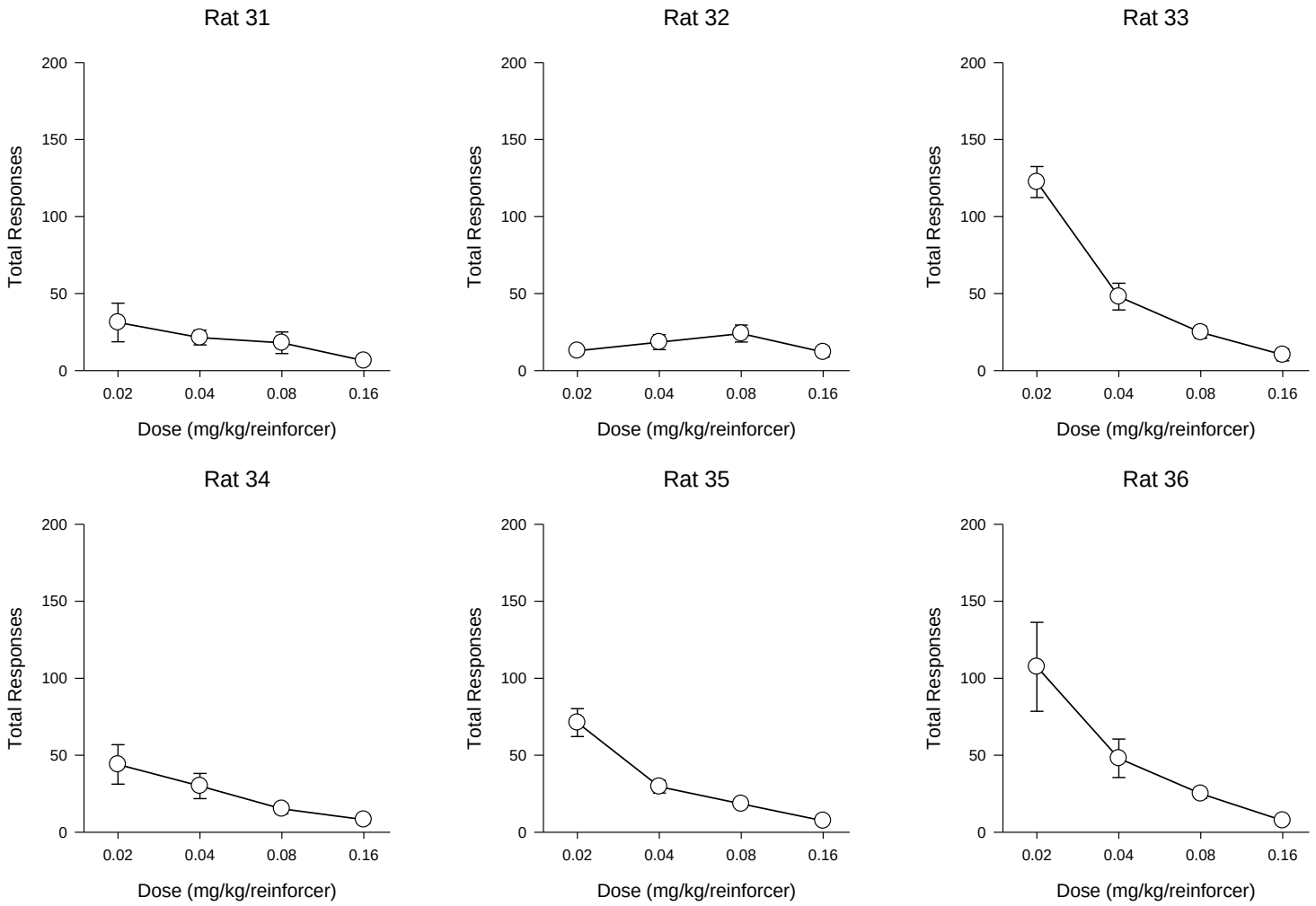

Figure 3.12: Dose-response curve for the oral self-administration of MDMA in 0.2% saccharin solution for 12 rats. Subjects were reinforced on a FR4 schedule of reinforcement. Error bars represent standard errors of the mean.

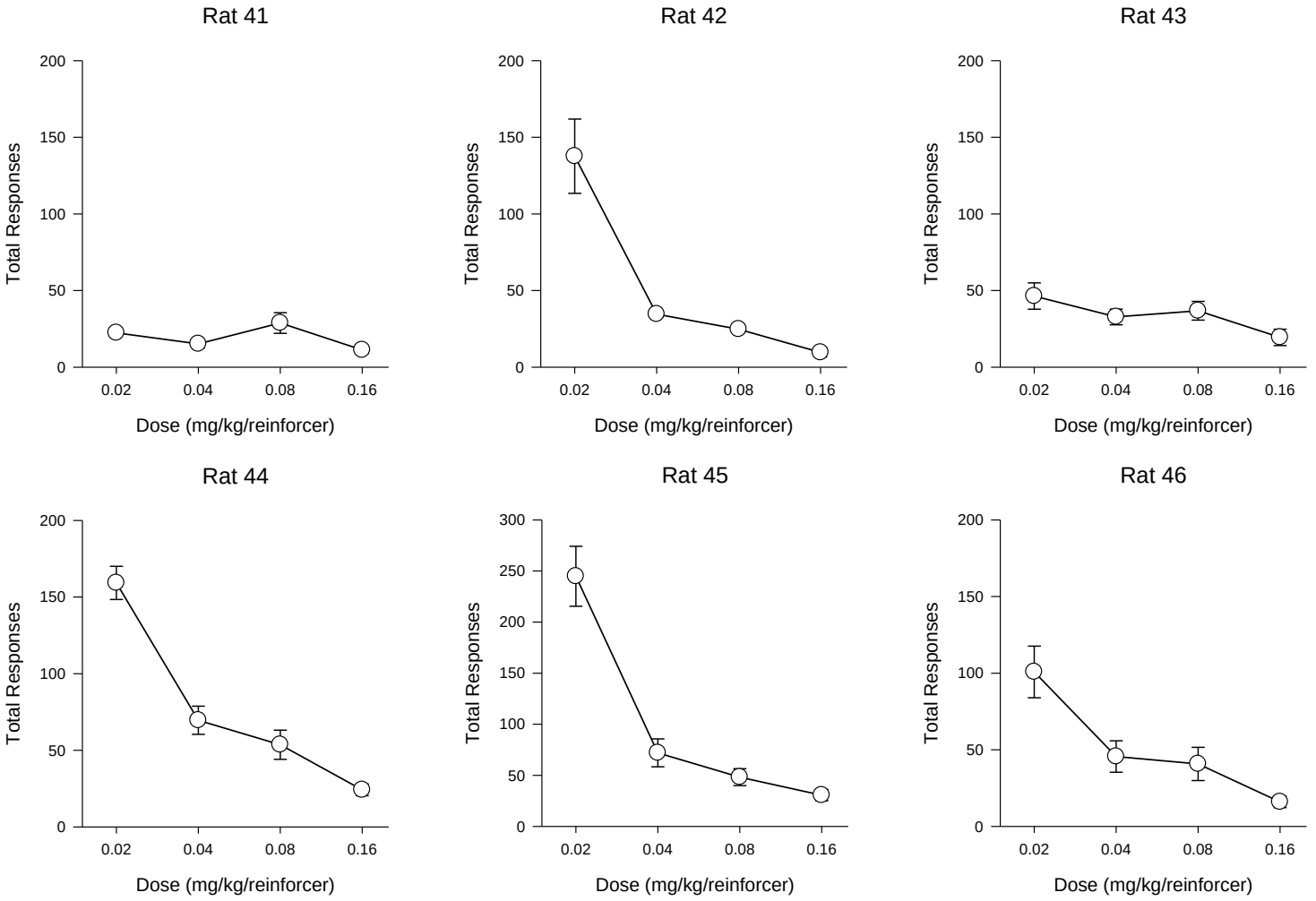

Figure 3.12: (cont) Dose-response curve for the oral self-administration of MDMA in 0.2% saccharin solution for 12 rats. Subjects were reinforced on a FR4 schedule of reinforcement. Error bars represent standard errors of the mean.

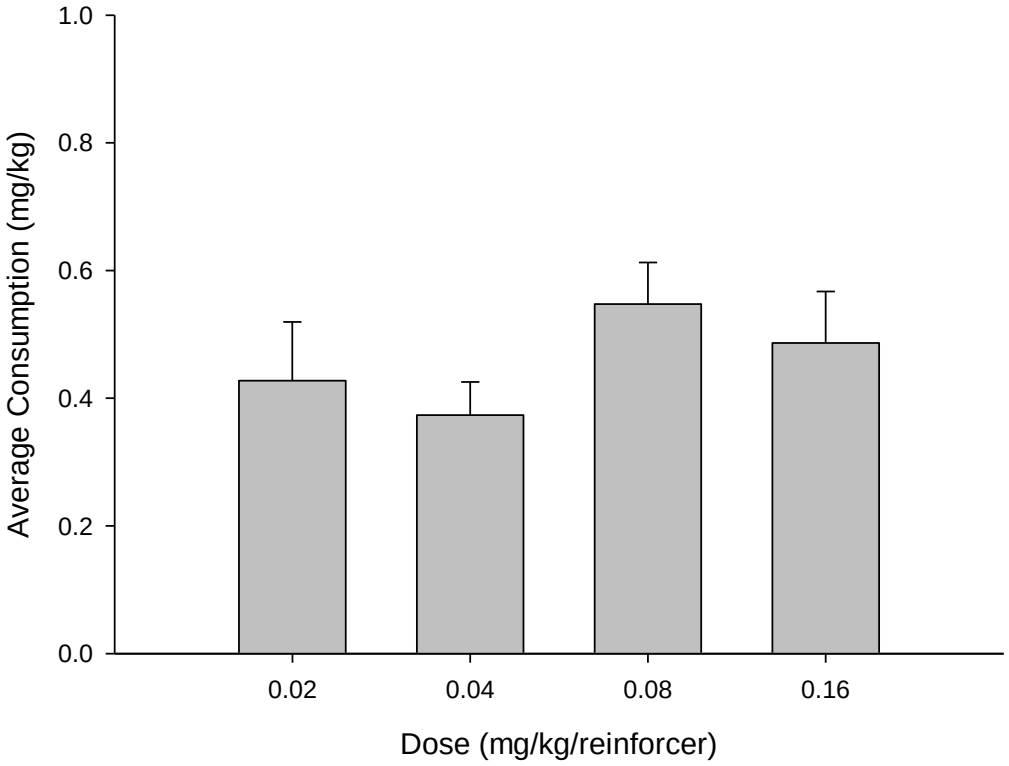

Figure 3.13: Average MDMA consumption plotted as function drug dose (n =12). Error bars represent standard error of the mean.

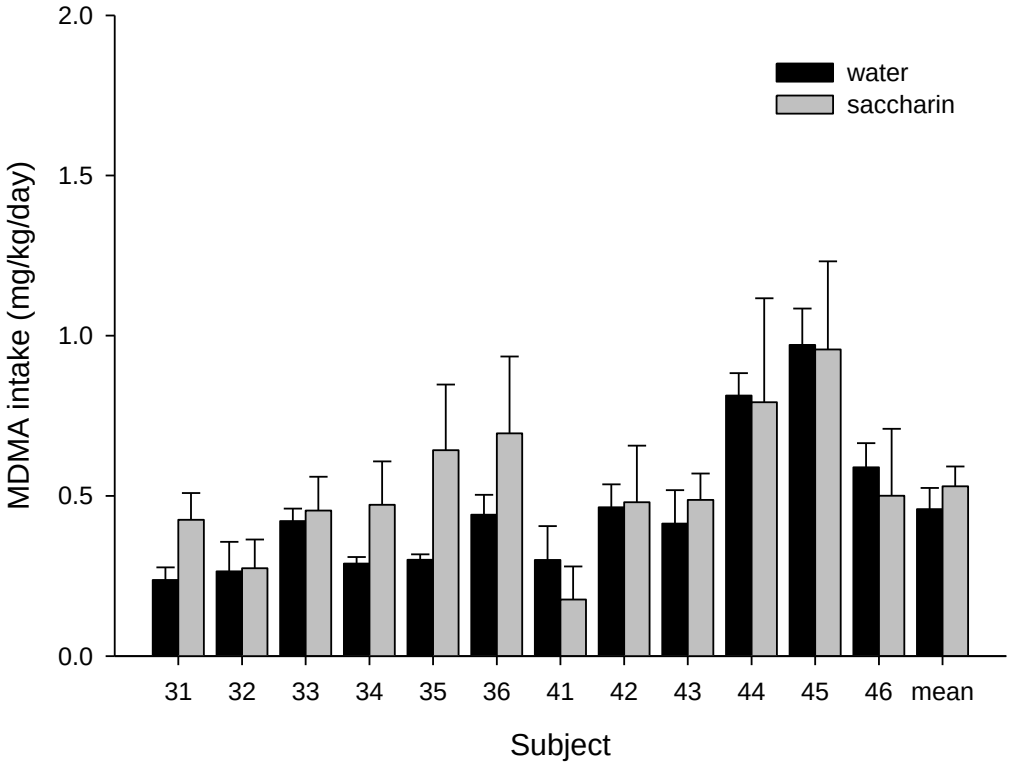

Figure 3.14: Average MDMA intake for individual subjects for water vehicle (black bars, Experiment 3.2) and saccharin vehicle (grey bars, Experiment 3.3) conditions. Error bars represent standard error of the mean.

Comparison of saccharin vs. water vehicle conditions

The current experiment used subjects who had already had significant experience with oral self-administration of MDMA in both the operant and free-choice paradigms (Experiments 3.1B & 3.2). Both water (Experiment 3.2) and saccharin vehicle solutions (current study) were generally indicative of similar responding for MDMA as a function of dose. The results in Experiment 3.3 were compared with those found in Experiment 3.2 in order to examine whether similar results were found for each vehicle condition (water vs. saccharin). The dose-response curve produced for the animals in Experiment 3.2 was compared with the curve obtained from the current study. Only those doses that were replicated across the experiments were included in this analysis. A two-factor repeated measures ANOVA utilising dose (3-levels: 0.2, 0.4 and 0.8mg/kg/inf) and vehicle (2-levels: water vs. saccharin) found a significant main effect of dose, F (2, 22) = 20.36, $p <$ 0.001, but no significant interaction (F (2, 22) = 0.94, ns) or main effect of the vehicle (F (2, 22) = 0.03, ns). Despite the differences in volume of reinforcer and vehicle there were no differences found between the dose-response curves for each experiment, at least across the common doses tested in these studies. Further analysis was conducted on MDMA intake (mg/kg) across each of the vehicle conditions. The average MDMA intake of each rat was averaged across doses for each vehicle and can be seen in Figure 3.14. The grey bars in Figure 3.14 show the average MDMA intake for rats in this study (saccharin vehicle) was 0.46mg/kg/session (SD = 0.22, range: 0.24 – 0.97mg/kg/session). There was substantial variation between intakes across rats, though generally variation was low for a given rat. Black bars in Figure 3.14 show corresponding intake levels for the same rats when water was the vehicle in Experiment 3.2. Mean intake during Experiment 3.2 was 0.53mg/kg/session (SD = 0.21, range: 0.18 – 0.96mg/kg/session). Analysis by t test found no significant differences between

drug intakes for the water or saccharin vehicle conditions. Individual analysis of each subject revealed that 7 out of 12 animals showed MDMA intake consistent across both vehicle conditions. Of the five remaining subjects, four subjects showed substantially higher responding when water was the vehicle, while only one subject showed the opposite effect. It is interesting to note that for those subjects that did show a change in intake across vehicle conditions tended to show a decrease in intake despite the addition of sweet tasting saccharin.

The similarities noted between the results for Experiments 3.2 and 3.3 suggest that the interpretation of these results as drug-mediated responding is more plausible, rather than being due to confounds evident within Experiment 3.2 and noted previously.

Experiment 3.4: Effect of SCH 23390 on the oral self-administration of MDMA

In light of the results found in Experiments 3.2 and 3.3 oral MDMA appears to promote and maintain at least a low level of self-administration behaviour in rats. However, it remains unclear how reinforcing the MDMA containing solutions are due to the high overall responding found for the vehicle conditions in both experiments using water and saccharin vehicles.

A large body of literature has implicated dopamine in the reinforcing effects of many drugs of abuse (Di Chiara & Imperato, 1988; Di Chiara et al., 2004; Self & Nestler, 1995). Specifically, Daniela et al. (2004) showed the involvement of dopamine in the self-administration of MDMA by finding that pre-treatment with the D1 receptor antagonist SCH 23390 shifted the dose-response curve for *iv* MDMA self-administration to the right. In addition, Brennan et al. (2009) showed increased responding for MDMA due to partial blockade of post-synaptic DA receptors when subjects were administered the D2-like receptor antagonist eticlopride. Fantegrossi et al. (2002) showed attenuation of MDMA self-administration through blockade of the 5-HT2 receptor using the antagonists ketanserin and MDL 100907, though this result was likely caused by the 5-HT2C receptor's ability to downregulate dopamine release (Alex & Pehek, 2007).

The dopamine D1 antagonist SCH 23390 was administered prior to daily self–administration sessions in order to test the extent to which MDMA contributes (via mediation of dopamine release) to the reinforcing properties of the oral solutions used in the current studies. If responding for oral MDMA solutions is mediated by dopamine release then administration of the antagonist SCH 23390 should partially block dopamine binding and shift the dose response curve for MDMA to the right.

Method

Subjects:

Twelve naïve male Sprague rats participated in this study. Animals were approximately four months old at the time of testing and weights ranged from 293 - 337 grams (*M* = 318, *SD* = 11.9) Animals were maintained at 85% of their free feeding weights with post-session feeding (Diet 86, Sharpes, New Zealand). Subjects were housed individually in polycarbonate cages with cage tops made of metal grating situated in the testing room. The testing room was maintained on a reversed 12:12 hour light/dark cycle with lights on at 6pm and maintained at a constant temperature between 19-21°C. Experimental sessions were conducted seven days a week. Research was approved and all animals were treated in accordance with ethical guidelines set forth by the Victoria University of Wellington Animals Ethics Committee.

Apparatus/materials:

Equipment:

Sessions were run in same chambers as those used during Experiments 3.2 and 3.3.

Solutions:

MDMA hydrochloride (ESR, Porirua, New Zealand) was mixed in a vehicle of 0.2% Sodium Saccharin (Sigma Aldrich, New Zealand) dissolved in tap water. The following doses were tested in this study; 0.003, 0.006, 0.0125, 0.025 and 0.05 mg/kg/reinforcer and vehicle (0.2% w/v saccharin solution). SCH 23390 (Sigma Aldrich, New Zealand) was dissolved in a sterile 0.9% saline solution and injected at a volume of 1ml/kg. SCH 23390 was injected at a dose of 0.01mg/kg *sc*.

Procedure:

Subjects were run seven days a week in daily 60-minute sessions. At the beginning of each experimental session the right hand lever was inserted into the chamber and the house light illuminated. Subjects were reinforced on an FR1 schedule with a single dip (0.02cc) of liquid reinforcer (MDMA + saccharin or saccharin-alone). Following reinforcement there was a 10-s ITI before the beginning of the next trial that was signalled by the insertion of the right hand retractable lever and illumination of the house light.

Subjects were initially autoshaped during daily sessions using a diluted sweetened condensed milk solution. Once reliable responding was established condensed milk was replaced with 0.2% saccharin solution and animals continued daily sessions. Daily sessions continued until baseline responding for 0.2% saccharin stabilised after which experimental testing began. Each dose condition consisted of 10 days of testing. Day 1-4 consisted of baseline for the current reinforcer (MDMA + saccharin solutions or saccharin-alone). Day 5-10 consisted of treatment days on which subjects received pre-treatment with either SCH 23390 0.01mg/kg or 0.9% saline injection administered *sc* in the homecage 15-min prior to daily sessions. In total each subject received pre-treatment with SCH 23390 or saline injections for three sessions each that were delivered in a pseudorandom order (determined individually for each rat by coin flip) such that SCH 23390 or saline were given for a maximum of two consecutive sessions. The saccharin-alone condition was conducted first followed by MDMA dose conditions conducted in descending order.

Results and Discussion

Baseline responding for MDMA

Mean total responding for saline pre-treatment conditions is shown in Figure 3.15. Data from the saline condition was analysed in order to examine the dose-response function for the oral self-administration of MDMA. A one-way ANOVA revealed a significant main effect of dose, F (5, 55) = 7.409, $p < 0.01$). Contrasts revealed that all MDMA doses were significantly different from one another (all $p < 0.05$), but there was no significant difference between responding for vehicle alone and the 0.003 mg/kg/reinforcer dose of MDMA. This replicates the findings, using naïve rats, of significant dose-response relationships found previously in Experiments 3.2 and 3.3. However it must be noted that doses used in the current study were lower than those used in previous experiments. Subjects had a mean MDMA intake across doses of 0.167 mg/kg (SE = 0.024). In comparison, subjects in Experiment 3.3 (which also used a saccharin vehicle) had a mean MDMA intake of 0.46 mg/kg (SE = 0.075). In both experiments, responding for the lowest dose was approximately equivalent to saccharin-alone responding despite the dose ranges used in each experiment being different.

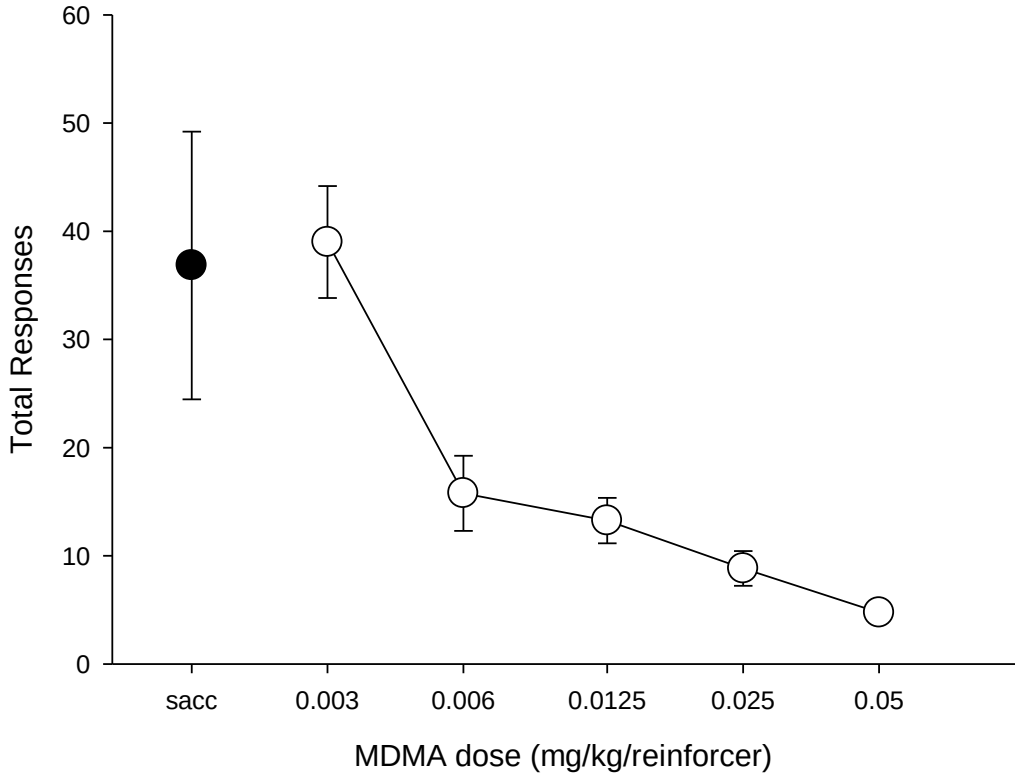

Figure 3.15: Dose-response curve for the oral self-administration of MDMA in a 0.2% saccharin vehicle solution. Rats (n = 12) were reinforced according to an FR1 schedule of reinforcement. Error bars represent standard error of the mean.

Figure 3.16 simultaneously plots the dose effects curves for both the current experiment as well Experiment 3.3 (plotted as reinforcers earned) and suggests that subjects in the current experiment appear to be more sensitive to the dose of the MDMA as evidenced by a shift in the dose-response curve to the left. It must be noted that subjects in the current study were naïve when experiments began and had much less experience with oral MDMA than did subjects in Experiment 3.3. This raises the possibility that subjects in Experiment 3.3 had developed a tolerance to MDMA due to their extended experience with the drug in prior experiments. The differences in dose-response functions across experiments are suggestive that the MDMA-experienced animals used in Experiment 3.3 may be more tolerance to the drugs reinforcing properties; however, care must be exercised as subjects in the

current study received reinforcement under a continuous reinforcement schedule, while subjects in the Experiment 3.3 were reinforced on an FR4 schedule. The effects of even subtle changes in ratio requirements have been shown to produce robust changes in responding across a range of reinforcers (see Chapter 4 for further discussion of this topic). Furthermore, it remains a possibility that subjects in the current study were more sensitive to the bitter taste of the MDMA + saccharin solution and that decreases in concentration make the solution more palatable.

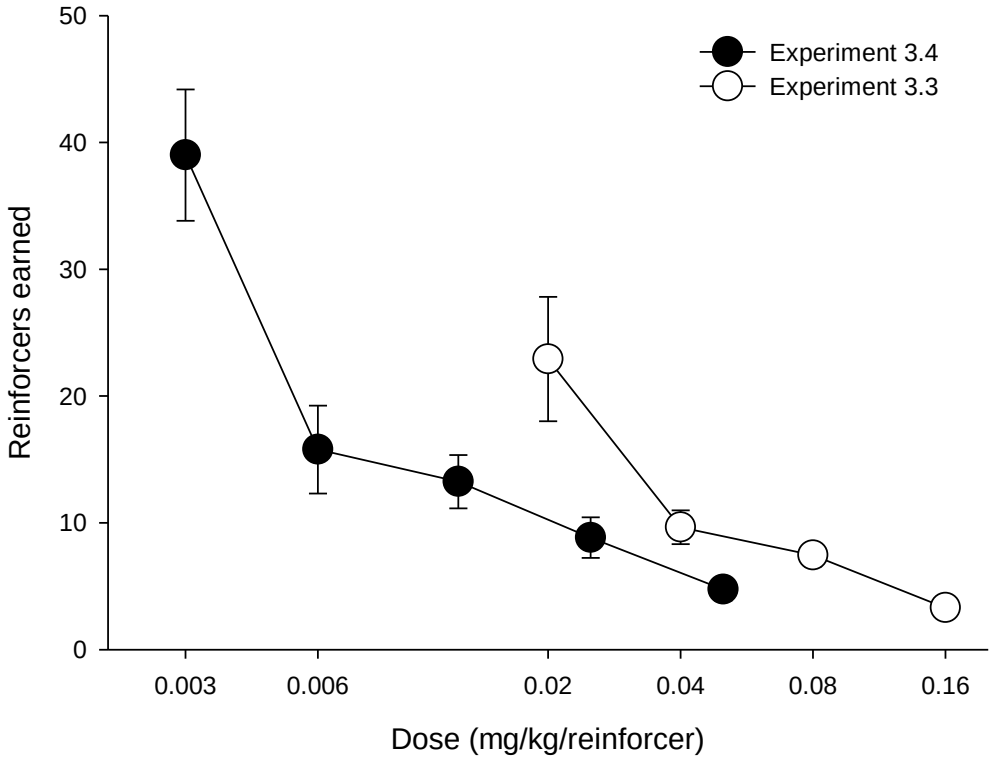

Figure 3.16: Comparison of the dose-response curves for oral MDMA in 0.2% saccharin solution obtained from Experiment 3.3 (open circles, n = 12) and Experiment 3.4 (filled circles, n = 12). Ordinate data represent average number of reinforcers earned during daily 1-hr sessions for Experiment 3.3 (FR4) and Experiment 3.4 (FR1). Error bars represent standard error of the mean.

Effect of SCH 23390 on oral self-administration of MDMA

The effect of SCH 23390 pre-treatment on the self-administration of oral MDMA is shown in Figure 3.17. SCH 23390 suppressed responding below baseline levels for every dose of MDMA tested. Pre-treatment with SCH 23390 also results in the suppression of responding for the saccharin-alone condition. A two-way repeated measures ANOVA between dose (6 levels) and pre-treatment (saline vs. SCH 23390) found a significant interaction, F (5, 55) = 7.087, $p < 0.001$. Paired-samples t test confirmed significant reductions in responding for saccharin-alone, 0.003, 0.0125, 0.025 and 0.05 mg/kg/reinforcer MDMA doses (all $p < 0.05$). However, there was no significant difference found for the 0.006 mg/kg/reinforcer MDMA dose.

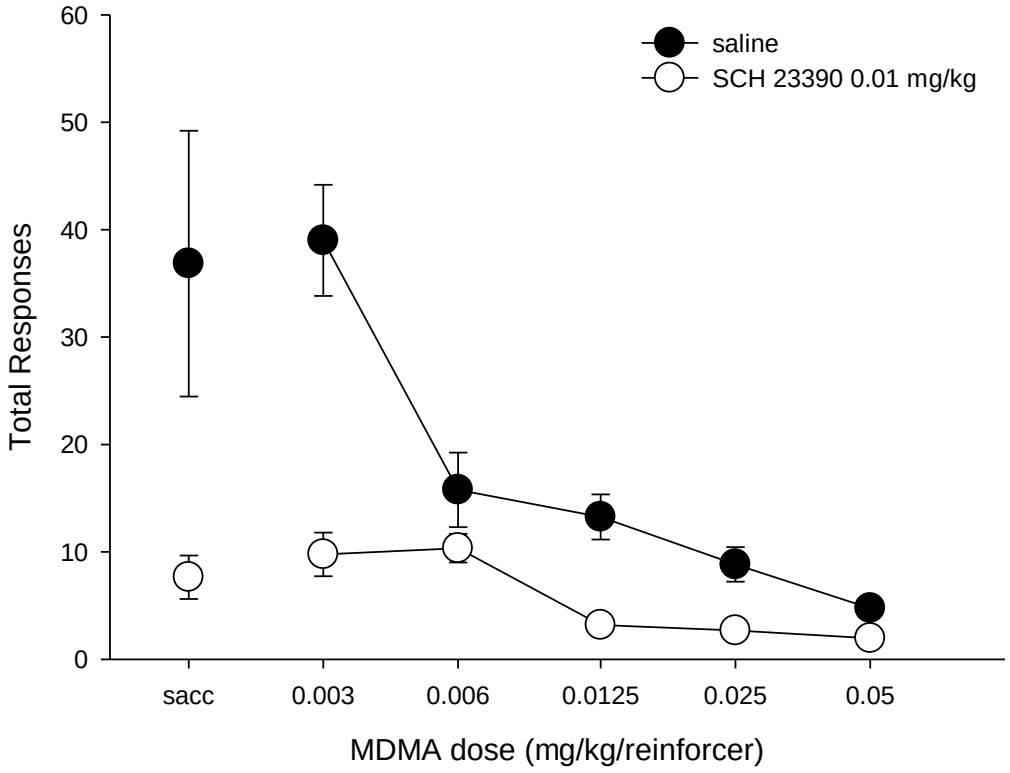

Figure 3.17: Effect of SCH 23390 (open circles) or saline (filled circles) on the dose-response curve for the oral self-administration of MDMA in a vehicle of 0.2% saccharin. Rats (n = 12) were reinforced according to an FR1 schedule of reinforcement. Error bars represent standard error of the mean.

Mean intake (mg/kg) for each MDMA dose is presented in Figure 3.18 as a function of saline or SCH 23390 pre-treatment. MDMA intake was higher for larger doses, and SCH 23390 decreased MDMA intake for all doses tested. Two-way repeated measures ANOVA revealed a significant interaction between treatment and dose; F (4, 44) = 3.369, p = 0.012. Post-hoc analysis revealed that MDMA intake was significantly lower after SCH 23390 pre-treatment for all doses except for the 0.06 mg/kg/reinforcer dose of MDMA. Inspection of the individual data revealed that in some rare cases while on the 0.06mg/kg/reinforcer dose subjects responded more when pre-treated with SCH 23390 than when they were pre-treated with saline.

However, this effect was not present in the either of the adjacent doses and is not suggestive of potentiated responding due to SCH 23390 pre-treatment, nor is there any clear indication of a shift in the dose-response curve for oral self-administration of MDMA.

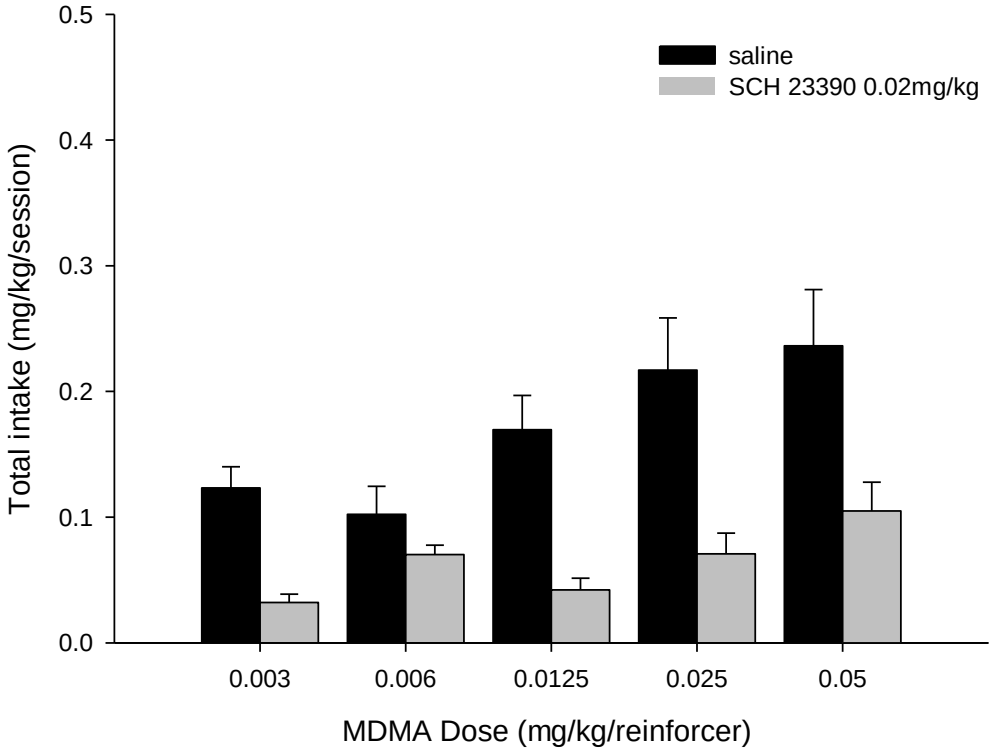

Figure 3.18: Average total MDMA intake (mg/kg) per day for saline and SCH 23390 conditions plotted as a function of dose (n = 12). Error bars represent standard error of the mean.

The current study sought to determine whether the reinforcing effects of oral MDMA were mediated through dopamine release. Of primary interest was whether the D1-like receptor antagonist, SCH 23390, would shift the dose-response function for oral MDMA to the right. A right-ward shift in the dose-response curve would be indicative of a decrease in the potency of MDMA as a result of partial blockade of dopamine receptors and suggest that responding for MDMA in this study was mediated by dopamine release. Instead, the current results showed a general suppression of operant behaviour across the range of doses tested, including the vehicle solution. It is possible that the decrease in responding noted in this study was a result of decreased motor activity due to the SCH 23390 pre-treatment. However, this seems unlikely for two reasons. Firstly, levels of responding were actually higher after SCH 23390 pre-treatment for the 0.003 and 0.006 MDMA doses than it was for 0.05 MDMA after saline pre-treatment suggesting the reduction in responding was not a case of not being able to respond due to a locomotor impairment. Secondly, Daniela et al. (2004) showed no effect of SCH 23390 pre-treatment on baseline locomotor activity counts using a 0.02 mg/kg dose, a dose that was twice the concentration of the dose used in the current study. Though in that study the 0.02mg/kg SCH 23390 attenuated MDMA-induced hyperactivity and the 0.01mg/kg dose did not. However, the reduction in responding noted in the current study indicates that 0.01mg/kg SCH 23390 is sufficient to cause significant decreases in reward-oriented behaviour. However, administration of SCH 23390 in this study resulted in a non-specific deficit in responding for all doses and most importantly the vehicle solution. It is well established that dopamine is integral to the rewarding properties of natural rewards, such as food and water (Wise, Spindler, De Wit & Gerber, 1978; Wise, 2006a, 2006b). In this study SCH 23390 pre-treatment appears to have decreased the rewarding properties of saccharin. Indeed, Nakajima (1986) found that administration of SCH 23390 dose-dependently decreased responding for water, food and saccharin. However, the D2

receptor antagonist sulpiride had no effect on the responding for any of the reinforcers tested. Similarly, Shimura, Imaoka and Yamamoto (2006) found that micro-injections of SCH 23390 into the ventral pallidum (VP) significantly decreased saccharin intake but not that of water or quinine, while the D2 antagonist sulpiride had no effect on fluid intake. The authors suggest that disruption of sweet-tasting saccharin, but not water or bitter-tasting quinine implicates D1 receptors in the ventral pallidum in the consumption of palatable tastes. Based on previous studies D1 receptors appear to not be an appropriate target for antagonism when dealing with oral solutions that combine multiple reinforcing properties together, including the saccharin/MDMA combination used in the current study. Antagonism of the D1 receptor decreased responding for all conditions which makes interpretation of the deficit in responding difficult. For example, the overall disruption across all doses (including saccharin-alone) may have been related to effects of SCH 23390 on saccharin responding and not on MDMA-mediated dopamine release as was intended. Further doses of SCH 23390 were not administered due to the complex interaction between saccharin-intake and antagonism of the D1 receptor which presented a confound that was unlikely to be resolved regardless of the doses tested. It remains unclear as to the contribution that MDMA-induced dopamine release has on the reinforcing properties of oral MDMA, especially when adulterated with saccharin. Indeed, there is no clear evidence that significant levels of dopamine were even being released as a result of oral self-administration of MDMA. The complex reinforcing profile of adulterated oral solutions of drugs may prevent further analysis using direct pharmacological techniques. It warrants mentioning that even using normally unobtrusive water as the vehicle solution may well also run into interpretation difficulties upon pharmacological intervention. One possibility for conducted future antagonist studies with oral MDMA would be using the intragastric (*ig*) method. By bypassing the mouth and taste buds and administering the drug via catheter directly to the stomach, confounds such as taste, would be avoided

allowing for more tightly controlled studies. An alternative approach would be to use behavioural (e.g., Behavioural economics or resistance to change) rather than pharmacological manipulations in order to delineate the differences between drug-maintained and vehicle-maintained behaviour.

Chapter Discussion

The previous studies reported in this chapter were designed to test the viability of oral methods of delivery of MDMA. Experiment 3.1 examined the rate of drinking MDMA when presented in drinking water. Rates of free drinking of MDMA in drinking water remained low throughout the duration of the study and plain water was consistently preferred to MDMA-containing solutions. Experiments 3.2 and 3.3 tested the operant self-administration of oral MDMA using either water or saccharin as a vehicle solution. Finally Experiment 3.4 sought to test the pharmacological impact of D1-receptor blockade using the antagonist SCH 23390 on the oral self-administration of MDMA. Reliable dose-response functions were found in Experiments 3.2 and 3.3 consistent with the descending limb of the of the dose-response curve despite minor differences in procedure and alternative vehicle solutions used across those studies. These results were further replicated in Experiment 3.4 with a further group of naïve rats.

Overall levels of MDMA intake were varied across experiments, with Experiment 3.2 (water vehicle) producing the highest mean daily intake (*M* = 0.53mg/kg, *SD* = 0.21). Experiments 3.3 and 3.4 using a sweetened saccharin vehicle solution had mean daily MDMA intakes of 0.46mg/kg (*SD* = 0.23) and 0.17mg/kg (*SD* = 0.058) respectively. It should be noted that Experiments 3.2 and 3.3 were conducted in the same animals and the results showed relatively consistent intakes (see Figure 3.14) across the two experiments despite different vehicle conditions and minor methodological changes between the studies. The results of Experiment 3.4 however show a marked reduction in intake compared with the equivalent results for Experiment 3.3. This result may be reflective of the experience of the previous subjects and a long history of MDMA intake across Experiments 3.2 and 3.1B prior to participating in Experiment 3.3. The water deprivation conditions of the earlier study may have contributed to the higher intakes noted in Experiment 3.3 relative to

the drug naïve subjects in Experiment 3.4 who did not have prior exposure to oral MDMA. Subjects in Experiment 3.4 appeared to be more sensitive to MDMA dose than those of Experiment 3.3 and this perhaps represents the development of tolerance due to long-term exposure to MDMA in the earlier study. However, the development of tolerance in this case is merely speculative as further studies are needed to ascertain the extent of which tolerance develops to the reinforcing properties of oral MDMA. It should be noted that the dose-response function as assessed during Experiment 3.4 used a range of lower doses in an attempt to produce both the ascending and descending limb of the dose-response function. However, like the earlier studies further reductions in dose did not reveal the ascending portion of the dose-response function as it was potentially obfuscated behind relatively high levels of intake for each of the different vehicle conditions. That both water and saccharin vehicle solutions both produced reinforcing effects may have occluded doses of MDMA that would normally form the ascending limb of the dose-response curve.

Intake levels shown in the previous studies are largely at odds with the range of MDMA intake reported across several other rodent studies using the *iv* route of administration. For example, the current studies showed extremely low levels of intake even compared with studies that showed low levels of *iv* MDMA self-administration such as those by Ratzenboeck et al. (2001) (3.5 mg/kg), Cornish et al. (2003) (7.5 mg/kg), Reveron et al. (2006, 2009) (6.5-6.6 mg/kg), Ball et al. (2007) (2.5mg/kg) and De La Garza (2007) (2.7 mg/kg). The current studies are even more at odds with studies conducted in the present laboratory: Schenk et al. (2003, 2007, 2008), Daniela et al. (2004, 2006) and Brennan et al. (2009) that have shown much higher rates of *iv* MDMA intake (range 21-24mg/kg). Unsurprisingly, the results of the current studies are most consistent with those of Reinhard and Wolffgramm (2005, 2006) who tested MDMA intake via the oral route. However,

intake of MDMA in the current studies was higher than found by Reinhard and Wolffgramm, though methodological differences may have contributed to this. In addition, Reinhard and Wolffgramm found decreased consumption of MDMA over time, which was not evident in any of the current studies.

The present results are difficult to reconcile with previous results due to the differences between *po* and *iv* administration. Intravenous administration will result in a rapid onset of action and little to no metabolism before producing its effect in the CNS. In contrast, the oral route will undergo significant metabolism that will significantly decrease both systemic and central bioavailability. This prevents direct comparison of intake levels between the studies conducted using the *iv* route and the current study using the oral route of administration.

Experiments 3.3 and 3.4 used an adulterated solution in order to make the drug more palatable. However, the effectiveness of oral drugs, most notably alcohol, can be affected by either food or liquids taken either before, concurrently with or even after drug administration. The effects of food or liquid are likely to modulate changes in a complex system involving absorption, metabolism and gastric emptying (Matthews, Overstreet, Rezvani, Devaud & Morrow, 2001). However, saccharin does not appear to play a role in this system, perhaps due to its non-nutritive nature. For example, Roberts, Heyser and Koob (1999) showed that saccharin plus ethanol produced similar effects on blood alcohol levels as did plain ethanol; however a sucrose plus ethanol solution resulted in decreased blood alcohol levels despite greater consumption for the sucrose-ethanol rats (Roberts et al., 1999). Matthews et al. (2001) found similar results indicating that blood alcohol levels were not reduced by adulteration with saccharin relative to ethanol alone; however, in contrast ethanol plus sucrose produced lower blood alcohol levels relative to ethanol intake. The effects of adulteration have not been specifically tested for oral doses of MDMA, though the research conducted with ethanol

suggests that the combination of MDMA and saccharin should not affect absorption or metabolism to any greater extent than using water as a vehicle.

Meisch (2001) suggests that in order to determine if a drug is reinforcing it must be shown that the drug be consumed at levels greater than its vehicle. Unfortunately, for the present experiments that is not the case. In this case both vehicle conditions (water or saccharin) acted as reinforcers in their own right. Sucrose fading procedures (e.g. Samson, 1986) have been used as a way to initiate high levels of drinking drug solutions; once established the sucrose concentration is subsequently decreased gradually across sessions. Subjects that undergo similar procedures will often maintain high levels of intake even after the sucrose concentration is reduced to zero. This procedure has been used extensively with alcohol, where the drug itself is cheap and easily replaced, attenuating any costs involved with wastage etc. However the fading procedure is not a viable option when the drug in question is more expensive or difficult to obtain (as in the case of the current experiments). Hence the present studies were unable to be conducted using a fading procedure and thus be tested under conditions whereby the vehicle itself produces minimal reinforcing effects.

An alternative method by which the reinforcing qualities of MDMA could be tested is to use the intragastric (*ig*) method of self-administration. This procedure is modelled on the *iv* self-administration procedure. Instead of intra-jugular catheters the catheters are instead inserted through an incision into the stomach. Intragastric administration lacks strong discriminative cues due to a lack of taste factors. In order to overcome this, the operant response is tied to drinking of flavoured solutions, rather than lever responding or some other operant (Fidler, Clews & Cunningham, 2006). Testing with intragastric self-administration would build upon the results found in the current studies and add to the literature concerning the oral reinforcing effects of MDMA. The *ig* method is more suitable for testing antagonist

and agonist based experiments where the reinforcing properties are not confounded with the reinforcing strength of the vehicle such as they were in the current studies. However, the *ig* method, much like *iv* self-administration, is much less suited to long-term testing due to issues of catheter patency. Regardless the *ig* method does provide numerous advantages such that future research into this method is warranted with MDMA.

The results of the current study found mixed evidence for the reinforcing properties of MDMA in rats when delivered orally. On the one hand, the current studies provided consistent results with regard to dose-response functions for MDMA using two distinct vehicle conditions, water and saccharin. However, high response rates for water and saccharin when presented without MDMA preclude any formal pronouncement of the reinforcing properties of MDMA itself. Further analysis of the reinforcing effects of MDMA-containing oral solutions is necessary to tease apart the relative contributions of MDMA and the vehicle solution. The analysis of relative reinforcer efficacy provides a potential solution to this problem. Measuring the relative reinforcer efficacy of MDMA + vehicle versus the relative reinforcer efficacy of the vehicle alone would provide a means to dissociate the reinforcing effects of MDMA from that of its vehicle. In addition, measurement of relative reinforcer efficacy forms the basis of a quantitative method of comparing reinforcing strength across drugs of abuse. The following chapter details several procedural and analytical approaches towards an analysis of the relative reinforcing efficacy of orally administered MDMA.

Chapter 4 BEHAVIOURAL ECONOMICS

Traditional self-administration methods only allow us to determine whether a drug acts as a reinforcer or not. The drug is considered be reinforcing if it initiates or supports the maintenance of self-administration behaviour (Spealman & Goldberg, 1978). In essence the self-administration procedure when tested using continuous reinforcement or fixed ratio requirements (i.e., FR1 or FR5) allows only a 'qualitative' measure of a given drug's reinforcing properties (Richardson & Roberts, 1996). However, these same traditional methods of self-administration do not allow us to determine how strong a reinforcer that drug is relative to other reinforcers, known as relative reinforcing efficacy. Different methods must be adopted that can instead provide a 'quantitative' measure of the reinforcing properties of a drug.

The term relative reinforcing efficacy refers to the behaviour-strengthening or behaviour-maintaining effects of drugs of abuse (Bickel, Marsch & Carroll, 2000). As relative reinforcer efficacy varies as a function of different drugs of abuse it has been used to refer to the rank ordinal relationship between reinforcers based on the strength of that reinforcer to maintain operant behaviour (Katz, 1990; Stafford, LeSage & Glowa, 1998). Due to the close correlation between drugs abused by humans and preclinical results with animals models (e.g. Schuster & Thompson, 1969; Griffiths et al., 1980) relative reinforcer efficacy of drugs of abuse has come to be analogous with the abuse liability of drugs of abuse in clinical settings and the real world. For the purposes of this thesis the terms relative reinforcer efficacy and abuse potential will be used interchangeably.

Reinforcing efficacy of a drug can be measured by assessing a subjects' 'motivation' to consume that drug. Motivation can be conceptualised as the amount of effort or "work" required to gain access to the drug. By manipulating the amount

of effort required to obtain drug reinforcers we can obtain behavioural measures for that drug's strength to act as a reinforcer. For example, a drug that maintains higher rates of responding as the difficulty of obtaining that reinforcer increases (e.g. increased FR schedules) would be considered a stronger reinforcer than a drug of equal potency that produced lower rates of responding. Hodos (1961) developed a procedure called Progressive Ratio (PR) in which motivation to consume reinforcers was measured as a function of incrementally increasing response ratios during a given session. Hodos showed that rats were willing to work longer and harder to obtain stronger concentrations of sweetened condensed milk and concluded that stronger concentrations of the reinforcer represented higher rewarding strength. The progressive-ratio procedure was later adopted by pharmacologists and saw increasing usage as a means of assessing relative reinforcing efficacy (see Richardson & Roberts, 1996; Stafford et al., 1998, for review).

Progressive Ratio Schedules

The primary attribute of the PR schedule is that the animal is subject to an incrementing FR schedule whereby each successive reinforcer requires an incrementally larger number of responses in order to obtain reinforcement. In a typical progressive-ratio experiment the FR requirement will be manipulated within-session by incrementing the FR after each successive reinforcer is obtained. In this way each reinforcer becomes progressively harder to obtain. For example, the FR may double with each successive reinforcer (e.g. 1, 2, 4, 8, 16) or may be incremented in some other incremental pattern (often exponential). The point at which reinforcers are no longer obtained is defined as the 'breakpoint' (BP), that is to say the point at which the subject is no longer willing to expend additional effort in order to obtain further reinforcement.

When drugs of abuse are considered we can potentially use relative reinforcer efficacy as a measure of a drug's abuse liability or addictiveness. Though

pharmacokinetic, pharmacodynamic and genetic factors are important in the overall assessment of abuse liability, drug seeking in the face of increasing costs reflects one definitive area with which we can quantify the addictive properties of drugs of abuse.

While it is generally acknowledged that MDMA is a weak reinforcer (at least relative to more prominent drugs of abuse such as cocaine or methamphetamine) only a small number of studies have attempted to quantify this by assessing MDMA's relative reinforcing efficacy. Progressive ratios have been used previously to measure relative reinforcing efficacy of MDMA in non-human primates (Lile et al., 2005; Wang & Woolverton, 2007), mice (Trigo et al., 2006) and rats (Schenk et al., 2007). Lile and colleagues compared the relative reinforcing efficacy of cocaine and MDMA in rhesus monkeys using a progressive ratio. They found that both drugs maintained higher BPs than saline; however, MDMA maintained lower peak BPs across a smaller range of doses than did cocaine. Similarly, Wang and Woolverton (2007) used a progressive ratio to study the relative reinforcing efficacy of Methamphetamine (MA), (±)-MDMA and each of its isomers ((+)-MDMA and (-)-MDMA). They concluded that MA produced the highest BPs irrespective of the potency difference (MA is more potent than MDMA by approximately a factor of ten) suggesting that MA is a stronger reinforcer than MDMA. MDMA showed differences in relative reinforcer efficacy as a function of condition, with (+)-MDMA producing the highest breakpoints and (-)-MDMA producing the lowest suggesting that the primary reinforcing properties are attributable to the (+)-MDMA isomer. Schenk and colleagues used a progressive ratio in MDMA self-administering rats and found that BP increased as a function of increasing dose suggesting that higher doses of MDMA were more efficacious (Schenk et al., 2007). Curiously, Trigo et al. (2006) found the opposite to be true when a progressive ratio was utilised in MDMA self-administering mice. That is, BP values decreased as a function of increased dose.

The result found in the Trigo et al. study is suggestive of the mice titrating their consumption of MDMA. It is possible that under high dose conditions, fewer responses were enough to satiate the mice, thus they ceased responding. Thus the Trigo et al. study may be confounded by drug satiation such that motivation to continue responding in the face of increased response cost is diminished. One way to avoid such a situation is increase the rate at which the ascending FR sequence increases such that an animal is never satiated during the course of a progressive ratio session. MDMA may be more susceptible to satiation of this type as its long half-life means that blood-levels will remain relatively constant throughout the session. Faster acting drugs such as cocaine tend to promote continued responding throughout the experimental session due to its much faster elimination, thus animals must return to responding in order to regulate desired drug effect levels. This idea is consistent with the notion that decreases in the interoceptive effects during a session (i.e. a dip below the preferred drug effect threshold) of the drug acts as a discriminative stimulus signalling it is time to return to responding (Panlilio, Thorndike & Schindler, 2008). In this case responding is under stimulus control such that when the animal is sated it signals a time of non-reinforcement. However, when the subjective effects drop below threshold level it signals that further drug infusions will be reinforcing.

Progressive ratios represent a relatively rapid method for the determination of relative reinforcer efficacy of drugs of abuse. Indeed as little as two sessions are enough to produce enough data with which to compare two different drugs. However the within-session determination of break points produces relatively little behaviourally relevant data. Only a single break point value is obtained for each session (Arnold & Roberts, 1997). In addition, the differences in potency and in elimination noted for different drugs makes direct comparisons of BP values difficult. In the case of long half-life drugs, such as MDMA, it may be more appropriate to

study the effects of increased response requirements across sessions rather than within a single session. Measuring behaviour across sessions and response requirements is a method commonly used in the field of *Behavioural Economics* which identifies and implements elements of microeconomic theory and applies it to the analysis of behaviour. Behavioural economic theory has provided a popular alternative to progressive-ratio schedule for measuring relative reinforcer efficacy.

Economic Concepts for the Analysis of Behaviour:

I. Price

In economic terms the number of responses (FR requirement) required to obtain a reinforcer can be considered to be analogous to *price* (Hursh, 1980). Consider that a subject's repertoire of behaviour is its own form of currency. It is able to expend that currency in the form of responses or effort. The more effort I am willing to expend to obtain a reinforcer the higher effective price I have paid. Hursh, Raslear, Shurtleff, Bauman and Simmons (1988) suggests that price is not simply a cost-benefit ratio between the number of responses and the number of reinforcers gained, but also takes into account other additional factors. For example, Hursh et al. (1988) manipulated both costs and benefits by changing the FR schedule and force required for lever depression (both cost factors) and also by changing the number of pellets obtained per ratio and the probability of reinforcement (both benefits). These factors together all contribute to changes in *unit price,* or "the amount of work required per unit of the commodity" (Hursh et al., 1988, pp 419). Rachlin (2003) presents a more complex view of price in that an animal subject is not a member of a 'money economy' where money is substitutable with any other good, he instead suggests that animals exist in a 'barter economy' whereby they trade leisure time (i.e. time not responding) for access to reinforcers. In this case,

the animal forgoes the ability to do other behaviours (cleaning, sleeping etc.) and instead responds on the lever, since lever pressing and leisure are considered mutually exclusive.

The concept of unit price lends itself well to the analysis of drug reinforcers whereby researchers manipulate the dose of drug available during self-administration sessions. Changes in dose reflect a change in the magnitude of the reinforcer and thus unit price may provide one way in which discrepant results might instead be reconciled to a simpler pattern of behaviour. To examine this concept, Bickel and colleagues (Bickel, DeGrandpre, Higgins & Hughes, 1990) re-evaluated past literature relating to drug self-administration studies that had manipulated both drug dose and schedule of reinforcement with respect to the notion of unit price. They found that of the ten studies they re-evaluated almost all produced data consistent with unit price. That is, when total consumption of drug (mg/kg) was plotted as a function of unit price (schedule of reinforcement/dose of drug) different doses of drug all conformed to the same positively decelerating function (called a demand function). This pattern of results was shown to be evident across multiple drugs, species and routes of administration indicating that unit price provides a convenient way of accounting for differences in dose.

After showing the functional equivalence of different drug doses and response requirements to control overall consumption in their earlier re-examination of the literature, Bickel and colleagues sought to provide support for unit price using a prospective study. Bickel, DeGrandpre, Hughes and Higgins (1991) tested this using human smokers while providing variable numbers of standardized puffs (1, 2 and 4) on the subjects preferred brand of cigarettes. In addition, response requirement was varied using FR 200, 400 and 1600 in order to produce six distinct unit prices. Of prime interest to the experimenters were those unit prices that were replicated across different number of puff and response requirement conditions.

This resulted in three distinct unit prices with replications and data showed that consumption (number of puffs) was equivocal for 4 out of the 5 subjects tested and that consumption was independent of number of puffs and response requirement. Similarly, DeGrandpre, Bickel, Hughes and Higgins (1992) reanalysed studies testing the nicotine regulation hypothesis using unit price. DeGrandpre et al. noted that studies on nicotine regulation (the changes in smoking behaviour as a result of changing nicotine yields) had shown often contrary results in the literature. However, when reanalysed using unit price (in this case the inverse of dose) there was a consistent positively decelerating function as a function of increasing unit price; that is consumption decreased as the unit price increased. The reanalysis conducted by DeGrandpre et al. indicated that unit price and a behavioural economic analysis helped to reconcile several sets of discordant data. The concept of unit price serves to integrate two different independent variables (schedule of reinforcement and reinforcer magnitude) previously thought to exert independent effects on behaviour into one single parsimonious measure that may help clarify areas in the literature previously thought to provide contradictory results (Bickel et al., 1990; Bickel, March & Carroll, 2000).

Early researchers (Lea, 1978; Allison, 1979) raised the issue of adopting economic concepts for the study of behaviour. This notion, later expanded upon by Hursh (1980, 1984) led to the adoption of several economic conventions such as using consumption (reinforcers gained) as the metric rather than the more traditional behavioural analytic measure of response rate (Hursh & Silberberg, 2008). In addition to the effects of price, other economic principles such as the effect of substitutable and complementary goods, open and closed economies and the effects of restrictions on income have been used. These concepts have all been successfully integrated into the field of behaviour analysis as a means of quantifying

behaviour and this led to the establishment of the field of *Behavioural Economics* (Hursh, 1980, 1984).

II. Consumption

Primarily behavioural economics is concerned with measuring consumption of a given commodity in the face of increasing costs (schedule of reinforcement). This is in contrast with 'real world' microeconomics where the key factor is the purchase of the commodity rather than its consumption. For animal subjects there exists little reason to purchase an item other than its consumption or use. Consumption is thus defined as the amount of commodity "X" consumed per day. Using the term consumption, however, can serve to misrepresent its purpose as behavioural economics is not just concerned with appetitive reinforcers like food (Hursh et al., 1988) but also various drugs of abuse (Bickel et al., 1991; Bickel, DeGrandpre & Higgins, 1993; Hursh, 1993; Rodefer & Carroll, 1996; Hursh, Galuska, Winger & Woods, 2005), and also for animal welfare issues including such diverse reinforcers like access to mates (Patterson-Kane, Hunt & Harper, 2002), and nesting litters or dust bathing substrates in hens (Gunnarsson, Matthews, Foster & Temple, 2000). A given commodity is usually only obtainable during experimental sessions (i.e. a closed economy) such that the number of reinforcers obtained per session is equivalent to the consumption of that reinforcer (assuming equal amounts are available per reinforcer). In the case of drug reinforcers the total dose consumed per kilogram can also be used and is particularly useful when testing multiple doses of the same drug as it allows for the analysis of consumption as a function of unit price (e.g. responses per milligram of drug). In behavioural economics, *Price* is most commonly manipulated by changing FR requirements, higher fixed ratios representing higher prices, though any manipulation that increases the 'work' or time required can be use to manipulate price. Basic economic theory dictates that

as price increases consumption of that commodity will decrease. The function describing the effects of price on consumption is called a *demand curve*.

III. Demand and Elasticity

Demand for a given commodity can be assessed by plotting total consumption of that commodity against a range of prices; the resulting demand curve describes the inverse relationship between consumption of that commodity and its cost (Vuchinich & Heather, 2003; Hursh et al., 2005).

Demand curves when plotted in log-log coordinates indicate two things. Firstly they indicate the intensity of responding at a given point, or how much of a commodity is consumed at various prices (Bickel, Green & Vuchinich, 1995). The height of the demand curve represents consumption and there is an inverse relation between consumption and price. Secondly, the slope of the demand curve measures how sensitive consumption is to changes in price. When price is low, consumption is at its highest and as price increases consumption typically decreases. In economics this is known as the *Demand Law* (Allison, 1979). At some point price become too high and responding will cease. The rate at which this change occurs is described by the slope of the demand curve. This sensitivity to changes in price is termed *elasticity of demand* (hereafter abbreviated to *elasticity*) and represents the ratio of proportional changes in consumption to proportional changes in price (Vuchinich & Heather, 2003). When changes in consumption are in direct proportion to changes in price this is termed *unit elasticity*, i.e. a doubling of price results in a halving of consumption. When consumption is defended such that consumption decreases to only a small degree with large changes in price this is termed *inelastic demand* (Hursh, 1984). For example, inelastic demand is evident when increases in price are met with near-proportional increases in responding, thus the animals response output increases as a function of price while consumption across those prices remains fairly stable (Hursh & Winger, 1995; Hursh et al. 2005). The opposite of

inelastic demand is termed *elastic demand* and represents when consumption decreases more than the proportional change in price; that is even small changes in price produce a larger than proportional decrease in consumption. In terms of total responses elastic demand produces a decrease in response rate or total expenditure as function of increase in price (Hursh, 1984).

In reality most commodities produce demand curves that span a continuum from inelastic to elastic demand; showing inelastic demand when prices are low and elastic demand at relatively higher prices (Hursh et al., 2005). The point at which the shift from inelastic to elastic demand occurs is a useful predictor of the strength of a given reinforcer analogous to how a break point provides a measure of 'motivation' to respond using progressive ratio schedules. The point at which responding shifts from being inelastic to elastic also corresponds with the peak effort of responding. Hursh (1984) proposes that inelastic demand is evident for essential commodities, like food, where the there is no other source of that commodity (or an alternative substitute), i.e. a closed economy. In contrast elastic demand is expected for non-essential items or commodities that have an alternative available source (an open economy). In general terms, inelastic demand is consistent with the definition of a 'need' or a necessity, while elastic demand represents demand for a 'want' or a luxury (Hursh, 1980; DeGrandpre et al., 1992).

IV. Modelling Demand

Demand curves can be modelled mathematically in order to quantify changes in intensity (level shifts) as well as changes in slope (elasticity of demand) for a given commodity. Hursh et al. (1988, 1989) conducted a series of studies manipulating unit price by changing FR, lever weight, number of pellets received and probability of reinforcement. They found that these manipulations of unit price were well described by a single unitary demand function. In addition this demand function was well described by Equation 4.1.

$$\ln(Q) = \ln(L) + b[\ln(P)] - a(P) \quad (4.1)$$

Equation 4.1 describes the relationship between *consumption* (*Q*) and *price* (*P*) with three free parameters, *a, b* and *L.* The *L* parameter represents initial demand at a minimal price. In most cases this is the equivalent of responding at FR1, the minimum work requirement. The *L* parameter will vary as function of reinforcer size and is the major determinant of the height or intensity of responding. For example, decreasing food size will result in an increase in consumption and this will affect the total number of reinforcers gained per session (consumption). In the case of drugs of abuse a decrease in dose has been shown to result in an increase in consumption (reinforcers per session) at low prices due to a decrease in the potency of the drug. This correlates well with increases in responding for drug doses on the descending limb of the dose effect curve when testing using single schedules of reinforcement. It has been suggested that when tested using single schedules (such as those employed in behavioural economic testing) the *L* parameter can predict choice between commodities in a concurrent schedule task (Bickel, Marsch & Carroll, 2000; Johnson & Bickel, 2006). Commodities that

produce higher consumption at low FRs as indicated by the parameter *L* should be the preferred choice when two or more options are concurrently available. However, preference does not always stay the same for all commodities as prices are increased. In many cases, such as the classic study by Elsmore et al. (1980), a shift in preference from one commodity to another has been shown as a function of increasing costs. In the Elsmore et al. study it was shown that when monkeys had many opportunities to gain reinforcers they preferred intravenous injections of heroin over food reinforcers; indicating a preference for heroin over food. However, as the number of choices given was decreased by increasing the inter-trial interval, subjects instead showed a preference for food over heroin. That is, demand for heroin was more elastic than responding for food despite initially preferring heroin. Similar results were found by Bickel, DeGrandpre, Higgins and Hughes (1991) and Johnson and Bickel (2006) using cigarette smokers working to obtain cigarette puffs or money. Both studies found preference for money at low FR requirements, but also showed a shift in preference to cigarettes at higher prices, indicating that demand for money was more elastic than demand for cigarettes.

Preference between commodities in concurrent schedules has been used as a measure of strength of a reinforcer in line with measures of relative reinforcing efficacy (Bickel et al., 2000); however, evidence of preference switching as a function of price indicates that care must be taken when using preference as measure of the abuse liability of drug compounds. The parametric nature of the behavioural economic analysis may reveal more complicated interactions than are possible when only limited prices are examined. The *L* parameter does however present a useful metric for the prediction of preference when two commodities are tested under concurrent schedules.

The *b* parameter describes the initial slope of the function at a minimal price. Because consumption should not theoretically increase as a function of price the *b*

parameter should be negative, that is there should be an initial downward slope to the function (Hursh & Silberberg, 2008). However as this parameter represents an infinitely low price the value of the *b* parameter should be both negative and close to zero. In cases where this is true then changes in elasticity are wholly caused by changes in the *a* parameter which describes the acceleration of the slope of the demand curve (Hursh & Silberberg, 2008). Higher values of the *a* parameter represent a faster acceleration of the downward sloping curvilinear function. Higher *a* values thus mean that demand is more elastic.

From Equation 4.1 the point at which demand switches from inelastic to elastic demand can also be calculated. The point at which the slope of the demand function is equal to -1 represents unit elasticity where proportional increases in price is met with precisely proportional decreases in consumption. Thus slopes shallower than -1 represent inelastic demand, while slopes steeper than -1 represent elastic demand. In addition, the point at which the slope of the demand function is equal to -1 is also the point at which maximal responding is found, thus this point has been termed P_{max} or the price yielding maximum output (Hursh et al., 1989). P_{max} values can be calculated using Equation 4.2.

$$P_{\max} = \frac{(1+b)}{a} \tag{4.2}$$

P_{max} directly relates to the rate at which elasticity changes, thus it provides a single measure with which to compare the elasticity of different demand curves. A useful function of P_{max} is that the units are expressed as prices (FR) at which responding becomes elastic. Thus direct comparisons of P_{max} are possible even if the range of prices used to fit the demand curve is different. This unique feature of demand

curves is a major benefit over studies using break point as a dependent measure as break points rely heavily on the range of ratios used and the size of the reinforcer being tested.

By fitting Equation 4.1 and using the calculated parameters to calculate P_{max} using Equation 4.2 it is possible to compare the relative reinforcing efficacy of multiple different commodities. Commodities with higher values of P_{max} are considered to be stronger or more efficacious reinforcers because the subject is willing to work longer or harder in order to obtain that reinforcer.

In addition to measures of consumption, behavioural economics can also be used to analyse total response output. Output function curves are typically bitonic and show increases in total responding as a function of increases in price followed by decreases in total responses at higher price values. The peak of the response output function represents the price at which maximal output is found and corresponds with the value of P_{max}. Best-fit non-linear regression using Equation 4.3 as proposed by Hursh et al. (1988, 1989) produces a bitonic function of responding and price. O_{max} is the predicted peak response output value at the point of P_{max} and is calculated using the parameters obtained by fitting Equation 4.1 to consumption data and then substituting those values into Equation 4.3 and substituting the calculated value of P_{max} for P.

$$\ln(O) = \ln(L) + (b + 1)\ln(P) - aP \tag{4.3}$$

V. Behavioural Economics and Drugs of Abuse

Behavioural economics has proven to be a useful tool in the study of drugs of abuse with principles from behavioural economics applied to areas such as measuring

abuse liability, examining complex interactions between drugs and other reinforcers and environmental conditions (such as open or closed economies), treatments for drug abuse (including agonist and antagonists therapies) and informing public policy (Hursh, 1991; Hursh, 1993; Bickel et al., 1993; Hursh et al., 2005). Behavioural economics, much like progressive ratio schedules, offers a way in which to measure 'motivation' to consume drugs of abuse. The study of abuse liability has become a popular field with increasing numbers of novel pharmacological compounds being abused every year. Thus having rigorous a framework in which different drugs of abuse can be characterised and empirically tested for their abuse liability is useful. Behavioural economics has proved useful for the study of the abuse potential of drugs of abuse and therefore it should not be considered strange that the vast majority of recent papers concerning behavioural economics belong in the behavioural pharmacology domain.

Central to a behavioural-economic approach to understanding abuse potential is that in humans drugs of abuse are often preferred in choice situations even when there are adverse effects from making those choices, including costs, withdrawal, addiction or other societal factors. The underlying assumption being that more than just acting as reinforcers, drugs of abuse promote maintenance of drug-taking through the process of addiction. One way to think of drugs of abuse in a behavioural economic context is that drugs are inelastic commodities. That is, either through extremely positively rewarding aspects of drugs of abuse, or through addiction mechanisms, many drugs of abuse fall would into the 'necessity' category typified by inelastic demand. Under this assumption access to drugs of abuse will be defended highly promoting increased responding in order to maintain levels of drug intake. However, not all drugs are created equal and thus different drugs of abuse with greater abuse potential should also promote inelastic responding to higher prices, consistent with high abuse potential drugs producing higher break

points under progressive ratio schedules. Behavioural economics allows for the quantification of abuse potential by measuring a drug's consumption in the face of rising costs. Like all commodities elasticity for a drug of abuse lies on continuum such at some point response rate ceases to increase as a function of price and thus consumption begins to decrease. P_{max} can thus serve as a quantitative measure of the abuse potential of a given drug of abuse. Research into unit price has indicated that the demand for drug reinforcers is independent of the individual replicable P_{max} value allowing for comparisons across drugs (Bickel et al., 1990; Hursh & Winger, 1995).

One potential pitfall in using P_{max} as a quantitative measure of abuse liability is that different drugs differ in their overall potency, and these differences confound any interpretations of the reinforcing strength of the drug. While manipulations of unit price aid in making within-drug comparisons, it does not allow for comparisons across different types of drugs or classes when potency is unequal. Consider a drug that produces very high level of initial consumption (high *L*), but is extremely elastic (*elasticity* < -1) in that consumption decreases rapidly with increased price. Then compare that drug with one with low initial consumption (low *L*) and relatively inelastic responding (*elasticity* > -1). According to behavioural economic theory, the latter is the more reinforcing compound because strength of the reinforcer is derived from its slope and not its intensity. It is possible for the first compound to produce a higher P_{max} value by virtue of a larger number of reinforcers consumed, despite it being more sensitive to changes in price. In order to eliminate this confound, Hursh and Winger (1995) proposed an alternative method whereby obtained data can be normalized for baseline consumption and each dose is presented as a percentage of consumption at FR1. This procedure has the advantage of eliminating intensity changes across commodities such that all demand curves begin at 100 (i.e. responding at FR1 is equivalent to 100% of baseline responding). In the case of

drug reinforcement this means that potency and dose no longer factor into the demand curve analysis (Hursh & Winger, 1995). Both consumption and price are calculated as a function of *q,* the normalized dose calculated using Equation 4.4 as previously reported by Hursh and Winger (1995) where *d* is the dose and *B* is average number of reinforcers gained at FR1; the cancelling out of the dose parameter (*d*) leaves the following simple equation for normalizing data, $q = B/100$. Thus price and consumption are normalized as a function of *q* where $P = FR \div q$ and $Q = Rq$ (Hursh & Winger, 1995).

$$q = \frac{d}{(dB)} \times 100 \tag{4.4}$$

Hursh and Winger (1995) reanalysed unit price data from two previous papers measuring demand for the stimulants, cocaine and methohexital (Winger, 1993) and the opioids alfentanil and nalbuphine (Winger, Woods & Hursh, 1996) in order to test the validity of the normalization procedure. Of note is that when fitting normalized data using Equation 4.1 the *L* parameter does not vary and is instead replaced by the constant value of 100, as that is the starting point for all normalized demand curves (Hursh & Winger, 1995). Hursh and Winger found that the normalization procedure accounted for more variance for alfentanil, similar R^2 values for nalbuphine and methohexital but poorer fits for cocaine. All of the drugs tested produced unitary demand functions using both the unit price and normalization procedures; with the exception of cocaine which was better described by multiple demand curves due to changes in demand as a function of dose (the lowest dose tested appeared more elastic compared with that of the other two doses tested). P_{max} values were calculated for each drug and indicated that alfentanil showed higher P_{max} values and thus more inelasticity than cocaine,

followed by a large decrease to nalbuphine and methohexital respectively. The normalization procedure thus allows for the assessment of the abuse liability across potency of drug but also across classes of drugs as shown by Hursh and Winger's analysis of both stimulants and opioids using the same procedure. When demand curves are normalized they are not confounded by the potency of the drug tested allowing for the direct comparisons of the elasticity of various compounds; this allows direct comparison of different drugs by using P_{max}, or the point that responding changes from inelastic to elastic responding as a quantitative measures of the abuse liability of drugs of abuse. However, it should also be noted that the normalization procedure can be used in the assessment of demand curves for all types of commodities and is not restricted to testing demand for drugs of abuse.

Behavioural economics, among other uses, has primarily been used for measuring the strength of a reinforcer or the 'motivation' to consume said reinforcer. However, the models that are used for this purpose, while providing appropriate fits to the data, are often abstracted such that the free parameters do not themselves represent the strength of reinforcement (Hursh & Silberberg, 2008). Recently, Hursh and Silberberg (2008) have published a new model based on using an exponential function to fit demand curves. The authors suggest that the exponential model shown in Equation 4.5 can be used as a method for measuring what they call 'essential value' or the strength of the reinforcer. Utilizing the normalization procedure referred to above, the exponential model has only a single free parameter, α which describes the rate of change of the exponential function. The value of Q_0 refers to the starting value or maximal level of consumption at minimum price (similar to *L* from Equation 4.1) and the parameter *k* is a constant that describes the range of the data and is set to the same value across comparisons. Price is normalized by default allowing for comparisons across commodities by the addition of $Q_0 \times C$, representing the independent variable *C*, or price, and

normalizing it in relation to Q_0, the maximum level of consumption at minimum price. This results in a single parameter that can be used to compare the demand for different commodities, α, representing the rate constant of the exponential. The parameter, α, is inversely related to elasticity, such that steeper functions representing more elastic commodities will have higher α values, conversely inelastic demand is represented by lower α values (Christensen, Silberberg, Hursh, Huntsberry & Riley (2008). Hursh and Silberberg applied the new exponential model to previously published data from a variety of sources and found reliable fits across almost all of the data tested ($R^2 > 0.95$).

$$\log(Q) = \log Q_0 + k(e^{-\alpha Q_0 C} - 1) \qquad (4.5)$$

Christensen et al. (2008) tested the new exponential equation using cocaine and food as reinforcers. In Experiment 1 of their study rats responded for both intravenous cocaine and food pellets during experimental sessions, though sessions arranged in blocks such that access was only available to a single commodity at a time. The results of this experiment produced larger α values when modelled using the exponential model for cocaine than for food, showing that the rats defended their access to food more readily than cocaine. However, when cocaine consumption was modelled alone (no access to food) α values were lower than when cocaine and food were available in the same session, indicating some interaction between the two commodities. However the opposite was true when food was tested alone as α values were smaller. This suggests that food acts as a complement for cocaine consumption, but cocaine acts as a substitute for food. Christensen et al. was the first prospective study to utilise the exponential model of

demand and in general fits obtained by the model were excellent, (mean group R^2 > 0.95) apart from the slight tendency for the exponential model to underestimate cocaine consumption when both food and drug were available.

So far the exponential model has been used in few studies and those studies have examined a range of phenomena and subjects. For example, Christensen, Kohut, Handler, Silberberg and Riley (2009) tested strain differences between Fischer and Lewis rats on demand for both cocaine and food using the exponential model. The authors found that Lewis rats demonstrated higher essential value for food than did Fischer rats, however this relationship was reversed when the commodity was changed to intravenous cocaine. Overall both strains of rats defended their access to food (in a closed economy) significantly more than they did for cocaine suggesting that food has higher essential value than cocaine. The authors suggest that the reversal of essential value for food and cocaine as a function of strain represents that the exponential model is sensitive to genetic factors that maybe be related to drug abuse. Two studies have used the exponential model of demand to test effects of history of exposure to cocaine and have shown that different histories of drug consumption lead to changes in the essential value of cocaine (Christensen, Silberberg, Hursh, Roma & Riley, 2008; Oleson & Roberts 2009). Foster and colleagues have recently used the exponential model to test the food preferences in hens across a range of three qualitatively different foods (Foster, Sumpter, Temple, Flevill & Poling, 2009). In addition to animal subjects the exponential model of demand has also been successfully used to measure relative reinforcing efficacy in humans. Murphy, MacKillop, Skidmore and Pederson (2009) used the exponential model to test the validity of an Alcohol Purchase Task in a college student sample as a measure of relative reinforcer efficacy. In all of these studies the exponential model of demand generally provided good fits to the data and was deemed suitable for measuring demand in all cases.

More prospective studies of the exponential model need to be completed in order to further test the predictions of the model and test the reliability of the α parameter as a measure of 'essential value' or relative reinforcer efficacy. As such, it remains prudent to test predictions of the linear-elasticity model (Hursh et al., 1988, 1989) and the exponential model concurrently. It stands to reason that should the predictions of the exponential model hold up in the face of further testing then the exponential model will provide the more parsimonious model for measuring or ranking the relative reinforcer efficacy of commodities due to it having only a single free parameter.

The current study

The following experiment has been designed to assess demand curves for MDMA when tested using the oral-self administration procedure presented previously (see Chapter 3). Obtained behavioural data will be used to test various behavioural economic models that have previously been used as a means of quantifying abuse potential or relative reinforcing efficacy. Data gathered will be analysed using two methods of equating drug dose, namely unit price and normalization of drug demand. In addition two models of demand will be utilised, the Linear-Elasticity model of Hursh et al. (1988, 1989) and the Exponential Model of Demand (Hursh & Silberberg, 2008). Model fits will be compared in order to assess the utility of both approaches and its relation to measuring relative reinforcer efficacy and abuse liability. As taste is a prominent factor in the oral self-administration of drugs of abuse, drug solutions will be a mixture of a sweetened saccharin solution and MDMA. Concordant with behavioural economic theory it is expected that as price increases consumption of both drug and vehicle solutions will decrease in a positively decelerating function describing the change from inelastic to elastic demand. It is expected that the MDMA-containing drug solutions will show more inelastic responding than the saccharin-vehicle condition indicating that the drug

containing solutions are stronger reinforcers than the vehicle alone providing support for MDMA's effectiveness as an oral reinforcer.

Experiment 4.1: Behavioural Economic Analysis of Oral Self-administration of MDMA

Method

Subjects:

Subjects were 11 male Sprague-Dawley rats who had extensive experience with orally delivered MDMA solutions. Subjects in this study had prior experience with oral MDMA though participation in Experiments 3.2 (self-administration in water vehicle), 3.1B (free access choice in the homecage) and 3.3 (self-administration in saccharin vehicle). Animals were aged approximately 12 months old at the beginning of testing and weights ranged from 388 – 510 grams (M = 435, SD = 33.7). Animals remained at 85% of their free-feeding weights throughout the duration of the experiment and water was freely available in the home cage. Subjects were housed individually in polycarbonate cages with cage tops made of metal grating situated in the testing room. The testing room was maintained on a reversed 12:12 hour light/dark cycle with lights on at 7pm. Animals were treated in accordance with ethical guidelines set forth by the Victoria University of Wellington Animals Ethics Committee.

Apparatus/materials:

Equipment:

Experimental sessions were conducted using the same equipment, housing and procedures as outlined previously for Experiment 3.3.

Solutions:

+/-3,4-methylenedioxymethamphetamine hydrochloride (ESR, Porirua, New Zealand) was mixed with tap water at the following doses; 1.624mg/ml, 0.812mg/ml and 0.40625mg/ml.

Procedure:

Immediately following the conclusion of Experiment 3.3 the procedure was modified to allow for daily manipulations of the FR value for each animal. An across session methodology was used to assess demand for oral MDMA self-administration such that each day animals' were subject to a different FR schedule of reinforcement. Each dose of drug was first tested using an ascending sequence of FR requirements in order to establish a suitable endpoint for each dose (highest FR ratio reached). The ascending sequence consisted of seven individual FR requirements that increased each day beginning at FR1 and continuing to double until a ratio was reached when no reinforcers were obtained. The arranged FR schedules used in this study were 1, 2, 4, 8, 16, 32, and 64. In some cases animals continued to respond at FR64 so an additional eighth FR schedule (FR96) was added to the sequence for those animals. After completion of the first ascending sequence each sequence was replicated twice more, however on the second and subsequent replications FR ratios were presented in a random order (individually for each rat) to produce a non-predictable sequence of ratios.

Demand curves were obtained in the following order for vehicle (0.2% saccharin), 0.02mg/kg/reinforcer, 0.04mg/kg/reinforcer and 0.08mg/kg/reinforcer MDMA solutions corresponding to the 0.40625, 0.812 and 1.624 mg/ml doses respectively. Reinforcers were delivered in 0.02cc dipper cups.

Results

Dose-effect Analysis

Figure 4.1 shows the average number of reinforcers obtained per day (consumption) plotted as a function of the FR requirement at which it was obtained for three doses of MDMA and the vehicle-alone (top panel). The same data is plotted in the bottom panel of Figure 4.1 using a log x-axis in order to more clearly differentiate the results. All subjects showed the highest average consumption of the saccharin vehicle at low and moderate FR requirements. Consumption of saccharin and, indeed MDMA for all doses, decreased as a function of increasing FR requirements. A two-way ANOVA using dose and FR requirement as factors found a significant interaction between both factors (F (18, 162) = 18.214, p < 0.001). Contrasts revealed significant differences between each of the three doses of MDMA and vehicle in the order of highest to lowest consumption: saccharin, 0.02, 0.04 followed by 0.08mg/kg/reinforcer MDMA. This pattern is consistent with the analysis of dose-response functions from earlier experiments in Chapter 3 with the exception that responding for saccharin was significantly higher than all doses of MDMA in the current experiment. In the previous experiments this was not the case and responding for saccharin was not significantly different from the lowest dose of MDMA tested (though it was significantly different from the other doses).

Total responding for MDMA and vehicle was analysed as a function of the FR requirements and are shown in Figure 4.2. In correspondence with the reinforcer

data total responding was highest for saccharin at all doses tested. There was a bitonic relationship between vehicle responding and FR requirement that peaked at FR8 before declining as a function of increasing FR. Responding for MDMA doses was inversely related to dose in that the lowest dose, 0.02 mg/kg/reinforcer, produced the highest responding followed by the 0.04 and 0.08 mg/kg/reinforcer MDMA dose. Peak responding for MDMA was not as marked as it was for the vehicle condition; however the peak responding for all three doses of MDMA was situated at FR16 which was higher than the peak responding for saccharin alone. Despite peak responding for saccharin being lower than that of MDMA the vehicle condition still produced more total responding at FR16 (the MDMA peak) than did any of the MDMA doses. The shift in the function to the right for MDMA suggests that MDMA responding might be less sensitive to changes in FR requirement than the saccharin was by itself which may be indicative of less elastic demand for MDMA relative to saccharin.

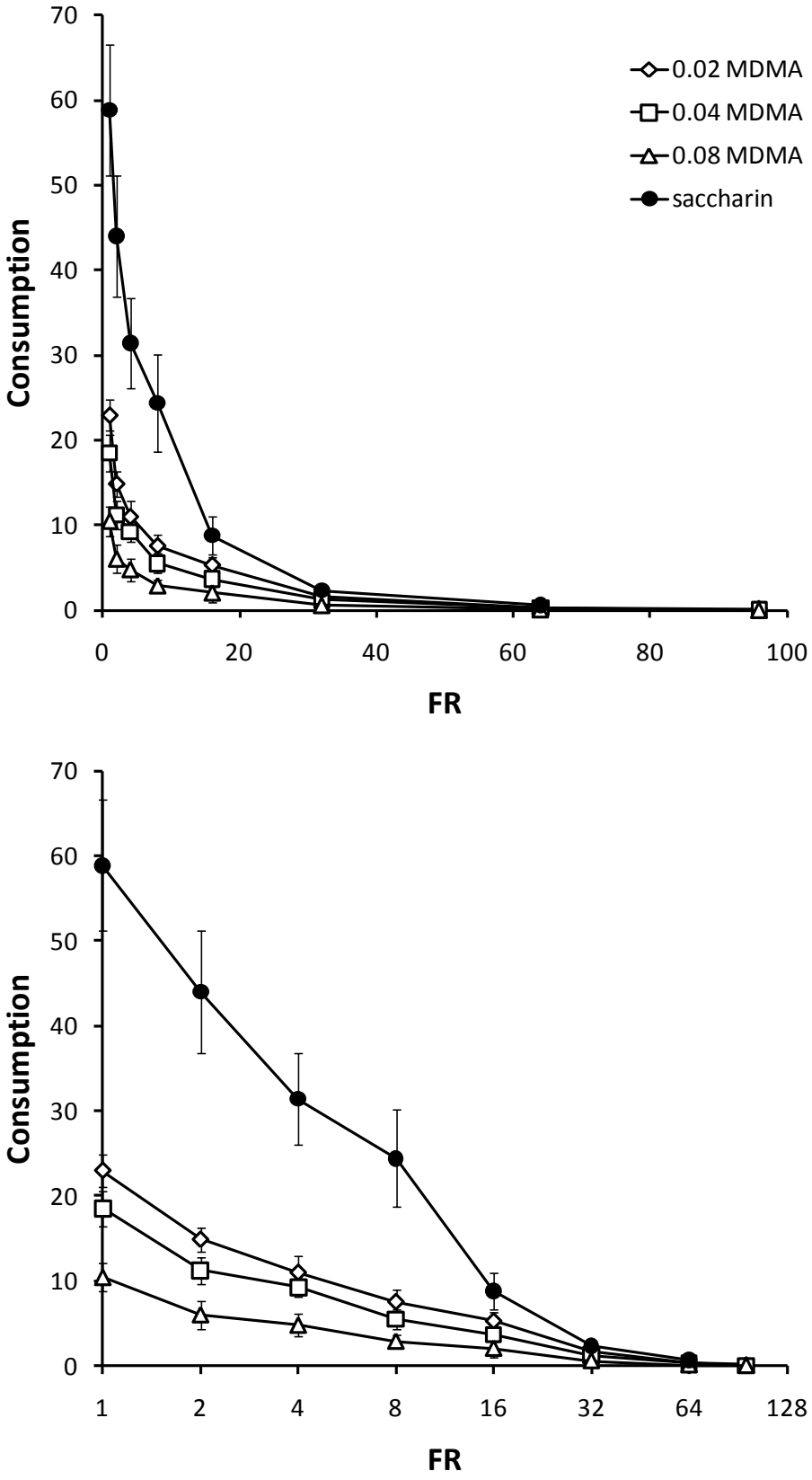

Figure 4.1: Group function (n= 11) for the oral self-administration of MDMA showing average reinforcers earned (consumption) of oral MDMA or vehicle as a function of FR requirement (top panel) or the same data presented on semi log-axes (*bottom panel)*. Error bars represent standard errors of the mean.

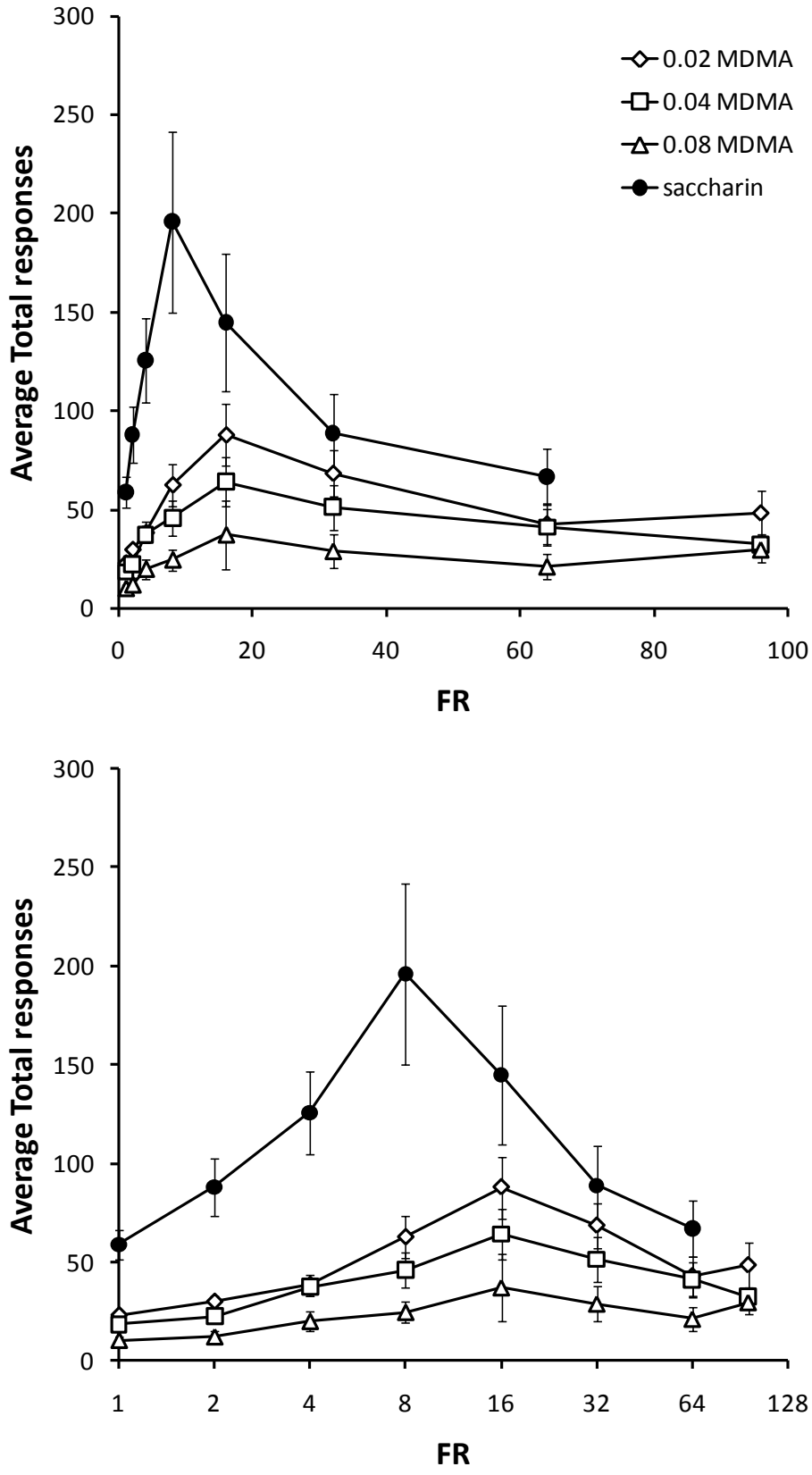

Figure 4.2: Group function (n= 11) for the oral self-administration of MDMA showing average total responding for oral MDMA or vehicle as a function of FR requirement (top panel) or the same data presented on semi log-axes (*bottom panel)*. Error bars represent standard errors of the mean.

Economic Analysis

In order to test differences in the sensitivity to price between MDMA-containing and vehicle solutions a behavioural economic analysis was conducted by fitting Equation 4.1 to the obtained number of reinforcers earned (consumption) as a function of the FR schedule (price). The resulting demand curves plot consumption as a function of price for each dose of MDMA and the vehicle-alone and are shown in Figure 4.3. As expected all doses of MDMA and the vehicle-alone show decreased responding as a function of increased price. Initially level of demand was highest for the vehicle-alone as evidenced by higher consumption at FR1. In addition, fits of the model described by Equation 4.1 show a higher *L* value (initial demand at minimum price) for vehicle-alone condition than for each of the drug doses. The demand curves for MDMA conditions reveal decreased values of *L* as a function of increasing dose, that is the highest *L* values were found for the lowest dose (0.02) followed by the 0.04 and 0.08 mg/kg/reinforcer MDMA dose respectively. Parameter estimates for the model are shown in Table 4.1.

When plotted on log-log coordinates the slope of the function describes the commodities elasticity or it's sensitivity to changes in price. Within the model elasticity is described by the *a* and *b* parameters that measure the rate of change and the initial slope of the curvilinear function respectively. By substituting the *a* and *b* parameters into Equation 4.2 the value of P_{max} can be calculated which corresponds to the price at which the slope of the function equals -1 and indicates the point at which responding changes from inelastic to elastic responding. P_{max} values are indicated on Figure 4.3 as bisecting vertical lines and are also reported in Table 4.1. P_{max} values for the low and medium doses were comparable producing values of 15.48 and 15.21 respectively indicating that both doses produced similar elasticity despite the lower dose producing a higher overall level of demand. The high dose MDMA condition produced a slightly lower P_{max} value ($P_{max\,(0.08)}$ = 12.28)

that was comparable to the vehicle-alone condition ($P_{max\ (saccharin)}$ = 11.91). A one-way ANOVA revealed no significant differences between the P_{max} values for any of the conditions (F (3, 30) = 0.408, p = 0.91).

The model was fit to data from individual subjects and resulting demand curves and parameter estimates can be seen in Figure 4.4 and Table 4.1 respectively. One subject, Rat 44, suffered significant weight loss during the course of the experiment and was removed before completing all three doses of MDMA. Generally individual results mirror the group results with saccharin producing the highest overall level of demand followed by the low, medium and high MDMA doses respectively. Peak P_{max} values were varied with 5 of 11 animals showing the highest P_{max} value for the low dose condition, 3 for the medium MDMA dose condition and 3 for the high dose condition. No animals produced their highest P_{max} value in the vehicle-alone condition, nor did it always produce the lowest P_{max} value as that was only the case for 4 out of 11 animals. Rats 32 (saccharin) and 33 (0.08 MDMA) produced negative values for P_{max} indicating that consumption was elastic even at a minimum FR for those conditions. Variance accounted for was generally high indicating that the model described the data well. The average R^2 value for all fits was M = 0.89, SE = 0.025. While some fits of the model produced acceptable R^2 values there were a number of fits that did not produce standard curvilinear demand functions generally as a function of variable data and a low number of data points. In particular, Rats' 32, 33 and 34 and produced atypical model fits and this occurred most often with the 0.08 mg/kg/reinforcer dose. Rat 41 produced atypical fits for the 0.02 and 0.04 mg/kg/reinforcer doses.

While MDMA doses were well described by the model several authors (e.g. Bickel et al., 1990; Hursh et al., 1988) have suggested that reinforcers with scalar properties such as drug dose can be described using a single demand function when consumption is plotted as function of unit price. Further analysis with unit

price will be presented below in order to assess if oral MDMA self-administration can also be described by a single demand function by utilising a unit price analysis.

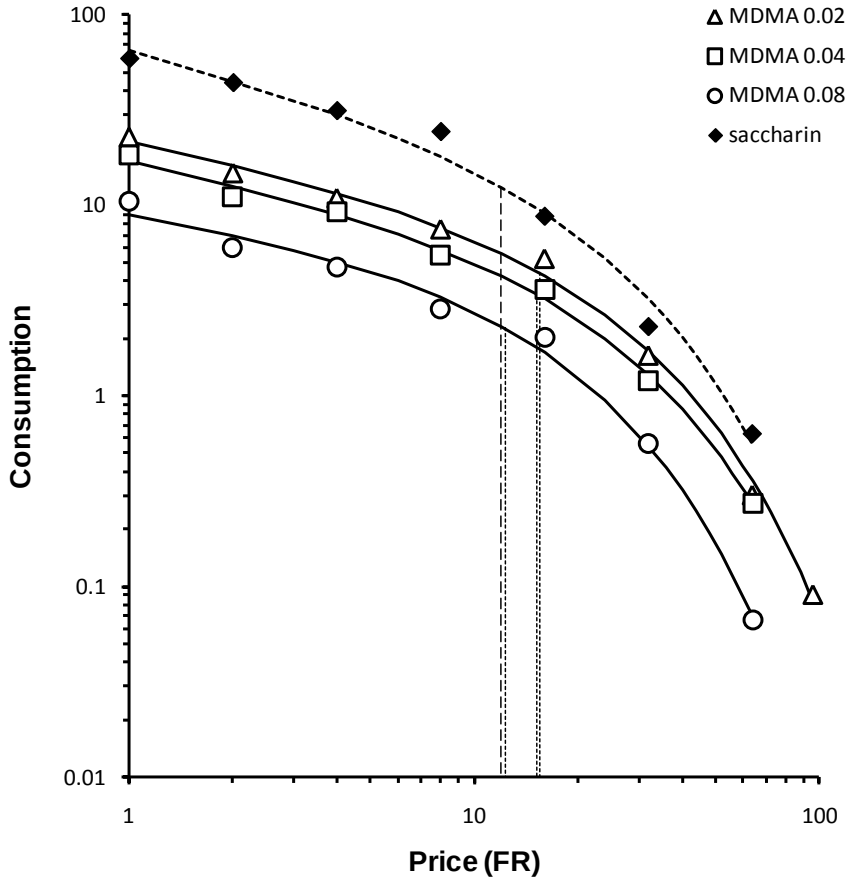

Figure 4.3: Demand for oral administration of 3 doses of MDMA and vehicle (0.2% saccharin). Demand curves represent consumption (average reinforcers earned) plotted as a function of price (FR ratio) on log-log axes. Best-fit functions were obtained by fitting Equation 4.1 to the data. Bisecting vertical lines represent P_{max} values for 3 doses of MDMA and vehicle-alone conditions and were calculated by inputting parameter estimates into Equation 4.2 for each condition.

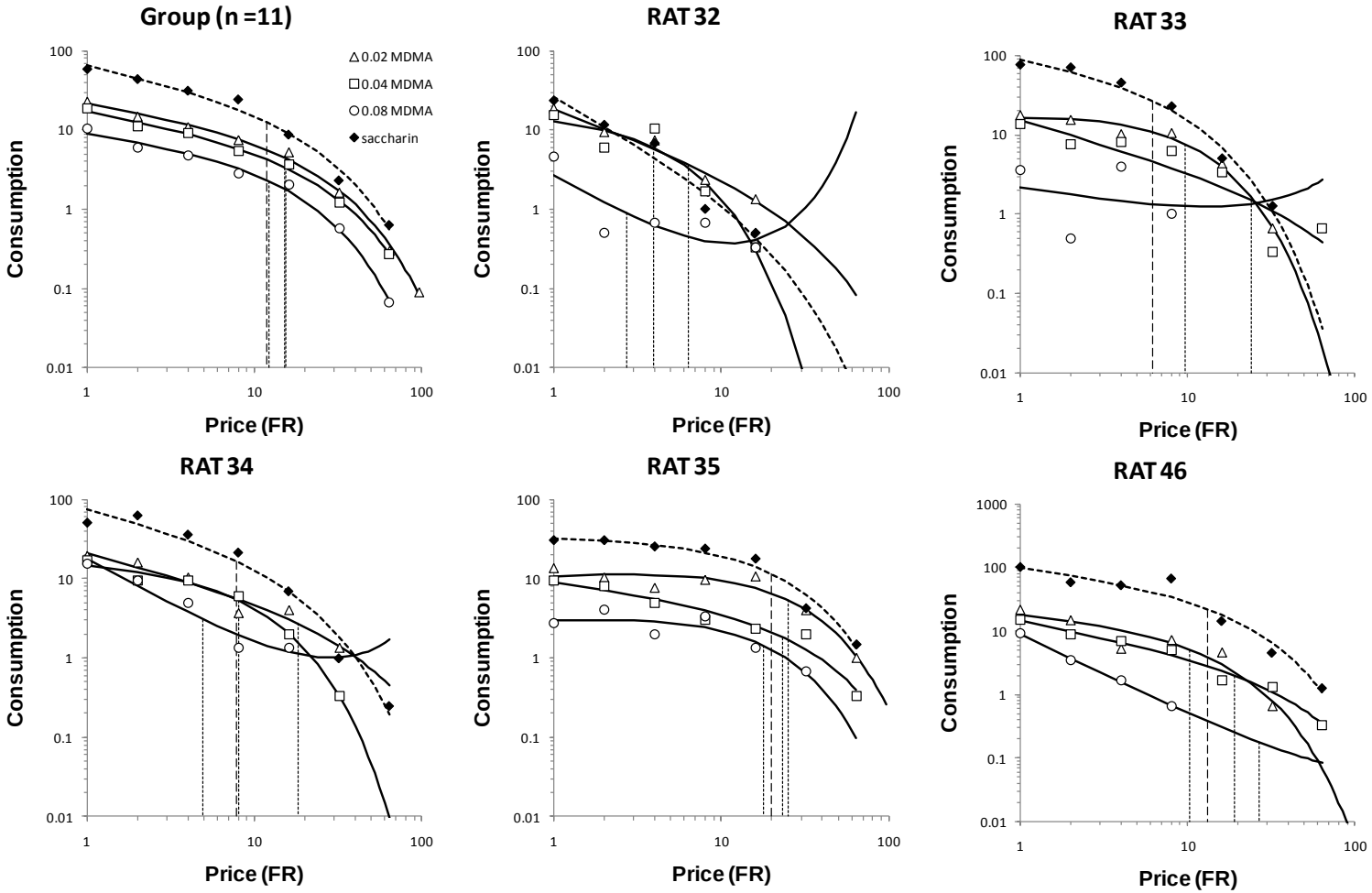

Figure 4.4: Demand for the oral self-administration for 3 doses of MDMA in 0.2% saccharin and vehicle-alone plotting consumption expressed as average reinforcers earned per day as a function of Price (FR) plotted in log-log coordinates. Panel 1 represents the group mean of all animals (n = 11). Successive panels represent individual subjects. Lines of best fit were calculated using Equation 4.1 for each condition. Bisecting vertical lines represent P_{max} values and were calculated by substituting parameter estimates into Equation 4.2. Figure continues next page.

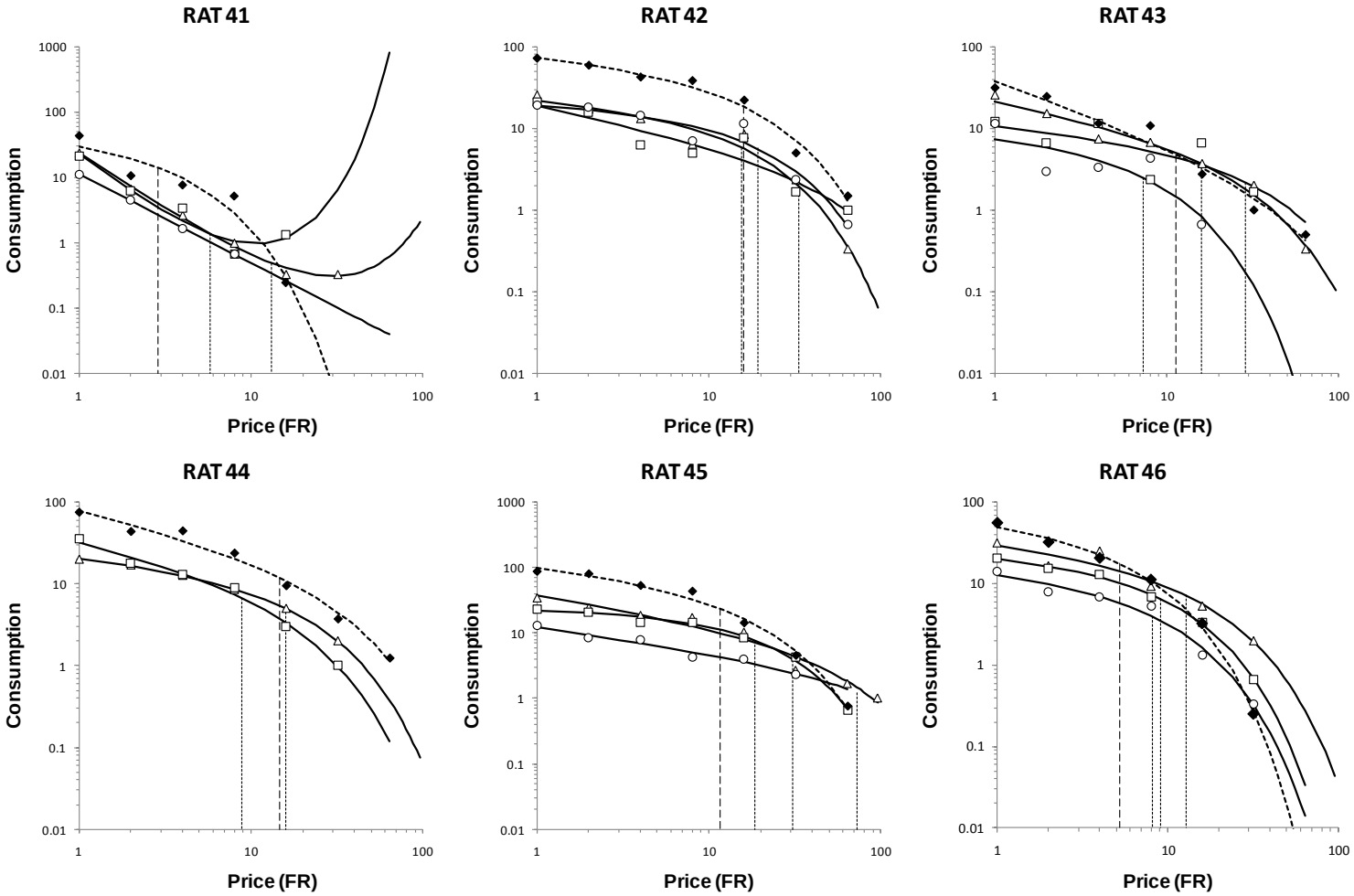

Figure 4.4 (cont): Demand for the oral self-administration for 3 doses of MDMA in 0.2% saccharin and vehicle-alone plotting consumption expressed as average reinforcers earned per day as a function of Price (FR) plotted in log-log coordinates. Panel 1 represents the group mean of all animals (n = 11). Successive panels represent individual subjects. Lines of best fit were calculated using Equation 4.1 for each condition. Bisecting vertical lines represent P_{max} values and were calculated by substituting parameter estimates into Equation 4.2.

Table 4.1: Parameter fits for demand curves for the oral self-administration of MDMA in 0.2% saccharin. Demand curves are expressed in terms of consumption (average reinforcers earned) as a function of unit price in log-log coordinates. Parameters were obtained through fits of Equation 4.1. P_{max} values were obtained by fitting Equation 4.2 to estimated parameters.

Subject	Dose (mg/kg/reinforcer)	*a*	*b*	*L*	P_{max}	R^2
Group	0.02	0.0412	-0.3614	22.52	15.48	0.99
	0.04	0.0405	-0.3835	17.83	15.21	0.98
	0.08	0.0583	-0.2836	9.45	12.28	0.96
	vehicle	0.0448	-0.4659	68.00	11.91	0.98
32	0.02	0.0342	-0.7822	19.17	6.36	0.98
	0.04	0.2359	-0.0645	16.54	3.97	0.75
	0.08	-0.1157	-1.3148	2.43	2.72	0.87
	vehicle	0.0611	-1.1434	27.80	-2.35	0.98
33	0.02	0.1132	0.1008	18.44	9.73	0.93
	0.04	0.0157	-0.6220	15.76	24.14	0.89
	0.08	-0.0258	-0.3365	2.11	-25.73	0.09
	vehicle	0.0988	-0.3845	99.24	6.23	0.95
34	0.02	0.0237	-0.5703	21.71	18.17	0.95
	0.04	0.1086	-0.1194	16.36	8.11	0.92
	0.08	-0.0422	-1.2092	17.27	4.95	0.96
	vehicle	0.0588	-0.5443	80.21	7.75	0.82
35	0.02	0.0450	0.1206	11.27	24.93	0.74
	0.04	0.0307	-0.2844	9.16	23.31	0.96
	0.08	0.0615	0.1051	3.18	17.98	0.66
	vehicle	0.0482	-0.0365	33.59	19.98	0.97
36	0.02	0.0724	-0.2521	19.37	10.33	0.87
	0.04	0.0245	-0.5311	15.11	19.16	0.98
	0.08	-0.0105	-1.2819	8.94	26.83	1.00
	vehicle	0.0486	-0.3561	105.09	13.24	0.84

Table 4.1 (cont):

Subject	Dose (mg/kg/reinforcer)	*a*	*b*	*L*	P_{max}	R^2
41	0.02	-0.0602	-1.7889	22.39	13.10	1.00
	0.04	-0.1979	-2.1467	18.97	5.79	0.99
	0.08	-0.0004	-1.3636	11.26	830.79	1.00
	vehicle	0.2582	-0.2534	38.36	2.89	0.81
42	0.02	0.0512	-0.2081	22.76	15.47	0.93
	0.04	0.0157	-0.4780	19.34	33.24	0.91
	0.08	0.0451	-0.1344	20.06	19.21	0.89
	vehicle	0.0476	-0.2436	77.00	15.89	0.98
43	0.02	0.0345	-0.4482	21.98	15.98	0.96
	0.04	0.0257	-0.2630	11.09	28.70	0.57
	0.08	0.1109	-0.1909	8.27	7.30	0.60
	vehicle	0.0202	-0.7714	38.57	11.33	0.95
44	0.02	0.0467	-0.2558	21.50	15.95	1.00
	0.04	0.0546	-0.5187	33.93	8.82	0.97
	0.08	-	-	-	-	-
	vehicle	0.0310	-0.5475	80.80	14.61	0.95
45	0.02	0.0171	-0.4712	38.44	30.84	0.97
	0.04	0.0509	-0.0571	23.22	18.53	0.97
	0.08	0.0082	-0.4022	12.54	72.80	0.96
	vehicle	0.0564	-0.3424	105.83	11.65	0.96
46	0.02	0.0543	-0.3031	31.38	12.85	0.83
	0.04	0.0901	-0.1755	22.08	9.15	0.99
	0.08	0.0921	-0.2439	14.13	8.21	0.94
	vehicle	0.1393	-0.2720	58.00	5.23	0.98

Unit Price Demand Curve Analysis

A unit price analysis was conducted by examining demand curves for the oral administration of MDMA by plotting average daily intake (mg/kg/day) of MDMA against unit price (response requirement/reinforcer magnitude) and plotted in log-log coordinates. Equation 4.1 was fitted to the obtained data. Several dose/FR requirement combinations resulted in replication of a given unit price and these were treated as additional data points within the analysis. Figure 4.5 shows that consumption of oral MDMA decreased as a function of increases in unit price. Despite multiple doses of MDMA being tested they were generally described well by the same demand function (R^2 = 0.92) fitted using Equation 4.1. However, the function best described the data when unit price was low to moderate and variability was small. The largest dose (0.8 mg/kg/reinforcer) of MDMA in particular appeared to be more elastic than the other doses of MDMA as it produced lower consumption at higher unit prices relative to the other two doses. P_{max} for the unit price analysis for the group function was 650.68. P_{max} values ranged from a minimum of 475.87 to a maximum of 1516.18 for the individual subjects (see Table 4.2 for parameter estimates for individual subjects). Individual demand functions for the consumption of MDMA for each animal are shown in Figure 4.6. All subjects' data is representative of the group function. Fits of the model to individual subject data produced generally good fits with 7 out of 11 animals producing fits greater than R^2 = 0.75, (*M* = 0.74, *SE* = 0.056, *min* = 0.35, *max* = 0.96).

Using a unit price analysis precludes a comparison with the vehicle-alone condition. An MDMA dose of 0.0 for the vehicle-alone condition is undefinable when converted to unit price. However, unit price analysis is a useful metric because it eliminates dose as a variable in the demand for a given drug commodity. The results for the unit price analysis of the self-administration of MDMA reported here show that

MDMA produces a single unitary demand function that was well described by Hursh's linear elasticity model (Equation 4.1).

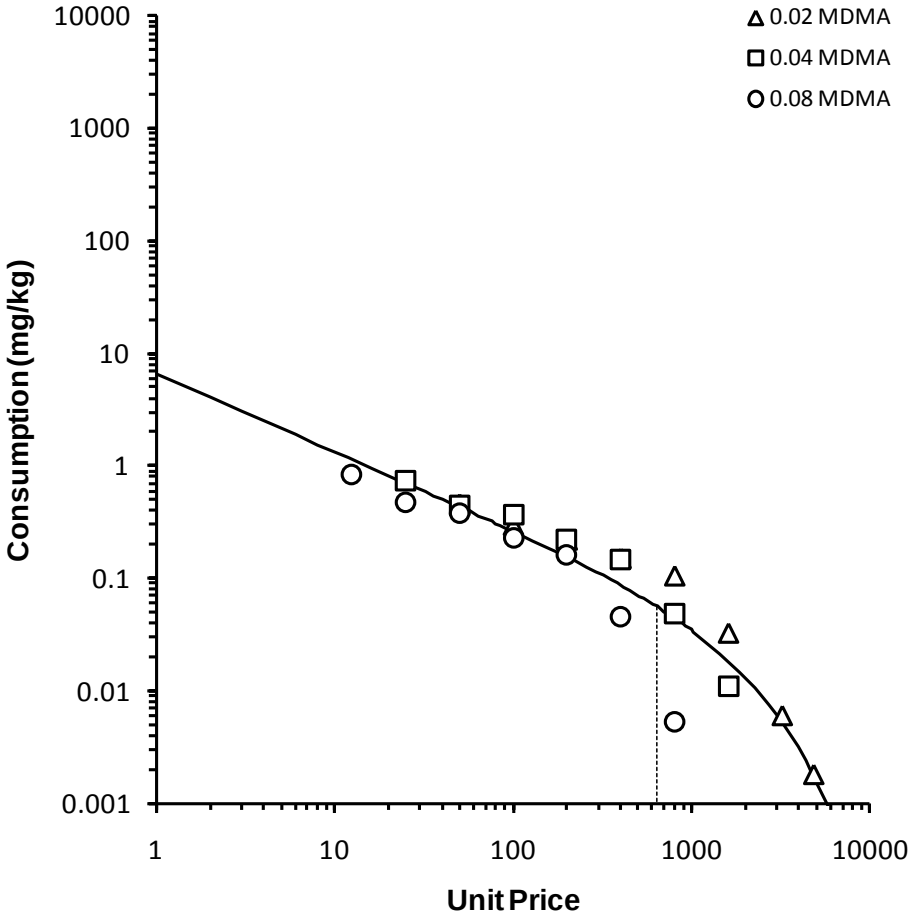

Figure 4.5: Demand for oral administration of 3 doses of MDMA in 0.2% saccharin vehicle. Demand curves represent consumption expressed in mg/kg/day plotted as a function unit price. Best fit functions were obtained by fitting Equation 4.1 to pooled data across MDMA doses. Bisecting vertical lines represent P_{max} values.

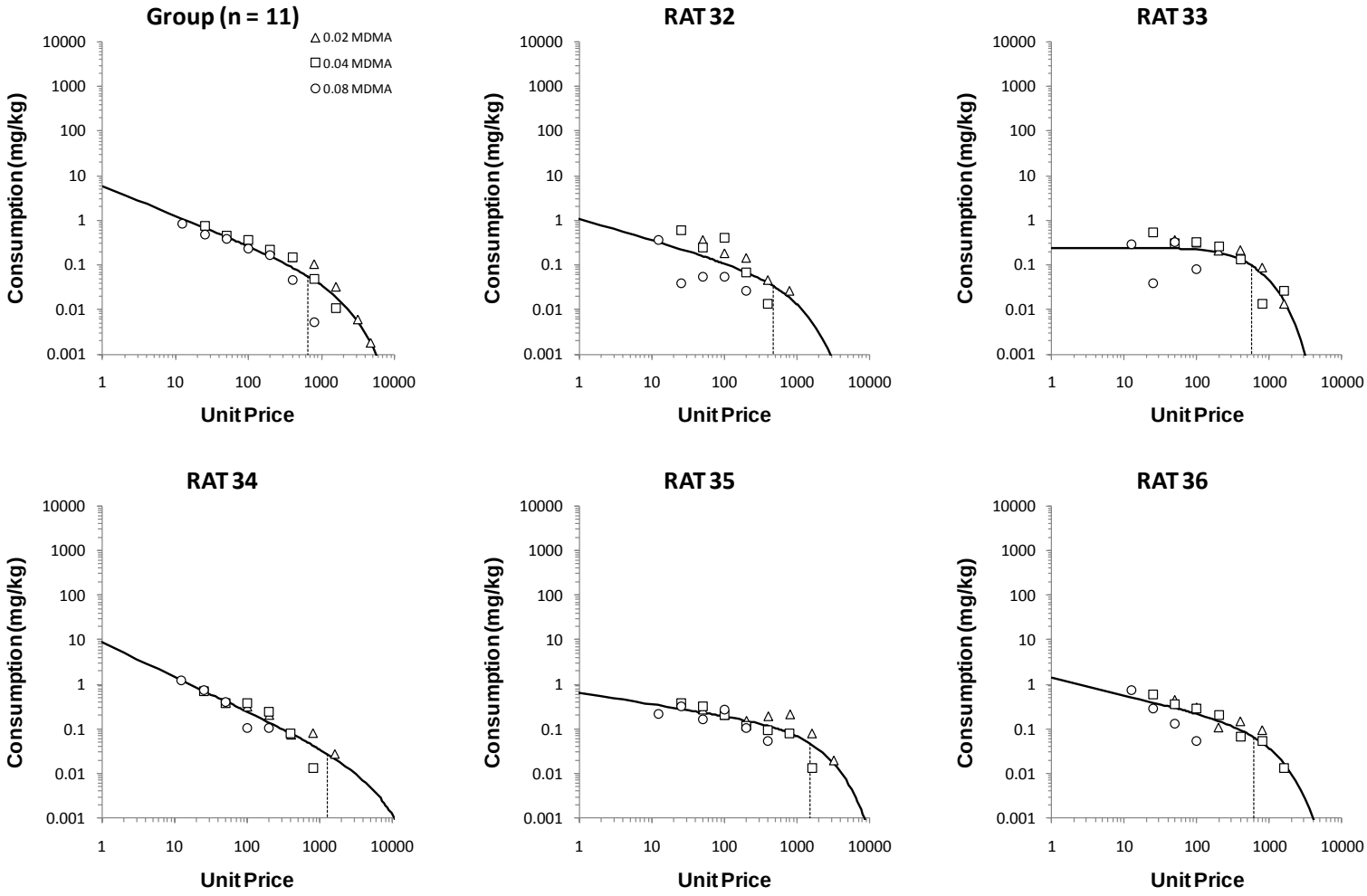

Figure 4.6: Demand for the oral self-administration for 3 doses of MDMA in a 0.2% saccharin vehicle plotting consumption expressed as mg/kg/session as a function of Unit Price. Panel 1 represents the group mean of all animals (n = 11). Successive panels represent individual subjects. Demand curves Lines of best fit were calculated using Equation 4.1 using pooled data across doses. All figures are plotted on log-log axis. Bisecting vertical lines represent P_{max} values. Figure continues next page.

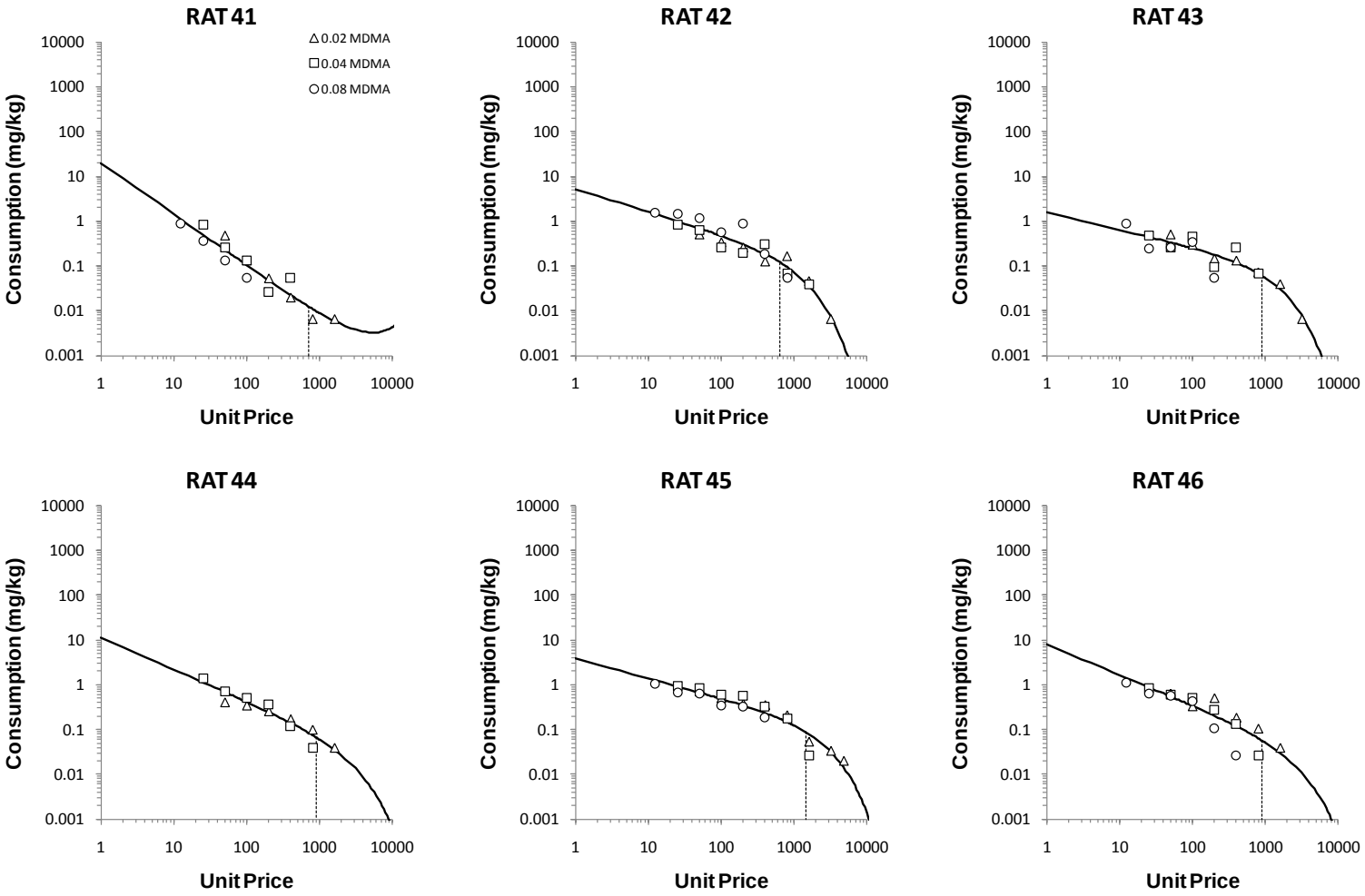

Figure 4.6 (cont): Demand for the oral self-administration for 3 doses of MDMA in a 0.2% saccharin vehicle plotting consumption expressed as mg/kg/session as a function of Unit Price. Panel 1 represents the group mean of all animals (n = 11). Successive panels represent individual subjects. Demand curves Lines of best fit were calculated using Equation 4.1 using pooled data across doses. All figures are plotted on log-log axis. Bisecting vertical lines represent P_{max} values.

Table 4.2: Parameter fits for demand curves for the oral self-administration of MDMA in 0.2% saccharin. Demand curves are expressed in terms of consumption (mg/kg/day) as a function of unit price in log-log coordinates. Parameters were obtained through fits of Equation 4.1. P_{max} values were obtained by fitting Equation 4.2 to estimated parameters.

Subject	condition	*a*	*b*	*L*	P_{max}	R^2
Group	MDMA	0.0005	-0.6654	5.83	650.68	0.92
32	MDMA	0.0011	-0.4847	1.11	475.87	0.34
33	MDMA	0.0018	0.0246	0.23	559.92	0.50
34	MDMA	0.0002	-0.7742	8.69	1282.37	0.96
35	MDMA	0.0005	-0.2530	0.64	1516.18	0.63
36	MDMA	0.0010	-0.3804	1.37	605.10	0.75
41	MDMA	-0.0002	-1.1490	20.16	701.89	0.80
42	MDMA	0.0008	-0.5095	5.31	638.23	0.79
43	MDMA	0.0007	-0.3879	1.59	899.22	0.70
44	MDMA	0.0003	-0.7105	11.24	897.66	0.89
45	MDMA	0.0004	-0.4405	3.83	1452.10	0.89
46	MDMA	0.0004	-0.6793	7.93	912.76	0.87

Normalized Demand Curve Analysis

Data was normalized using Equation 4.4 per the method of Hursh and Winger (1995). Each dose was normalized according to the number of reinforcers obtained at FR1 by multiplying consumption and dividing price by the scaling parameter *q* where $q = B/100$, where *B* is the average number of reinforcers obtained at FR1. When scaled using *q* initial consumption values at FR1 are equal to 100. Thus fits of the linear-elasticity model substitute the constant 100 for the free parameter *L* in Equation 4.1 as shown in Equation 4.6.

$$\ln(Q) = \ln(100) + b[\ln(P)] - a(P) \qquad (4.6)$$

Normalized demand curves were obtained for group data (n = 11) by plotting average normalized consumption as a function of normalized price for three doses of MDMA (combined) and the vehicle-alone condition and are shown in Figure 4.7. Data for the combined drug condition was pooled across doses and Equation 4.6 was fitted to the resulting pooled group data and the vehicle separately. In addition, the model was fitted to group data for each MDMA dose separately. Parameter estimates for the each individual dose as well as the combined and vehicle conditions can be found in Table 4.3.

Figure 4.7 shows the demand curves for the oral self-administration of MDMA and indicates that consumption of MDMA decreased as a function of price. Normalization of the data produces a unitary function ($R^2 = 0.88$) of the effects of price on the consumption of MDMA. However Figure 4.7 indicates that the vehicle-alone condition produced a shallower demand curve than that of the MDMA condition suggesting that subjects treated drug and vehicle-alone conditions

differently. However it appears that saccharin produced more inelastic responding than did the drug-containing solutions. Data for the combined drug condition and the vehicle-alone were well described by the model and accounted for 88% ($R^2 = 0.88$) and 96% ($R^2 = 0.96$) of the variance respectively.

P_{max} was calculated for each condition by substituting the obtained parameter estimates into Equation 4.2. P_{max} was lower for the MDMA (combined) condition ($P_{max\ (combined)} = 2.74$) than it was for the vehicle-alone condition ($P_{max\ (vehicle\ alone)} = 6.04$) suggesting that responding for saccharin-alone was more inelastic than responding for the vehicle-alone condition.

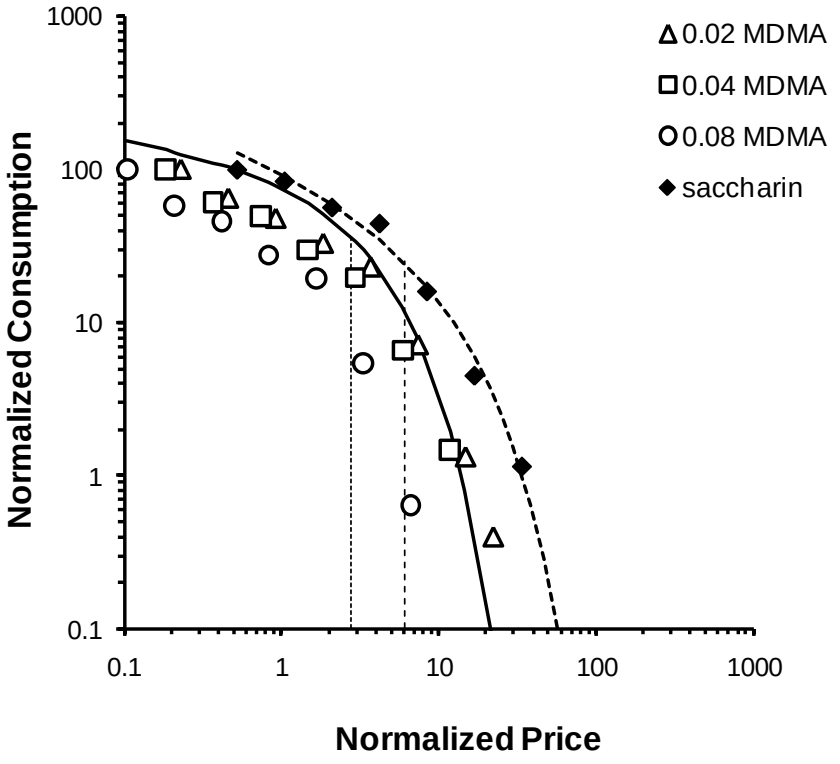

Figure 4.7: Normalized demand curves plotted in log-log coordinates for the oral self-administration for 3 doses of MDMA in 0.2% saccharin vehicle (open symbols) and saccharin vehicle alone (closed symbols). Fitted functions were obtained by fitting Equation 4.6 to normalized data. Bisecting vertical lines represent P_{max} values for each condition.

Analysis of Figure 4.7 indicates that the demand function for the self-administration of MDMA appears to overestimate demand for MDMA despite producing a reasonable fit of the model (R^2 = 0.88). Specifically the normalization procedure sets the initial level of demand (*L*) to 100 and this value is substituted into the Equation 4.1, resulting in the modified form shown in Equation 4.6. However, when fitting Equation 4.6 *L* is treated as a constant representing consumption at minimum price. In lieu of actual values of initial price at infinitely low price responding at FR1 is substituted instead. In the current study *q* (the normalization parameter) is greater than 1 for all conditions, thus normalized demand at minimum price occurs at prices lower than 1. The equation however calculates *L* as a function of normalized FR1 responding which leads to overestimation of consumption at prices less than 1. Note that when *q* is less than the minimum FR schedule, normalized price does not drop below 1 and thus *L* will accurately represent initial demand levels as 100%. In order to counter this phenomenon the model was refitted to the normalized data using Equation 4.1 and all three free parameters were allowed to vary; results are shown in Figure 4.8. Allowing *L* to vary in the model produced less overestimation of the demand for the oral self administration of MDMA. The variance accounted for when fitting normalized data to Equation 4.1 was R^2 = 0.88 and thus resulted in similar levels of R^2 when *L* was free to vary than to when it was fixed at a constant value of 100.

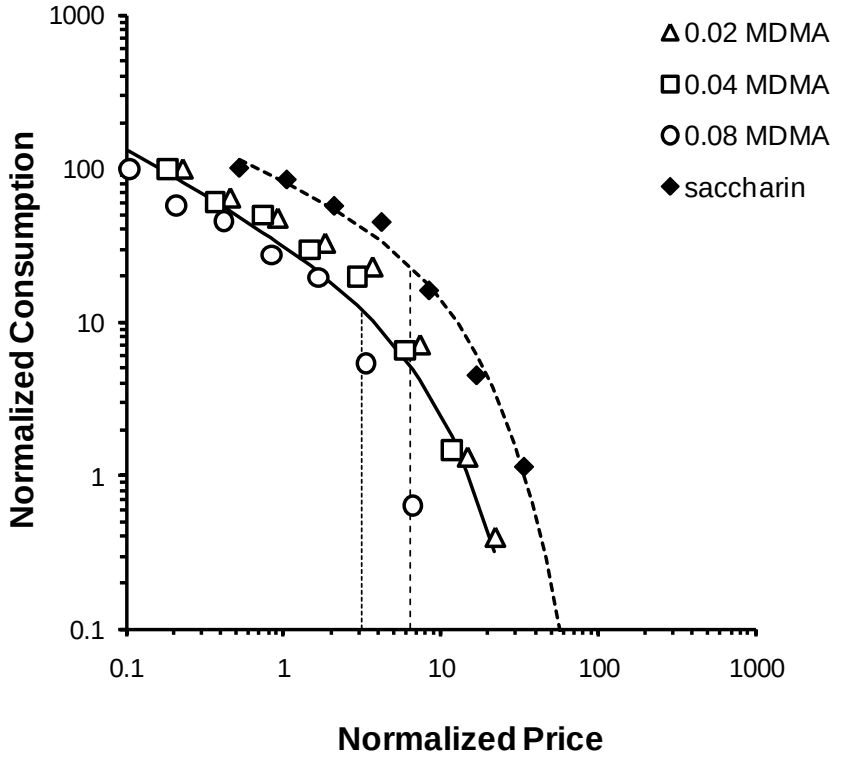

Figure 4.8: Normalized demand curves plotted in log-log coordinates for the oral self-administration for 3 doses of MDMA in 0.2% saccharin vehicle (open symbols) and saccharin vehicle alone (closed symbols). Fitted functions were obtained by fitting Equation 4.1 to normalized data. Bisecting vertical lines represent P_{max} values for each condition.

Normalized demand curves for oral MDMA administration for individual subjects are shown in Figure 4.9. Parameter estimates and R^2 values are displayed in Table 4.3. As the variance accounted for did not differ between fits of either Equation 4.1 or 4.6 for the group function the data from individual subjects was analysed using Equation 4.6 as used by Hursh and Winger (1995) such that the *L* parameter is entered as a constant. In general, individual subjects showed similar patterns of responding to that of the group function; that is all subjects showed decreased responding for MDMA and saccharin as a function of increased price. Like the group function saccharin resulted in more inelastic responding (as measured by P_{max}) for 8 of 11

subjects tested. Some animals, for example Rats' 32, 41, 43 and 46 produced equivalent P_{max} values across conditions, though Rats' 32 and 43 produced (albeit marginally) higher P_{max} for MDMA than saccharin-alone. A paired-samples t-test revealed that P_{max} for saccharin (M = 6.31, SE = 1.36) was significantly higher than the P_{max} for MDMA (M = 2.68, SE = 0.46), t (10) = -3.113, p = 0.011. Model fits were highly variable across subjects with variance accounted for varying between $R^{2 =}$ 0.24 - 0.93, M = 0.59, SE = 0.075) for the MDMA condition. Variance accounted for the saccharin condition was higher with R^2 values ranging from 0.72 to 0.98 (M = 0.92, SE = 0.026).

Table 4.3: Parameter fits for normalized demand curves for the oral self-administration of MDMA in 0.2% saccharin vehicle.

Subject	Condition	*a*	*b*	P_{max}	R^2
Group	MDMA	0.2909	-0.2026	2.74	0.88
	vehicle	0.0742	-0.5172	6.51	0.97
32	MDMA	1.4032	0.3422	0.96	0.24
	vehicle	1.1558	-0.0538	0.82	0.94
33	MDMA	0.5387	0.1682	2.17	0.51
	vehicle	0.1353	-0.2843	5.29	0.97
34	MDMA	0.6610	0.0588	1.60	0.77
	vehicle	0.1153	-0.5097	4.25	0.83
35	MDMA	0.3970	0.0212	2.57	0.54
	vehicle	0.1525	-0.0483	6.24	0.96
36	MDMA	0.5419	0.2417	2.29	0.32
	vehicle	0.0490	-0.3328	13.63	0.85
41	MDMA	0.9103	0.2764	1.40	0.35
	vehicle	0.7503	0.0661	1.42	0.72
42	MDMA	0.2681	-0.0535	3.53	0.81
	vehicle	0.0666	-0.2465	11.31	0.98
43	MDMA	0.3456	0.1205	3.24	0.38
	vehicle	0.1344	-0.6633	2.51	0.96
44	MDMA	0.3398	-0.0830	2.70	0.93
	vehicle	0.0394	-0.5934	10.32	0.95
45	MDMA	0.1053	-0.2941	6.70	0.87
	vehicle	0.0678	-0.2606	10.90	0.98
46	MDMA	0.4155	-0.0539	2.28	0.81
	vehicle	0.2524	-0.3018	2.77	0.98

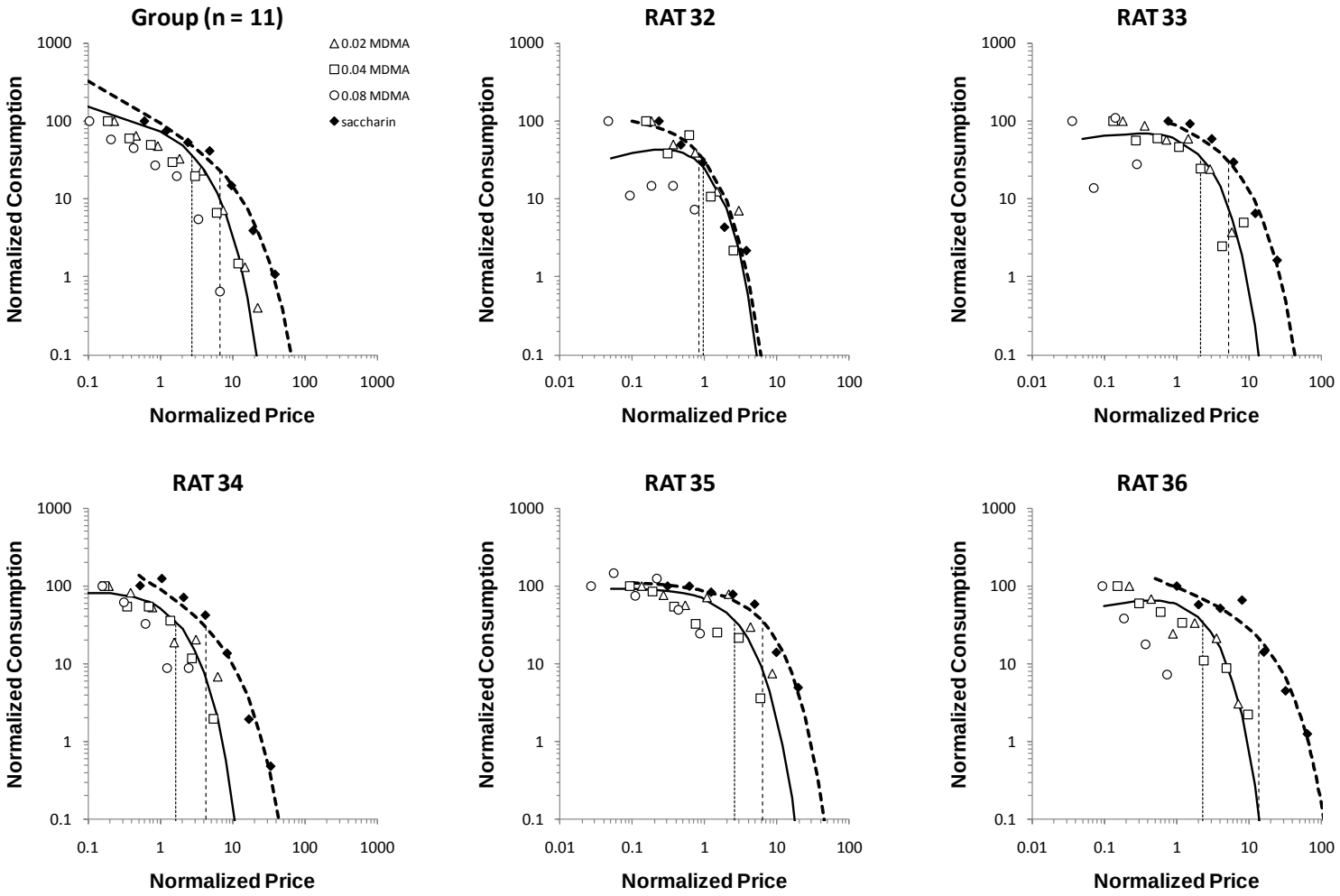

Figure 4.9: Normalized demand curves for the oral self-administration of 3 doses of MDMA in a 0.2% saccharin vehicle. Demand curves for the combined MDMA (open symbols) and vehicle-alone (closed symbols) conditions have been plotted separately. Panel 1 represent the group mean of all animals (n = 11). Successive panels represent individual subjects. Lines of best fit were calculated using pooled data across doses and fitted using Equation 4.6 plotted on log-log axis. Vertical dotted lines represent values of P_{max} fitted using Equation 4.2. See Table 4.3 for parameter estimates. Figure continues next page.

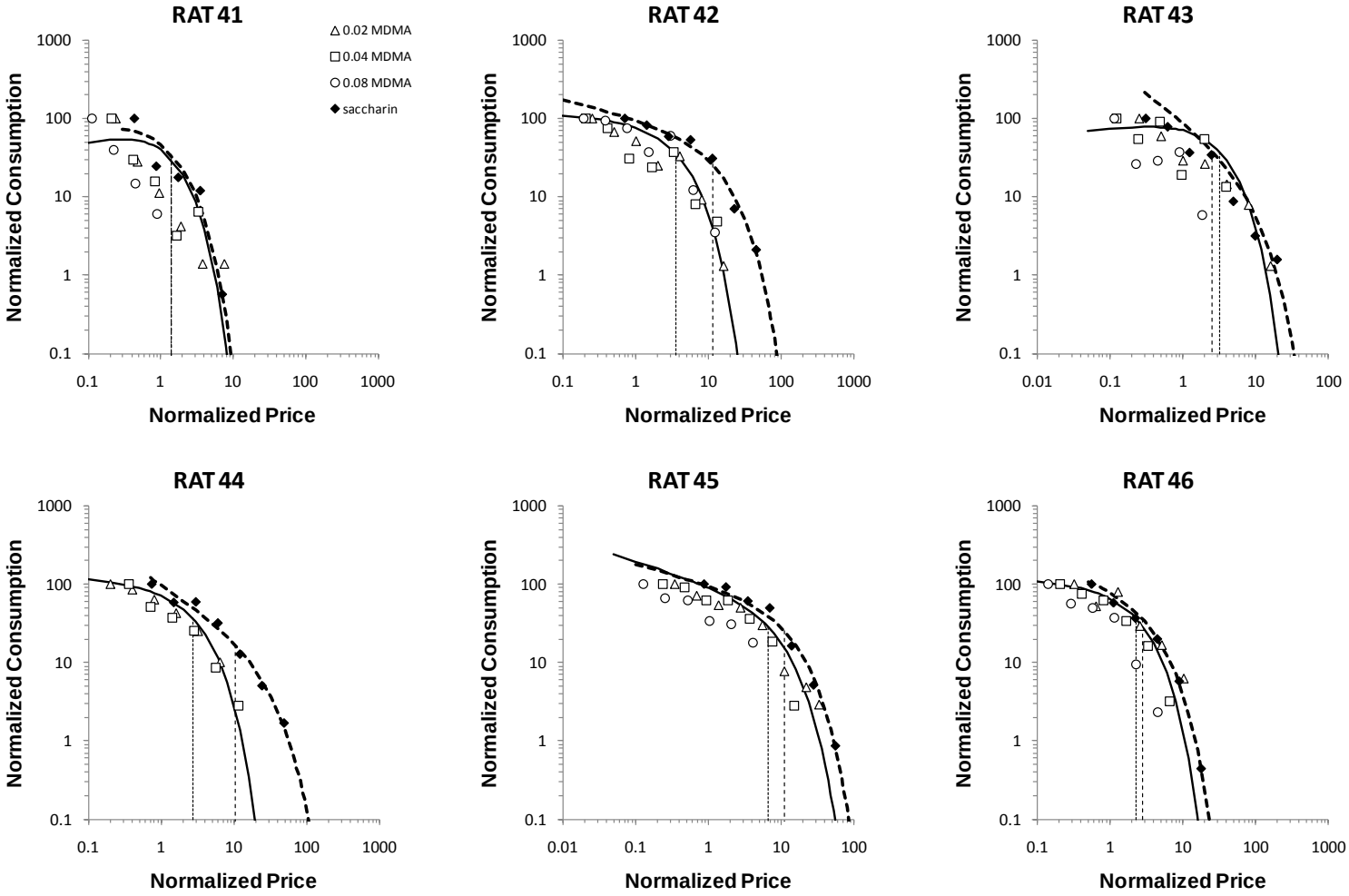

Figure 4.9 (cont): Normalized demand curves for the oral self-administration of 3 doses of MDMA in a 0.2% saccharin vehicle. Demand curves for the combined MDMA (open symbols) and vehicle-alone (closed symbols) conditions have been plotted separately. Panel 1 represent the group mean of all animals (n = 11). Successive panels represent individual subjects. Lines of best fit were calculated using pooled data across doses and fitted using Equation 4.6 plotted on log-log axis. Vertical dotted lines represent values of P_{max} fitted using Equation 4.2. See Table 4.3 for parameter estimates.

Exponential Model of Demand Curve Analysis

Further analysis was conducted by fitting the exponential model proposed by Hursh and Silberberg (2008) to the data obtained during this experiment. The model, shown in Equation 4.5, uses a single free parameter (α, the rate constant of the exponential) to describe differences in what Hursh and Silberberg term 'essential value' which corresponds to differences in elasticity between commodities. Much like the normalized demand model discussed earlier, the exponential model plots demand in normalized space in order to facilitate comparisons between commodities. In order to accomplish this, the exponential model uses the parameter *k* in order to constraint the range of the exponential function. For the current analysis *k* was set to the logarithm of the largest range of consumption for each individual fit.

Demand function and exponential models fits for the group data are shown in Figure 4.10. The top panel of Figure 4.10 shows demand for each dose of MDMA and the vehicle-alone plotted in log-log coordinates. Demand for both MDMA and vehicle-alone decrease as a function of increased price. Individual exponential functions were fit to each dose of MDMA and the results indicate that the 0.02 and 0.04 doses of MDMA produced similar functions indicating that those doses produced similar essential value for the self-administration of oral MDMA. This result is confirmed by an examination of the α parameters for each condition (see Table 4.4) indicating that the 0.02 condition produced an α of 7.95×10^{-4} while 0.04 MDMA condition was similar with an α value of 9.97×10^{-4}. In contrast the 0.08 MDMA condition had an α value of 2.09×10^{-3} indicating a faster decline in demand as a function of price and thus lower essential demand. The difference in drug conditions was further highlighted by P_{max} values with 0.02 and 0.04 MDMA doses producing normalized P_{max} values of 2.26 and 1.80 respectively. P_{max} for the high dose MDMA condition (0.08) was 0.86 and overall lower than either of the other MDMA doses. Of all the

conditions however the vehicle alone condition produced the lowest α and highest normalized P_{max} with α = 3.30 x 10^{-4} and P_{max} = 5.45. Overall fits of the exponential model to the group data were extremely good with variance accounted for greater than 98% for all conditions.

The bottom panel of Figure 4.10 shows the same data plotted on log-linear axis and highlights the differences across conditions where the slope of the exponential function indicates sensitivity to price. Figure 4.10 indicates that subjects defended their access to saccharin more strongly than they did for any of the MDMA doses tested.

Exponential model fits to individual subject data are shown in Figure 4.11 (log-log plots) and Figure 4.12 (log-linear plots). Overall fits of the exponential model were good with mean R^2 = 0.90 (*SE* = 0.02). A one-way ANOVA on the α values found a significant main effect of dose, F (3, 30) = 10.147, $p < 0.001$. Post-hoc contrasts revealed significant differences between all doses including the vehicle condition (all $p < 0.01$). Additionally, a one-way ANOVA on P_{max} also revealed an identical pattern of results, F (3, 30) = 17.645, $p < 0.01$). The results for α and P_{max} calculated using the exponential model indicates that elasticity decreased systematically as a function of increasing dose. In this case the vehicle condition (saccharin alone) produced the most inelastic responding suggesting that subjects defended their access to saccharin more so than they did for saccharin containing MDMA. Furthermore both α and P_{max} values varied systematically across MDMA doses indicating that MDMA consumption was not well characterised by a single demand function using the exponential model. The differences in α values suggest that each individual dose of MDMA has a different essential value and as such differ in elasticity.

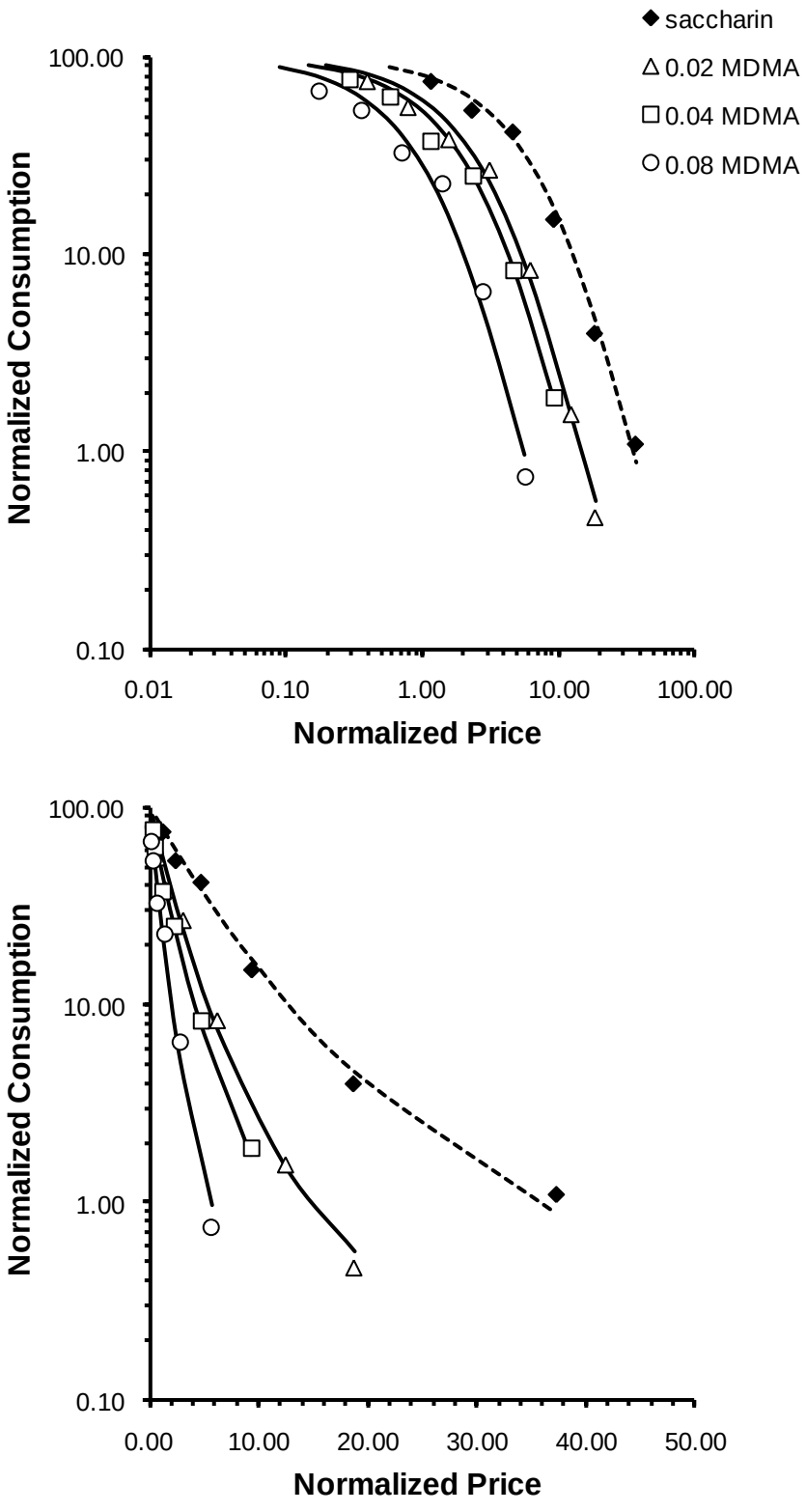

Figure 4.10: Group (n = 11) normalized demand functions for the oral self-administration of 3 doses of MDMA in a 0.2% saccharin vehicle or vehicle-alone. Demand curves for each MDMA dose (open symbols) and the vehicle-alone (closed symbols) conditions have been plotted separately. The top panel represents data plotted in log-log space while the bottom panel represent s the same data plotted on log-linear axes. Lines of best fit were fitted using the exponential equation shown in Equation 4.5.

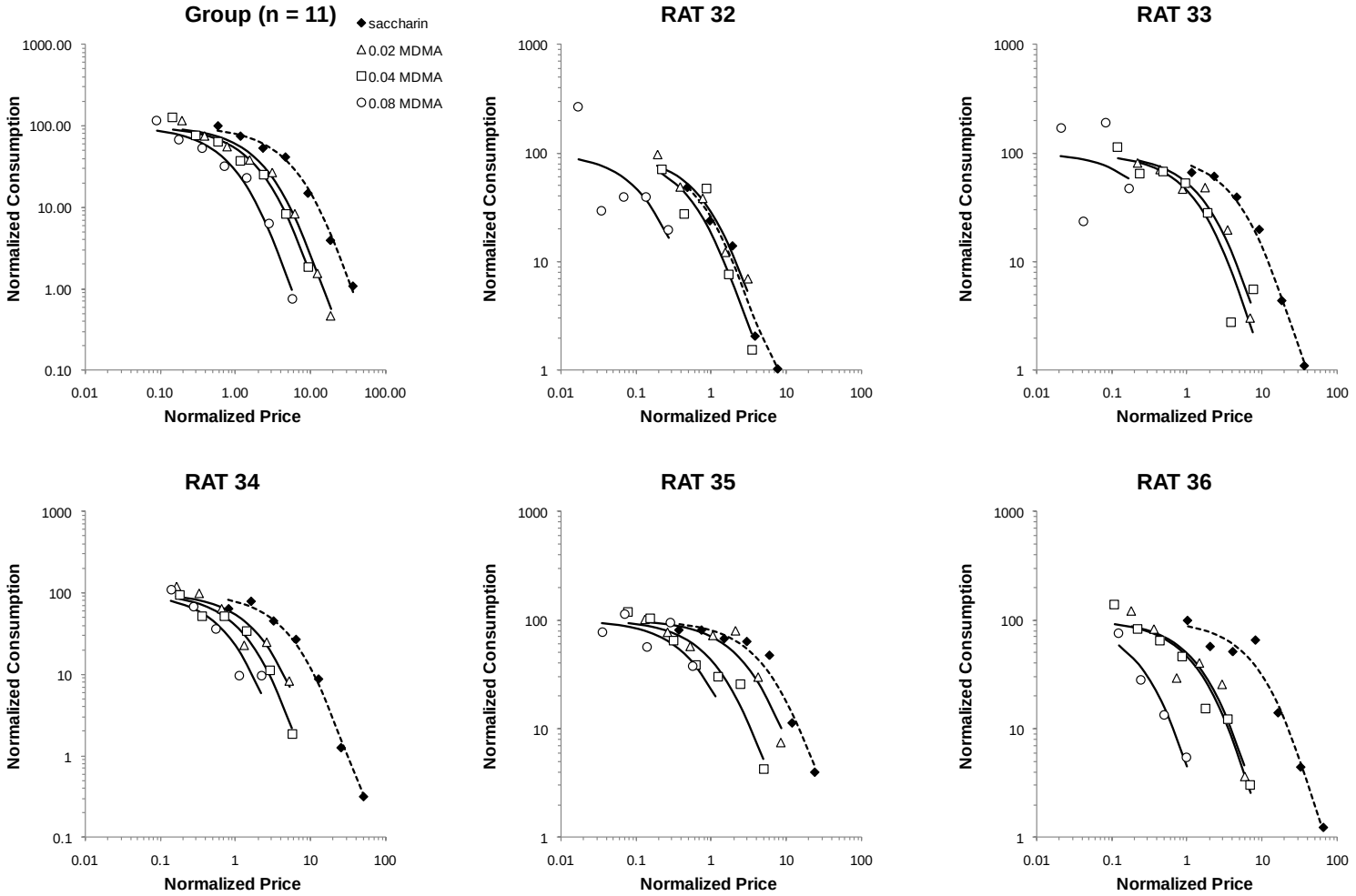

Figure 4.11: Normalized demand curves for the oral self-administration of 3 doses of MDMA in a 0.2% saccharin vehicle. Demand curves for each MDMA dose (open symbols) and vehicle-alone (closed symbols) conditions have been plotted separately. Panel 1 represent the group mean of all animals (n = 11). Successive panels represent individual subjects. Lines of best fit were fitted using the exponential equation shown in Equation 4.5 and are plotted on log-log axes. Figure continued next page.

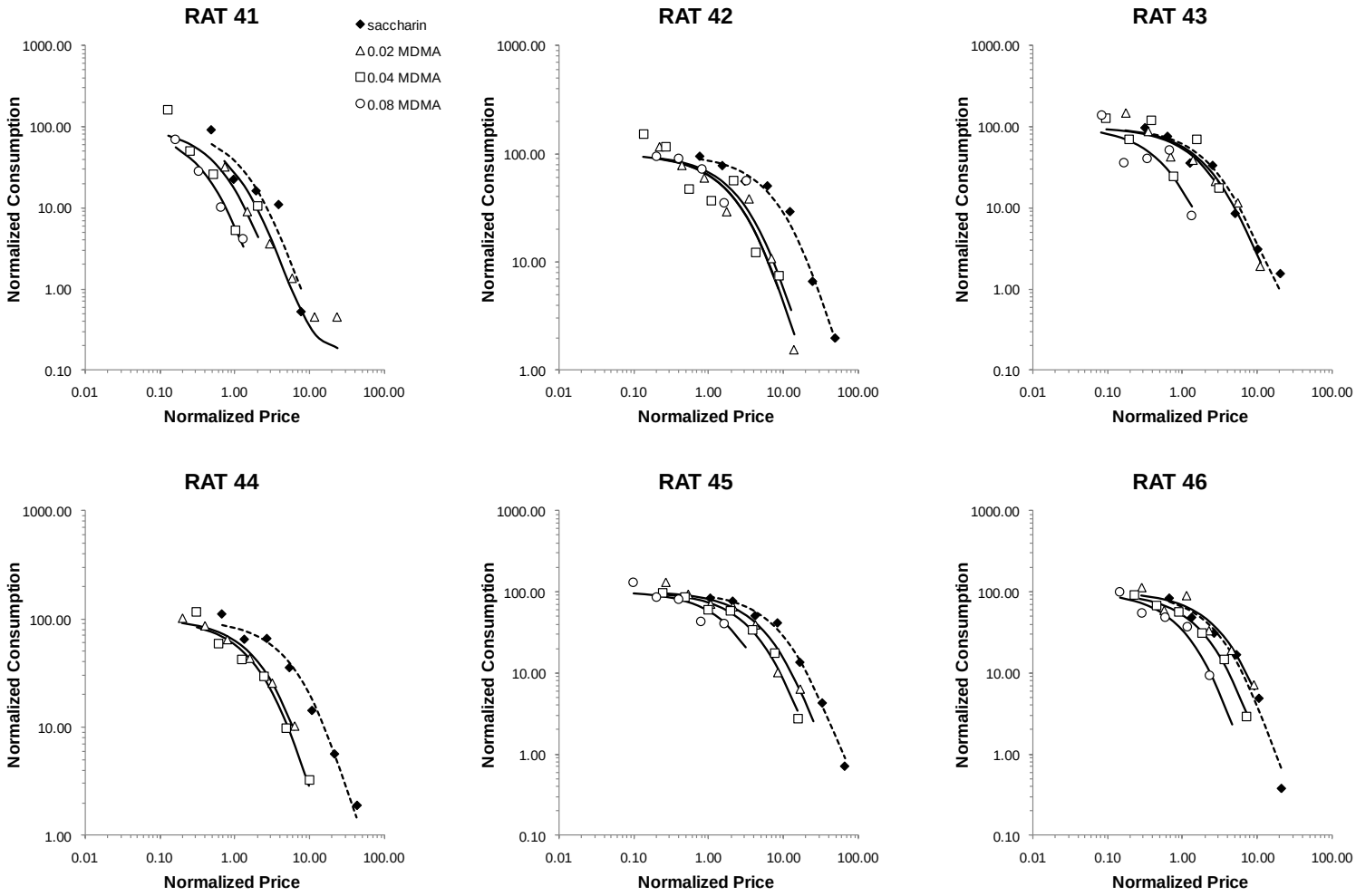

Figure 4.11 (cont): Normalized demand curves for the oral self-administration of 3 doses of MDMA in a 0.2% saccharin vehicle. Demand curves for each MDMA dose (open symbols) and vehicle-alone (closed symbols) conditions have been plotted separately. Panel 1 represent the group mean of all animals (n = 11). Successive panels represent individual subjects. Lines of best fit were fitted using the exponential equation shown in Equation 4.5 and are plotted on log-log axes.

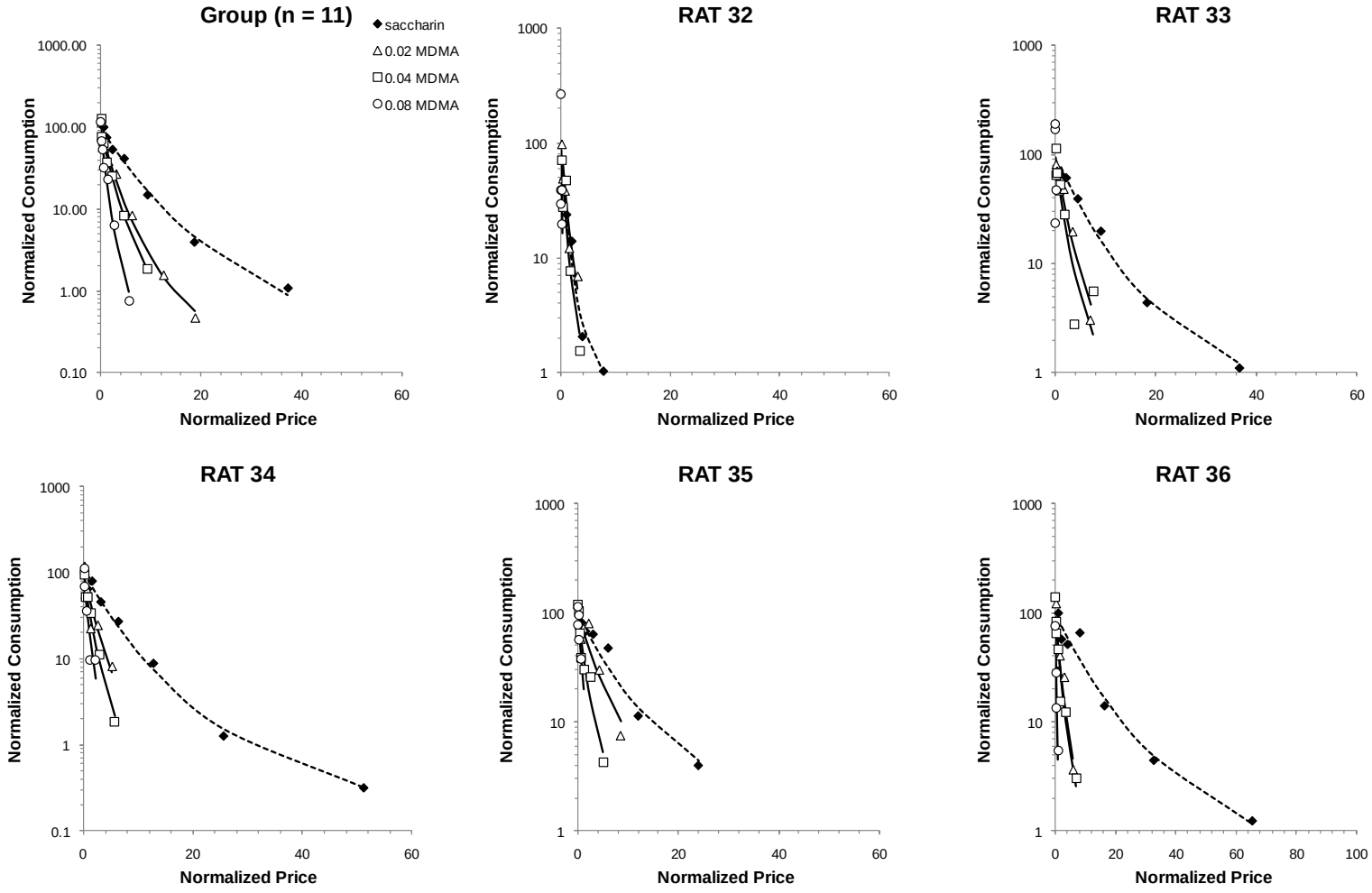

Figure 4.12: Normalized demand curves for the oral self-administration of 3 doses of MDMA in a 0.2% saccharin vehicle. Demand curves for each MDMA dose (open symbols) and vehicle-alone (closed symbols) conditions have been plotted separately. Panel 1 represent the group mean of all animals (n = 11). Successive panels represent individual subjects. Lines of best fit were fitted using the exponential equation shown in Equation 4.5 and are plotted on log-linear axes. Note: a modified scale was use to plot the data for Rat 36. Figure continued next page.

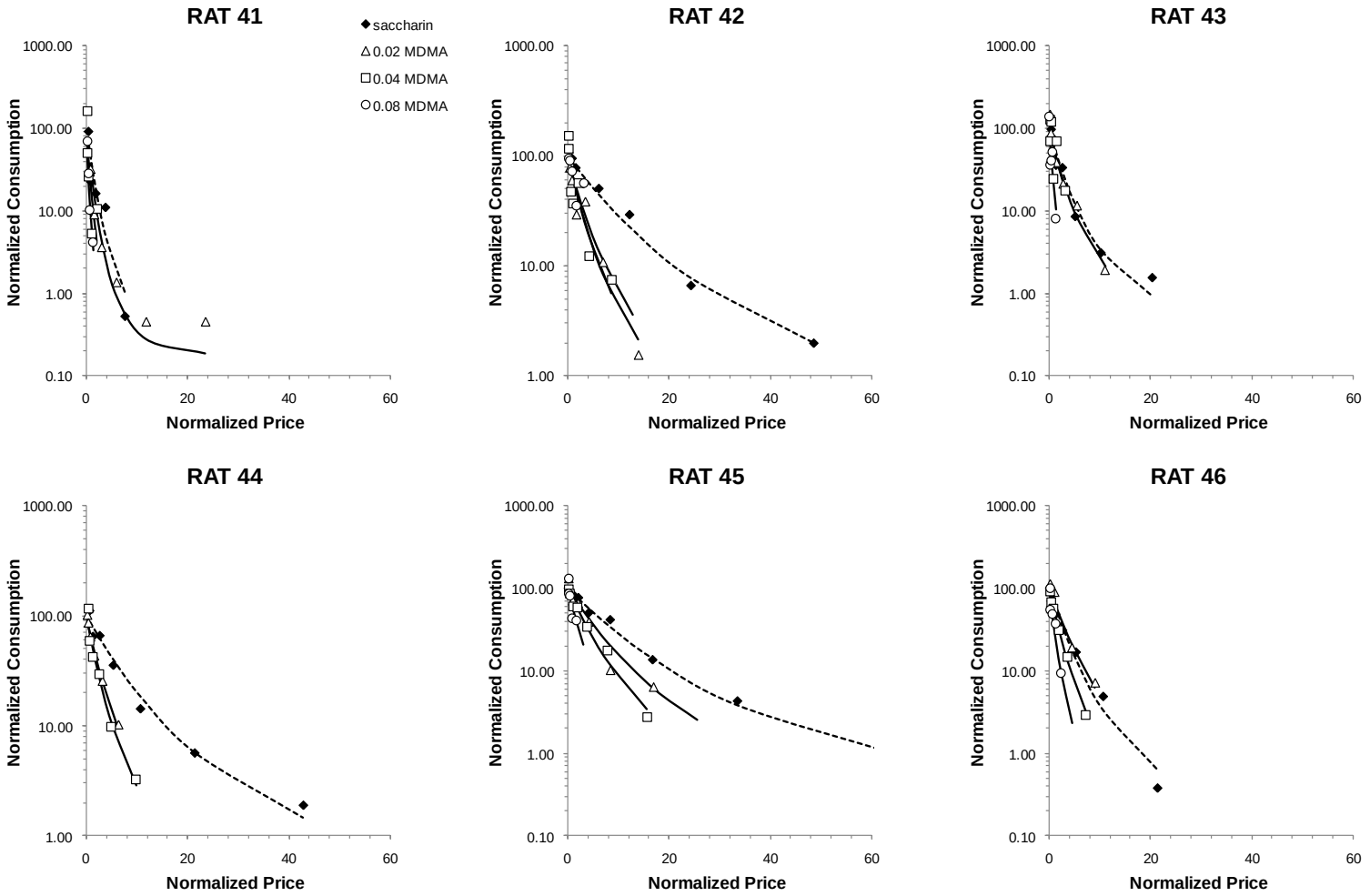

Figure 4.12 (cont): Normalized demand curves for the oral self-administration of 3 doses of MDMA in a 0.2% saccharin vehicle. Demand curves for each MDMA dose (open symbols) and vehicle-alone (closed symbols) conditions have been plotted separately. Panel 1 represent the group mean of all animals (n = 11). Successive panels represent individual subjects. Lines of best fit were fitted using the exponential equation shown in Equation 4.5 and are plotted on log-linear axes.

Table 4.4: Parameter estimates for exponential model fits to the oral self-administration of 3 doses of MDMA and 0.2% saccharin vehicle. Model fits were obtained by fitting Equation 4.5 to the data for the group mean and to each individual subject.

					P_{max}		
Subject	Condition	Q_0	k	α	C	$\frac{C \times Q_0}{100}$	R^2
Group	0.02	19.6	2.902	7.95×10^{-04}	11.54	2.26	0.99
	0.04	14.6	2.902	9.97×10^{-04}	12.32	1.80	0.99
	0.08	8.9	2.902	2.09×10^{-03}	9.72	0.86	0.98
	vehicle	58.2	2.902	3.30×10^{-04}	9.36	5.45	0.99
32	0.02	19.1	2.168	2.88×10^{-03}	4.70	0.90	0.94
	0.04	21.8	2.168	4.13×10^{-03}	2.87	0.63	0.91
	0.08	1.7	2.168	1.64×10^{-02}	9.24	0.16	0.70
	vehicle	48.3	2.168	3.26×10^{-03}	1.64	0.79	0.98
33	0.02	22.1	2.383	1.22×10^{-03}	8.44	1.86	0.96
	0.04	11.9	2.383	1.55×10^{-03}	12.37	1.47	0.83
	0.08	2.1	2.383	6.06×10^{-03}	17.84	0.38	0.27
	vehicle	114.4	2.383	4.42×10^{-04}	4.50	5.15	0.99
34	0.02	16.3	2.903	9.77×10^{-04}	11.29	1.84	0.88
	0.04	17.9	2.903	1.49×10^{-03}	6.77	1.21	0.98
	0.08	13.9	2.903	2.48×10^{-03}	5.20	0.72	0.82
	vehicle	79.9	2.903	3.85×10^{-04}	5.85	4.67	0.99
35	0.02	13.3	1.981	$8.24 \times 10_{-04}$	26.81	3.57	0.91
	0.04	7.8	1.981	2.09×10^{-03}	18.08	1.41	0.92
	0.08	3.5	1.981	3.88×10^{-03}	21.41	0.76	0.83
	vehicle	37.5	1.981	4.80×10^{-04}	16.33	6.13	0.97
36	0.02	18.3	2.506	1.30×10^{-03}	8.98	1.64	0.89
	0.04	10.9	2.506	1.45×10^{-03}	13.51	1.48	0.94
	0.08	12.3	2.506	7.77×10^{-03}	2.23	0.28	0.96
	vehicle	102.0	2.506	2.27×10^{-04}	9.22	9.41	0.97

Subject	Condition	Q_0	k	α	P_{max}		R^2
					C	$\frac{C \times Q_0}{100}$	
41	0.02	73.3	2.744	2.29×10^{-03}	1.14	0.84	0.90
	0.04	12.8	2.744	3.37×10^{-03}	4.46	0.57	0.64
	0.08	16.1	2.744	5.98×10^{-03}	1.99	0.32	0.96
	vehicle	48.2	2.744	1.71×10^{-03}	2.33	1.12	0.90
42	0.02	21.8	2.380	8.66×10^{-04}	12.08	2.64	0.95
	0.04	13.5	2.380	8.62×10^{-04}	19.63	2.65	0.85
	0.08	20.0	2.380	7.29×10^{-04}	15.68	3.13	0.95
	vehicle	75.8	2.380	2.59×10^{-04}	11.63	8.82	0.99
43	0.02	17.3	2.380	1.09×10^{-03}	12.05	2.09	0.95
	0.04	9.6	2.380	1.18×10^{-03}	20.14	1.93	0.56
	0.08	8.3	2.380	4.03×10^{-03}	6.84	0.57	0.74
	vehicle	31.9	2.380	9.46×10^{-04}	7.56	2.41	0.97
44	0.02	19.7	2.373	8.95×10^{-04}	12.99	2.56	0.99
	0.04	30.7	2.373	1.07×10^{-03}	6.99	2.15	0.98
	0.08	-	-	-	-	-	-
	vehicle	66.8	2.373	3.47×10^{-04}	9.87	6.59	0.98
45	0.02	26.6	2.568	3.81×10^{-04}	20.48	5.45	0.95
	0.04	24.4	2.568	5.38×10^{-04}	15.81	3.85	0.98
	0.08	9.9	2.568	9.84×10^{-04}	21.30	2.11	0.86
	vehicle	104.6	2.568	2.41×10^{-04}	8.21	8.59	0.99
46	0.02	28.3	2.852	6.05×10^{-04}	10.71	3.03	0.94
	0.04	22.7	2.852	1.03×10^{-03}	7.84	1.78	1.00
	0.08	14.5	2.852	1.84×10^{-03}	6.88	1.00	0.98
	vehicle	66.9	2.852	6.86×10^{-04}	3.99	2.67	0.97

Discussion

An economic analysis was used in order to ascertain the relative reinforcer efficacy of orally self-administered MDMA. Of interest were three key factors, firstly could the responding (and thus reinforcing strength) for MDMA-containing solutions be separated from its parent vehicle compound, in this case saccharin solution. Initial studies (see Chapter 3) revealed that responding for the vehicle alone promoted higher rates of responding than did the drug-containing solutions thus further analysis was necessary to determine whether saccharin represented more a desirable commodity than MDMA when a subject was forced to defend access to that commodity. Secondly, of interest was whether the relative reinforcing efficacy of orally administered MDMA would conform to a single function of reinforcing strength that could be best described by a single demand function. That is, does the relative reinforcing efficacy represent a measurable property of the drug itself when factors such as dose and potency are eliminated? Finally, recent advances in the field have led to a novel model of economic demand. To date few studies have tested the suitability of the model, thus a comparison was made between the exponential demand model and that of models and analyses used previously in the literature.

Results from Chapter 3 indicated that response rates for saccharin were higher or at least comparable to levels of responding for MDMA-containing solutions. However it was expected that when exposed to economic constraint, such as increased FRs used in the current experiment, that responding for MDMA would be more resistant to increased constraint than would the vehicle alone. In economic terms, MDMA responding should be more inelastic thus animals will defend their access more strongly than they would for the drug-free solution.

Initial analysis of the data revealed that both reinforcers earned, and responses per session as a function of dose, were higher for saccharin than they were for any of

the MDMA doses. However, the FR value at which peak responding occurred was greater for MDMA than it was for saccharin suggesting that subjects were defending their access to MDMA more strongly than saccharin alone. Economic analyses using the linear elasticity model (Equation 4.1) showed that MDMA at the 0.02 and 0.04 doses did indeed show a higher P_{max} than did the vehicle alone, though the differences were relatively small and not significant.

Further analysis was conducted using the normalized demand model (Hursh & Winger, 1995) in order to directly compare the elasticity of both MDMA and saccharin. The normalization procedure had been shown to be a robust method for comparing elasticity across different commodities. By eliminating overall level of demand and plotting data as a proportion of baseline for that commodity at minimal price it allows for the direct comparison of elasticity by using normalized P_{max} to compare demand functions. The normalization procedure had been demonstrated to facilitate comparison of relative reinforcer efficacy across drugs of differing potency as well as drugs of different classes (e.g. stimulants versus opioids) (Hursh & Winger, 1995) and to compare demand for qualitatively different foods such as normal, puffed and honey puffed wheat in hens (Foster et al., 2009) or food and fat in rats (Madden, Smethalls, Ewan & Hursh, 2007). The current study sought to use the normalization procedure to disentangle the reinforcing properties of MDMA from the reinforcing properties of the vehicle solution it was presented in. Doing so would add a valuable tool that would further help in studies of oral drugs of abuse where drugs are sometimes presented in a compound reinforcer made up of drug and non-drug components. Differentiating between the relative contributions of each part of this compound reinforcer can sometimes be difficult, so a quantifiable method of elucidating these differences would be a promising step.

It was expected that the addition of MDMA to the saccharin vehicle would increase the reinforcing efficacy of the solution and that this increase would manifest as an

increase in inelastic demand measured by P_{max}, a measure that has been used to quantify relative reinforcer efficacy for drugs of abuse previously. However, normalized demand plots analysed using Equation 4.6 showed that saccharin produced a higher P_{max} than did MDMA indicating that despite predictions to the contrary subjects defended their access to saccharin more strongly than they did for MDMA.

The same data was subsequently analysed with the more recent exponential model proposed by Hursh and Silberberg (2008) to the same ends. Saccharin produced higher normalized P_{max} values than did each of the MDMA-containing solutions. Concordant with the P_{max} data, α values (the rate constant of the exponential) were lower for saccharin (indicating slower decay) than they were for MDMA. Much like the normalization procedure, the exponential model examines demand as a function of normalized space, so it not surprising that the results from fits of Equation 4.6 and Equation 4.5 produced similar results.

Contrary to expectations MDMA produced a lower relative reinforcer efficacy as measured by P_{max} in the linear-elasticity model and α (Hursh and colleague's 'essential value') in the exponential demand model than did saccharin when consumption and price were normalized. Rather than the addition of MDMA to the solution increasing demand it instead decreased demand by both decreasing overall levels of intake (approximated by L in the linear model and Q_0 in the exponential model) but also by increasing elasticity. That is, MDMA-containing solutions produced lower overall intake and were also more sensitive to price increases. A decrease in overall levels of intake was somewhat expected due to MDMA-containing solutions producing drug effects that the vehicle condition did not. Evidence from both the current study and Chapter 3 suggested that the animals were titrating their intake of MDMA as a function of dose, that is consumption was increased as dose was decreased and vice versa. Titration of MDMA dose was

supported by unit price analyses where subjects maintained similar rates of consumption of MDMA (mg/kg) such that consumption (mg/kg) plotted as function of unit price (price per mg/kg) conformed to a single function. However, relatively more elastic responding evidenced during MDMA conditions compared with vehicle was unexpected and difficult to reconcile. It seems unlikely that aversive drug-specific consequences are the cause of the increased elasticity as consumption is decreased more so at larger FR requirements thus aversive effects of the drug would be lower than responding at low FR requirements. One explanation for MDMA's lower elasticity may be that rather than the supposed drug effects, subjects are in fact responding as a function of taste. Though the MDMA solutions were adulterated with saccharin to ameliorate taste related responding it remains plausible that some identifying taste remains. If the taste of the solutions were indeed aversive and this aversiveness changed as a function of concentration of MDMA then it would be expected that different doses of MDMA would produce distinct demand curves (when normalized) rather than conforming to a single function. There is some evidence to suggest that this may be case with both the unit price and normalized demand functions indicating that the 0.08 dose of MDMA was generally not as well described by the fitted functions. However in both cases the functions for the 0.02 and 0.04 dose of MDMA were well described by the model and seem to produce a single unitary function of demand. However, inspection of demand curves for the exponential analysis reveals that there was a significant systematic decrease in P_{max} and an increase in α as a function of dose, suggesting that as dose increased elasticity also increased. This systematic change in elasticity for dose may be indicative of the animals' responding being sensitive to the increases in the concentration of MDMA that resulted in a change in the taste rather than being sensitivity to the reinforcing effects of MDMA per se. It must be noted that the doses of MDMA used in this study were substantially smaller than those used previously so it cannot be confirmed that the drug itself was producing

reinforcing effects with the concentrations tested. Due to the relatively low doses of MDMA used in this study it is possible that MDMA intake falls along the ascending (rather than the descending) portion of the dose-response curve. If this were the case then MDMA reinforced behaviour could possibly be masked by the saccharin when MDMA doses were below threshold for the occurrence of reinforcing effects; leaving taste of the resulting solutions as the sole differentiating factor. However it should be noted that a five-fold change in concentration did not appreciably change the dose-response curve for oral MDMA between Experiments 3.2 and 3.3 (though in the first case MDMA was mixed with water and in the second case MDMA doses were adulterated with saccharin that may have aided in palatability despite the change in MDMA concentration). It seems likely that taste would have a larger effect during the course of Experiment 3.2 when solutions were not adulterated, yet total intake of MDMA stayed relatively constant across each of those experiments.

An alternative and more likely explanation for the current findings is that oral MDMA represents a weak reinforcer and thus does not promote greater inelastic responding than saccharin (a reinforcer in its own right) as was predicted.

The second point of interest for this study was how well each of the behavioural economic models would fit the self-administration data for orally delivered MDMA. Specifically of interest was whether data from multiple doses could be extrapolated and described with a single function which might represent a unitary measure of relative reinforcer efficacy for MDMA when delivered orally. To serve this purpose various models and analyses were fitted to the obtained data. Bickel and colleagues (Bickel et al., 1990; DeGrandpre, Bickel, Hughes, Layng & Badger, 1993) suggest that unit price presents a “useful metric” for studying drugs of abuse as it allows for reinforcer magnitude (i.e. dose) to be removed from the equation on the assumption that changes in dose (reinforcer magnitude) and changes in response requirement are equivalent operations. Unit price, operationalised in self-

administration studies as responses per mg/kg allows for multiple doses of drug to be plotted simultaneously as a single demand function. The advantage of data that can be plotted as a single function is that it indicates that all doses of a drug share what is effectively the same relative reinforcer efficacy and allows a direct quantification of relative reinforcer efficacy for the purposes of comparisons within and across other drugs of abuse.

Obtained data from the oral-self administration of MDMA when transformed into average total intake per day and plotted as function of unit price did indeed produce a function that was well described by the model ($R^2 = 0.92$) (see Figure 4.5). However, unit price represents an absolute measure of the reinforcing efficacy of a given commodity and assumes that the commodity is scalar such that the unit price model assumes that consumption changes linearly as a function of the scalar variable (e.g. drug dose, grams of food, concentration etc.). For drug doses these assumptions only hold true as long the drug being examined is measured in the same part of the dose response curve. For example, it might be expected that drug doses measured from the ascending and descending sequences of the dose-response curve would produce differing values for reinforcer efficacy that might be more appropriately modelled as separate functions. For example, Winger (1993, see also Hursh & Winger, 1995) found that a low dose of *iv* self-administered cocaine produced a unit price demand function that was not consistent with two larger doses tested in the same rhesus monkeys. The current data (see Figure 4.5) also provide some support for this as the 0.08 MDMA dose ($P_{max\ (0.08)} = 153.51$, $R^2 = 0.96$) appears to more elastic than the other two doses ($P_{max\ (0.02+0.04)} = 659.02$, $R^2 = 0.99$).

Unfortunately while unit price presents a nice conceptualisation of the reinforcing efficacy of MDMA and other drugs of abuse it is difficult to directly compare the elasticity of one drug with another (or other commodity) due to overall differences in

potency (level of demand) across drugs. Hursh and Winger (1995) instead proposed a normalization procedure as an alternative to unit price that used consumption relative to baseline rather than absolute measures such as unit price. By normalizing demand across commodities it provides a way of directly comparing elasticity of a drug without potency contributing as a factor. This allows a direct comparison of the sensitivity to changes in price and allows P_{max} values to be used as a direct measure of relative reinforcer efficacy.

Much like the unit price analysis the normalization procedure produced a relatively good fit ($R^2 = 0.88$) to the obtained oral MDMA self-administration data collected during this study and ultimately does a good job of representing the relative reinforcing efficacy of oral MDMA when tested under these conditions.

$$\text{Elasticity} = b - aP \tag{4.7}$$

In a recent contribution to the literature Hursh and Silberberg (2008) proposed an exponential model as an alternative to the conventional linear elasticity model. The primary advantage according to the authors was that elasticity is described through the use of a single free parameter, α. In contrast, the linear elasticity model uses two parameters to describe elasticity as shown in Equation 4.7, the initial slope *b* and the rate of change of the curve, *a*. Elasticity changes as linear function of price (*P*) since the parameters *a* and *b* are fixed for each demand curve (Hursh & Silberberg, 2008). While the linear elasticity model can be summarised to produce a single estimate of elasticity by using P_{max}, Hursh and Silberberg suggest that the single parameter exponential model fulfils the goal of a producing a more parsimonious model with a single measure of elasticity of demand. This measure,

α, is described by Hursh and Silberberg as representing the ‘essential value’ of a commodity thusly due to its close correlation with other demand measures can be substituted as a measure of relative reinforcer efficacy.

The current study sought to test and compare the linear elasticity and the exponential models of demand. The exponential model produced a good description of the data with fits of the group data accounting for at least 98% of the variance. The data was also in accordance with fits of the normalized demand model and similarly produced the highest relative reinforcer efficacy for the vehicle-alone condition compared with MDMA-containing solutions.

In fitting the exponential model to the oral MDMA self-administration data *k* was set to the log of the maximum range of consumption for each of the analyses. The *k* parameter represents a scaling parameter and effectively constrains the asymptotes of the exponential function. In order to allow the direct comparison of each demand curve Hursh and colleagues suggest that the *k* value for those comparisons should be the same. In all cases this resulted in the *k* value being set to the range of the vehicle condition as it possessed the greatest range in consumption as a function of price. While this solution produced adequate fits of the both group and individual data it should be noted that variations in the *k* parameter can have a direct effect on the results obtained. Foster et al. (2009) tested exponential model as a means of measuring demand for qualitatively different foods in hens and found that fitting the model with a range of *k* values resulted in changes not only in the α parameter itself but also in the orders with which the three commodities were arrayed. As a comparative tool for measuring relative reinforcer efficacy this presents a potential problem as fits within studies, and certainly across studies, will vary based on the *k* value with which the functions are fit. This may prevent α values being directly compared across studies which would be a benefit in the process of cataloguing relative reinforcer efficacy across a range of drugs and classes (a function

potentially more suited to the normalized demand model where no scaling parameter is necessary). Indeed the range of *k* values used in published studies varies widely across studies. In order to compensate for this potential shortcoming it seems prudent that where possible that raw data be published in order to allow for subsequent authors to refit analyses with *k* values appropriate to both new and old data sets where comparisons are necessary or warranted.

Christensen et al. (2008b) present an interesting test of the exponential model of demand. In their study the authors sought to examine cocaine escalation data previously reported by Ahmed and Koob (1998). In Ahmed and Koob's study they found that rats given 6-hours access to cocaine self-administration (long access) produced a markedly different profile of self-administration than those animals that had only a 1-hour (short access) daily access to cocaine. The animals in the long access group showed escalation of cocaine intake over the course of training and showed an upward shift in the dose-response for cocaine in contrast with those in the short access group. Christensen et al. (2008b) reanalysed Ahmed and Koob's data within and economic context and found that cocaine produced a higher essential value (lower α) in the long access subjects compared with those in the short access group. They further studied this effect by re-determining demand curves for food and cocaine for rats who had previous experience from a prior study (Christensen et al. 2008a). Interestingly they found that essential value for cocaine increased as a function of the second demand curve analysis while essential demand for food remained the same. This illustrates an interesting approach to the disambiguation of the differences between drug reinforcers and biological necessities like food. Within the context of the current study this approach may provide a way to further examine the reinforcing effects of MDMA and provide support for the reinforcing effects of MDMA independent of the reinforcing effects of the vehicle. Specifically, if replicating the demand analysis resulted in increases in

essential value for MDMA, but not the vehicle conditions then those changes would be attributable to MDMA's effect as drug reinforcer and not to the effects of taste or the vehicle solution it was presented in.

Though still it's in infancy, the exponential model provides another robust tool for the economic analysis of behaviour. It appears that the new model shares a strong correspondence with the earlier model in that the results and predictions remain largely similar across applications of each model. That the exponential model achieves this despite using a single parameter is a distinct advantage of that model over previous iterations. Though desirable it may be too early to tell whether the exponential demand model can be repurposed specifically for providing rank-ordered and detailed analysis of the relative reinforcing efficacy of multiple compounds and across many classes of drugs of abuse. As recommended by Bickel and colleagues (Bickel, Marsch & Carroll, 2000; Johnson & Bickel, 2006) adopting behavioural economic models as standard practice for the analysis of the relative reinforcer efficacy is worthy and allows for comprehensive testing of the abuse liability of drugs of abuse across a variety of dimensions.

In this chapter behavioural economic analysis was conducted on the oral self-administration of MDMA. The analyses revealed that care must be taken when conducting economic analyses as different methods and models can provide different conclusions and support different interpretations of the data. Initial analysis of the data indicated that MDMA functioned as a stronger reinforcer by supporting responding to higher FR requirements than did the saccharin-vehicle alone. However, when data was normalized in order to allow direct comparison across commodities this effect was instead reversed, suggesting that the vehicle itself functioned as a stronger reinforcer. Further research is necessary to more clearly understand the relationship between the reinforcing properties of oral MDMA and that of saccharin. Of particular importance is research investigating demand for

MDMA and saccharin when available under concurrent schedules. Under such conditions more conclusive results might be found with regard to the reinforcing efficacy of oral MDMA in comparison with the separate schedules method employed during the current study.

Chapter 5 General Discussion

Meisch and Carroll (1987) identify three key factors in determining whether an oral drug serves as a positive reinforcer or not. Firstly, the drug must maintain behaviour consistent with that shown under intermittent schedule performance. Secondly, the drug should produce orderly dose-response functions. That is, the drug should show evidence of an inverted U dose-response function or at the very least evidence of the ascending or descending portions of the dose-response curve. Finally, behaviour for the drug solution should exceed that of its component vehicle solution. The current thesis sought to examine MDMA as oral reinforcer and the results found herein generally conform to the guidelines outlined by Meisch and Carroll.

The current thesis finds support for intermittent schedule performance in two key ways. Behaviour in all operant experiments was maintained on fixed ratio schedules of performance and subjects responded across all conditions tested in order to gain access to reinforcers (irrespective of dose). Crucially, when FR was manipulated during Experiment 4.1 subjects produced FR-dependent responding; that is, as the FR was increased total session responding increased then decreased as a bitonic function of FR as expected. Overall response rates for the experiments tested above were relatively low when tested across the entire session however; in general, subjects showed differences in response rate across the course of the daily sessions that was both dose- and FR-dependent. Responding primarily occurred during the first part of daily sessions with lower response rates during the latter portions of each session (see Figure 3.10). It might be expected that in Experiment 3.2 that responding for water-alone would decrease during the course of each session due to satiation of thirst-induced responding. The decrease in responding over the latter portions of daily sessions for water-alone suggests that that may have occurred. By the same token it may have been expected that animals in

Experiment 3.3 responding for saccharin-alone would continue to respond to respond at high rates throughout the session for access to saccharin because responding would not be inhibited by accumulation or titration of drug dose in that condition. This was not the case and responding for saccharin-alone decreased throughout the session at much the same rate as the 0.02 dose of MDMA (data not shown).

Meisch and Carroll's (1987) second proposition posits that orderly concentration response curves must be demonstrated in order firmly conclude that that a given oral drug is functioning as a reinforcer. Experiment 3.2 clearly demonstrated that orderly dose-response functions were found for the oral self-administration of MDMA. Group averages (and to a lesser extent individual subject data) produced orderly dose response functions indicating increased responding as a function of decreases in dose. Individual-subject data was more variable but generally subjects showed orderly functions of dose (with the exception of a single animal who no evidence of dose-dependent responding). The data obtained from Experiment 3.2 is indicative of titration of drug levels during daily sessions and is representative of the descending portion of the dose-response curve. Experiment 3.2 did not produce an inverted U-shaped function of dose and found no evidence representative of the ascending portion of the dose-response function. It is likely that the ascending portion of the dose-response function was masked by responding for the vehicle condition (water) which remained high due to animals being tested under water-deprivation conditions. Hence any further decreases in dose served to make the solution more vehicle-like. Because responding for water-alone was high, lower concentration MDMA solutions would be expected to maintain rates similar to those seen for the vehicle-alone. It is likely that the animals would continue to respond for access to water component of the solution even as the reinforcing magnitude of MDMA is decreased (as would be expected for doses along the ascending portion

of the dose-response curve). A similar pattern of results was found during Experiment 3.3 when subjects were instead tested with various doses of MDMA in a vehicle of 0.2% saccharin solution. Again both group averages and individual subjects showed orderly dose-response functions indicating increased responding as a function of decreases in dose. Furthermore, MDMA intake as a function of dose was highly correlated across Experiment 3.2 and 3.3 for individual subjects (r = 0.798, n = 12, p = 0.01) despite a number of fundamental changes in experimental procedure. For example, in Experiment 3.3 subjects responded for MDMA in a saccharin vehicle solution whereas Experiment 3.2 used water as the vehicle (in water-deprived rats). The use of a saccharin vehicle allowed for subjects to have free access to water in the home cage, removing the necessity of water deprivation during the earlier experiment. In addition, higher dose, but smaller magnitude (0.1cc versus 0.02cc) reinforcers were used during Experiment 3.3. Despite these fundamental differences subjects showed generally similar MDMA intake across the experiments. Like Experiment 3.2 before it, Experiment 3.3 also found orderly dose-response functions representing only the descending portion of the dose-response curve. When tested with low FR values (FR4) it appears that saccharin is a highly regarded reinforcer in its own right, thus much like Experiment 3.2 the saccharin vehicle may have masked the ascending portion of the dose-response curve. Experiment 3.4 served two purposes; (1) examine the effect of the D1 antagonist SCH23390 on oral MDMA self-administration and (2) test a lower range of MDMA doses for oral self-administration in order to more fully examine the ascending portion of the dose-response curve. Daniela et al. (2004) showed that pre-treatment with of SCH 23390 shifted the dose-response curve for *iv* self-administration to the right. However, pre-treatment with SCH 23390 to rats orally self-administering MDMA instead produced a non-specific decrease in responding across all doses, and crucially, the vehicle-alone condition. Rather than shifting the dose-response curve as expected SCH 23390 abolished responding for all doses

suggesting that SCH 23390 may have decreased motivation to respond for all conditions. However, pre-treatment with SCH 23390 did demonstrate that responding for oral MDMA in saccharin was sensitive to challenge by pharmacological and not just behavioural methods. Experiment 3.4 used a different dose range from used in Experiments 3.2 and 3.3. The purpose was to further examine whether oral MDMA in saccharin would demonstrate an inverted U-shaped dose-response curve. Unfortunately, there was still no definitive evidence for the ascending portion of the dose-response curve with doses as low as 0.003mg/kg of MDMA. However, Experiment 3.4 did produce data consistent with the descending arm of the dose-response curve and indeed consistent with those produced previously for Experiments 3.2 and 3.3 despite a different dose range and inexperienced animals.

In contrast to the operant self-administration methods, Experiments 3.1A and 3.1B used concurrently available water and MDMA during a free-access task. All subjects developed a clear preference for the water-containing bottle over that of the MDMA-containing solution. Though animals continued to sample from the MDMA containing solution it is unclear to what extent the taste of the unadulterated solution affected this preference. In addition, only a single dose of MDMA was tested in conjunction with plain water, thus no dose-effect evaluations were able to be conducted. An adapted form of the task used in Experiment 3.1 might prove useful for demonstrating dose-effect relationships for consumption of oral MDMA in the homecage. Specifically, the procedure should use a three-bottle test rather than two. In one bottle, plain water would be available. The second bottle would contain MDMA solutions in a saccharin vehicle (to aid in palatability). The final bottle would contain plain saccharin and would serve as a control for drinking from the MDMA-containing solution. The presence or absence of the third bottle would allow for the analysis of changes in MDMA consumption as a function of availability of an

alternative reinforcer that may act as a partial substitute. The key effect of using easily flavoured solutions for the drug and alternative reinforcer is that the animal is free to consume water necessary for survival independent of the manipulated drug solutions. In addition multiple doses of MDMA should be made available in order to measure dose-effect relations for consumption of oral MDMA.

The final feature key attribute outlined by Meisch and Carroll (1987) is that rates of drug maintained behaviour for the drug-containing solutions must exceed that of the parent vehicle solution whether presented concurrently (as they were in Experiment 3.2A & B) or sequentially such as in Experiments 3.2, 3.3, 3.4 and 4.1. Chapter 3 provided mixed evidence for this proposition. In the free-access choice paradigm water was always preferred to MDMA; though in that case drinking of water was related to survival while drinking of the drug solution was not. The expectation that drinking of drug solutions should be higher in that case would be erroneous. Typically the lowest dose of MDMA used in Experiments 3.2, 3.3 and 3.4 approached but did not exceed the rate of vehicle maintained behaviour and other doses produced total responding lower than that of the vehicle-alone. In all three studies the vehicle solution acted as a reinforcer in its own right and maintained generally high rates of responding. On the surface using Meisch and Carroll's criteria suggest that oral MDMA does not function as a reinforcer under the conditions tested during Chapter 3. Meisch and Carroll's third criteria can be examined as rather simplistic view of the strength of a reinforcer as studies of the overt behaviour can sometimes mask underlying elements that are revealed only under conditions of challenge. In Experiment 4.1 reinforcement with oral MDMA and vehicle alone was challenged under economic constraint. Behavioural economic demand functions were fit to data obtained when FR (price) was varied. In this way elasticity (sensitivity to changes in price) was used as a measure to distinguish between demand for MDMA + saccharin from saccharin-alone. If indeed

MDMA functioned as a reinforcer it was expected that MDMA-containing solutions would show more relative elasticity than the vehicle-alone. However, using a variety of analyses and two different behavioural economic models this proposition was shown to be tenuous at best. Though simple forms of analysis revealed that MDMA may indeed represent more inelastic demand than saccharin-alone, these effects were reversed when data was analysed using normalization procedures. When normalized demand curves were compared for MDMA and saccharin-alone, it was the saccharin-alone rather than the MDMA-containing solutions that were defended more strongly as a function of increasing price.

Alternative Approaches and Future Directions

Concurrent Schedules

An alternative method not utilised in the current thesis that warrants further examination would be the analysis of the effect of economic constraint under concurrent schedule performance. That is study the effect of price increases to either or both MDMA and vehicle when both commodities are available during the same session.

To illustrate, we again turn to the classic study of Elsmore et al. (1980). In their study Baboons were given concurrent access to intravenous heroin or food. Using a discrete trials procedure income (number of opportunities to choose between the two reinforcers) was manipulated by increasing the inter-trial interval (ITI) between choices. When the ITI was low and income was plentiful subjects chose heroin more often than food. However as the ITI was increased and income was decreased subjects responded more for food at the expense of heroin. In effect the subjects showed a preference reversal where the initially favoured alternative (heroin) decreased as a function of economic constraint in favour of the other alternative (food). In this case food was more inelastic than heroin. This study

illustrates that the presence of alternative reinforcers can change the elasticity of other reinforcers. Environmental events (including the presence of other reinforcers, economy etc.) can thus affect the elasticity of commodity and as such elasticity cannot be thought as inherent property of that commodity (Petry & Heyman, 1995). Thus far this thesis has only considered elasticity of demand as a function of changes in price for itself, more correctly termed *own-price elasticity* (Allison, 1979; Petry & Heyman, 1995). However, elasticity can also be measured as a function of proportional change in consumption as a function of changes in price of an alternative commodity, termed *cross-price elasticity* (Allison, 1979; Hursh, 1980, 1984; Bickel et al., 1995; Petry & Heyman, 1995). Interactions between reinforcers form a continuum spanning three categories: substitutes, complements and independents (Bickel et al., 1995). A commodity that acts as a substitute would exhibit increased consumption as function of increased price in the alternative commodity while its own price remained fixed. For example, price increases in a commodity (e.g. Coca-cola) would result in an increase in consumption for a different alternative (e.g. Pepsi) that provides similar effects (Bickel et al., 1995). Conversely a complement would exhibit decreased consumption as a function of increasing price of the alternative reinforcer and vice versa. Finally independent commodities show no changes in consumption as result of changes in price of an available alternative. Note that the relationships between concurrently available commodities are not always reciprocal and can produce asymmetrical relationships between commodities (Bickel et al., 1995).

Petry and Heyman (1995) examined the effects of concurrently available ethanol in sucrose and sucrose-alone by manipulating the price of either the ethanol mix, sucrose or both. When the price of the ethanol mix was manipulated by increasing the VR schedule subjects evidence of increased responding, suggesting that responding for ethanol was inelastic (at least for modest increases in price). As the

price of ethanol increased sucrose intake remained relatively constant suggesting that sucrose responding was independent of the price changes for ethanol. When the price of sucrose was manipulated, sucrose responding declined even with modest increases in price suggesting sucrose responding was elastic, at the same time responding for the fixed price alternative, ethanol, increased suggesting that the ethanol mix acted as a substitute for plain sucrose. When the price of both ethanol and sucrose was increased subjects typically showed inelastic demand for both commodities at low prices but as price continued to increase responding predominantly on the ethanol-associated lever suggesting that the ethanol mix was inelastic and responding for sucrose was elastic. Similarly, Williams and Woods (2000) offered concurrent access to ethanol or water in Rhesus monkeys when baseline responding for ethanol was either higher (2% EtOH), equal (8% EtOH) or lower (32% EtOH) than water-maintained responding. They found that as price increased for both commodities ethanol responding was more resistant to increased price than was water for all three conditions. When baseline ethanol responding was lower than baseline water consumption increasing price resulted in a preference reversal from water to ethanol. This highlights the importance of measuring preference across a large range of prices as making a single measurement at a low fixed price would lead to the assumption that water was more reinforcing than ethanol. However, measuring preference over a higher price range revealed that the subjects were more willing to defend their consumption of ethanol than water and indicating that in fact ethanol was the more efficacious reinforcer.

Similar methodology could be utilised to further explore the relationship between MDMA and saccharin and may in fact provide support for MDMA's function as an oral reinforcer in rats. If it can be shown that price increases for both commodities results in a preference reversal from saccharin to MDMA as a function of increased economic constraint then it would illustrate that MDMA does provide reinforcing

properties over and above that of the vehicle alone. Additionally, by increasing the price of MDMA it can be examined whether saccharin acts as a substitute for MDMA and vice versa. However, use of concurrently available reinforcers obfuscates attempts to measure relative reinforcer efficacy across drugs as preference reversals make choice procedures inadequate for producing consistent and reliable measures of reinforcer value (Madden et al., 2007).

Resistance to Change

Resistance to change is an alternate method through which differences in strength of reinforcers can be assessed, one which could be further used to examine differences in reinforcer strength between MDMA-containing and vehicle solutions. Resistance to change is a measure of how insensitive behaviour is to disruption by an extraneous source. Behavioural momentum theory (Nevin, Mandell & Atak, 1983; Nevin & Grace, 2000) uses the analogy of Newton's laws of motion and relates momentum to reinforced behaviour whereby baseline response rate is analogous to *velocity* and resistance to change is analogous to *mass*. Using this analogy, reinforcers that maintain similar response rates can be differentiated in strength by instead measuring disruption caused by change (e.g. extinction, non-contingent reinforcer presentation) relative to baseline response rate. For example, Nevin and Grace (2000) use the analogy of a two concrete walls. One wall is reinforced with steel rods (equivalent to increased mass). On the surface both walls appear as strong as one another (by analogy both have the same velocity); however it is not until a disruptive force is applied such as a wrecking ball that the differences in strength of the walls become apparent. The wall that has been reinforced with steel is more resistant to the effects of the wrecking ball and thus represents the stronger of the two walls. By further analogy if both walls were reinforced with steel the wall that has more steel reinforcing will be more resistant to demolition. Behaviours are said to work the same way and "…more frequently or generously

reinforced behaviour becomes more resistant to challenge or disruption, and this increase in its resistance need not imply an observable increase in the rate or probability of currently observed behaviour. Instead, the strengthening effects of reinforcement may be evident only when responding is disrupted in some way" (Nevin & Grace, pp 75). Resistance to change is proportional to response strength, that is, reinforcers that are more frequent, larger or less delayed produce more persistence, or more resistance to disruption. For example, response strength can be increased either as a function of an increase in the rate of reinforcement or by a change in the magnitude of reinforcement (Nevin, 1974).

Results from studies using drugs as reinforcers are consistent with previous research on resistance to change. For example, rats trained to self-administer ethanol showed more resistance to extinction in the rich component of a multiple schedule than they did in the lean schedule (Jimenez-Gomez & Shahan, 2007). In a related experiment, Shahan and Burke (2004) showed that non-contingent presentation of food in one component of a multiple schedule for ethanol self-administration resulted in decreased response rates, but also an increase in resistance to extinction. Recently, Quick and Shahan (2009) showed that these results generalised to *iv* self-administration of cocaine.

Resistance to change could be used as a way of distinguishing the strength of MDMA from that of the vehicle itself. If the additive effects of MDMA plus vehicle produced a more efficacious reinforcer (i.e. a higher magnitude) than just vehicle-alone it should be possible to delineate those differences by examining changes in persistence after disruption (e.g. extinction). Traditionally resistance to change is measured using multiple schedules where subjects will respond under alternating schedules accompanied by unique discriminative stimuli. In this way response rates and reinforcer magnitudes can be varied relative to one another and the presence of the disrupter will be equal for both components of the multiple schedules. However,

in order to use resistance to change the current paradigm for the oral self-administration of MDMA would need to be further adapted. Traditionally resistance to change research has utilised variable interval schedules of reinforcement over ratio schedules. The advantage of variable interval schedules is that reinforcer rate is not confounded with response rate as it is in FR schedules (Nevin, 1995).

Behavioural momentum theory suggests that what is important is not absolute changes in response rate, but instead changes in response rate relative to baseline. If MDMA solutions had higher response strength it would manifest as a difference in the proportional change from baseline responding (for a given drug dose) versus response rate during extinction at that given drug dose. Specifically, if MDMA is acting as a reinforcer, then proportion of change (baseline versus extinction) should be smaller for higher versus lower doses. In a comparison between drug-containing solutions and vehicle-alone it would be predicted that the addition of the drug to the solution would make it a stronger reinforcer than the solution without MDMA. Thus, it would be expected that MDMA would produce higher resistance to change, manifesting as higher responding during extinction relative to the vehicle on its own. Furthermore, tests of concurrently available solutions including drug/drug and drug/vehicle will likely provide stronger evidence for response strength as measured by resistance to change.

Conditioned Place Preference

A large body of literature has used Conditioned Place Preference (CPP) to measure the motivational effects of drugs (Tzschentke, 2007), including MDMA (Cole, Sumnall, O'Shea & Marsden, 2003; Meyer et al., 2002; Schechter, 1991). Briefly, CCP has been used to assess the reinforcing properties of drugs of abuse. Rats are given a free choice (preference test) between two sides of a chamber that are equipped with unique and distinct environments (contexts) separated by a wall that incorporates a removable door. Each context is then paired several times with

either a drug's effects (an MDMA injection prior to the conditioning trial for instance) or with saline. The conditioning trials then alternate between drug and saline injections with animals always confined to the same compartment for drug pairings and the other compartment for saline pairings. After multiple drug/context pairings a further preference test is conducted. During this test the door is opened and rats are free to move between both compartments of the CPP chamber and should choose the side of the chamber associated with the most positive effects, in this case those of the side paired with exposure to the drug. Conditioned place preference is shown by a shift in preference towards the drug-paired location, such that the animal spends more of its time in the compartment that was paired with the subjective effects of the drug.

As part of the data collected for this thesis, CPP was seen as a suitable way to directly compare the reinforcing effects of MDMA when delivered across a variety of routes of administration. To this end initial studies were conducted to examine the suitability of the task. Initially, CCP was examined using *ip* injection and three doses of MDMA; 0.25, 5.0 and 10.0mg/kg. Each drug dose received four pairings and saline received an identical number on alternate days in the opposite compartment. The results indicated a dose-dependent decrease in preference as a function of MDMA dose. That is, rather than animals spending more time in the drug-paired compartment during the preference test they instead showed less time in the drug-paired compartment, i.e. a conditioned place aversion. It was thought that the speed of onset of the *ip* injection might produce more aversive consequences due to the rapid increase in MDMA-plasma levels (see Baumann et al., 2009). CPP for MDMA was further examined by using the *sc* route of administration and comparing it that of the *ip* route of administration. MDMA 5mg/kg administered *ip* or *sc* for four conditioning trials each again indicated the presence of a conditioned place aversion. However, in a subsequent preference

test subjects were administered with a further dose of MDMA and allowed to freely choose between the two compartments. Administration of MDMA during the preference assessment resulted in a preference toward the compartment that had previously been paired with MDMA. Though these results were not consistent with the existing literature, the finding of conditioned place aversion after administration of MDMA suggests that the subjective drug effects may be aversive, at least under some circumstances. MDMA-induced CPP seems to be extremely sensitive to experimental manipulation. For example, Diller et al. (2007) found significant place preference for MDMA at 5.0mg/kg *sc*, but not 10mg/kg *sc.* Meyer et al. (2002) showed that MDMA-induced CPP was sensitive to housing conditions such that only rats that had been isolated from other rats prior to conditioning showed a significant MDMA-induced place preference.

It is unclear as to why these exploratory CPP tests failed to find evidence of MDMA-induced place preference. The failure to replicate previous literature on MDMA-induced CPP meant that testing the reinforcing properties of oral MDMA via place preference was never examined. However, further examination of CPP using oral administration of MDMA is warranted.

Behavioural Economics and Demand Curve Analysis

Bickel et al. (2000) suggest that relative reinforcer efficacy is not a homogeneous phenomenon and suggest that the heterogeneous elements typically used for measuring relative reinforcer efficacy (i.e. breakpoints on PR schedules, peak response rates in single schedules and preference in concurrent schedules) can be more appropriately modelled by demand curves that encapsulate differences in each of these methods for determining relative reinforcer efficacy. Measures of relative reinforcer efficacy as measured by different tasks should ideally produce a convergence across tasks; however often times this has not been the case. Using behavioural economics as a framework it can predicted not only when different

methods of measuring relative reinforcer efficacy should coincide, but also when there should be divergence (Johnson & Bickel, 2006). Bickel et al. suggest that PR breakpoints, peak response rates and preference correspond to P_{max}, O_{max} and relative position of demand curves (especially in cases where the demand curves cross) respectively. The use of demand curves for analysing drugs of abuse thus incorporates features of each of these alternative methods of measuring relative reinforcer efficacy and presents the most robust parametric analysis for comparing relative reinforcer efficacy by using behavioural economic methods.

In this thesis several behavioural economic models were compared. Each model tested produced stable demand curves and data that were mostly congruent with other analyses. The linear-elasticity model (Hursh et al., 1988) has been used previously for the analysis many behaviours, including measuring relative reinforcer efficacy. The exponential model of demand (Hursh & Silberberg, 2008) provides a fresh approach to the analysis of demand. The exponential model provides a useful theoretical addition to the literature by providing a single measure of 'essential demand'. To date few studies have tested the model and conducted valid comparisons between various models using the same data set. This thesis tested the predictions of both the linear-elasticity model with that of the exponential model and found an overall correspondence between the two. Essentially the models are based on the same theoretical framework and highly utilise obtained and predicted parameter in the similar ways. However, it is notable that the exponential model succeeds at doing this while using less free parameters is to its advantage, thus if Occam's Razor were to decide the victor in this case the exponential would be the victor. Further tested of cross-model comparisons, in addition to testing predictions related to the effects of substitutable and complementary goods are necessary.

The current study used the behavioural economic framework in order to analyse the relative reinforcing efficacy of MDMA. Oral doses of MDMA were analysed for two

reasons. Firstly, from previous experience it became apparent that within-subject parametric analyses of *iv* self-administration using behavioural economics was complicated by issues of catheter patency. The limited ability to maintain effective catheters (at least in rats) for long periods of time prevented effective use of the self-administration procedure for this purpose. Development of rapid methods for demand curve analysis for *iv* self-administration is both enviable and encouraged (though somewhat complicated by long–acting drugs such as MDMA). The benefits of oral administration of MDMA (or indeed) other drugs were that issues of catheter patency were removed and thus long-term studies are more tenable. Secondly, MDMA is an oral drug of abuse and thus it merits analysis of how its relative reinforcing efficacy changes as a function of route of administration. The current studies indicate that MDMA at least when presented orally is a relatively weak reinforcer. Subjects defended access to plain saccharin more so than saccharin solutions containing MDMA. It is unclear to what extent this result is a factor of taste of the MDMA containing solutions. However, rats will readily consume drugs such as ethanol despite a relatively strong and aversive taste (Ator & Griffiths, 2003).

Although the current study attempted to quantify the relative reinforcing efficacy of MDMA it should be noted that no comparisons were made to other abused drugs. Though significant advances in quantification of have been made with approaches such as behavioural economics, a comparison with a known quantity (such as alcohol via the oral route or cocaine via *iv*) is important with regard to easing the use of across study comparisons of relative reinforcer efficacy.

Also of significant importance is the comparison of relative reinforcer efficacy across routes of administrations. A comparison of the demand curves for *iv* and orally self-administered MDMA would give an indication of how differential pharmacokinetics of

each route of administration affects relative reinforcing efficacy and thus MDMA's abuse potential.

Intravenous versus oral administration of MDMA

The current results on the assessment of the relative reinforcing efficacy of oral MDMA have proven inconclusive and more research is needed to more clearly understand the dynamic interaction between oral MDMA and the vehicle in which was delivered. The studies in the current project were designed to replicate those of the intravenous self-administration of MDMA in which MDMA has been shown to be an effective (if somewhat low efficacy) reinforcer. In the *iv* self-administration paradigm cumulative small doses are experienced and the resulting cumulative drug effects (and associated positive affective effects) are appropriate for modelling the pharmacodynamic effects of MDMA in animals. An important contrast lies in the way that human users normally take the drug, which is large oral doses a small number of times per session. Instead of trying to replicate the animal condition with regard to patterns of *iv* consumption it may be that analyses of oral MDMA exposure would be more appropriately based on a more human oriented pattern of use. That is provide the animals with fewer, but larger doses that are more likely to result in onset of pharmacodynamic processes and less exposed to attenuation due to excessive exposure to pharmacokinetic processes involved in oral administration.

The present studies indicate that taste may have powerful effects that interfere with the analysis of the positively reinforcing effects of MDMA. The doses used in this study were chosen by necessity as higher doses supported little to no responding at all. It is possible that the reinforcing subjective effects of MDMA are not apparent at the doses used and that analysis of higher doses of MDMA is necessary. However the taste factor prevents testing of higher doses than those used in the current study by the methods chosen. It may be necessary to adopt induction procedures (such as SIPs) in order to increase initial drug intake such that the animals fully

experience the reinforcing effects of MDMA. A number of studies have shown that behaviour induced in this way is maintained when the inducing manipulation is subsequently removed (Falk & Lau, 1995). Intragastric administration of MDMA may provide a promising avenue of research of the oral effects of MDMA not confounded by taste. In a way, the intragastric self-administration more closely resembles the human experience as there is no effect of taste involved. In humans swallowing a pill (the most common method of MDMA use) does not generally induce a taste response since any aversive taste is removed almost immediately and (generally) not repeated throughout the night. Also in some cases the pills do not even invoke an aversive taste either by adulteration of the pills (i.e. the pill are sometimes mixed with sugary or sweet adulterants) or by bypassing of the taste buds. Using intragastric self-administration allows for the use of higher doses than those used in the procedure used in the current thesis and can be conducted in an identical procedure to that of *iv* self-administration allowing for direct comparisons between the two. However, like *iv* self-administration the intragastric method would likely prove less reliable for long-term studies like those examining behavioural economics.

Final Thoughts

Despite MDMA being an oral drug in humans relatively little research has focussed on the effects of MDMA when delivered orally. This thesis presents the first use of an operant methodology for the study of the reinforcing effects of MDMA in rats. While MDMA maintained dose-response functions typical of other drugs of abuse delivered both intravenously or orally, it did not engender higher rates of responding than the vehicle in which it was presented. These results suggest that MDMA may act a relatively weak reinforcer in rats when delivered orally.

In addition, this thesis has been the first to examine the reinforcing effects of MDMA using a behavioural-economic analysis. The behavioural-economic framework

provides a powerful analytic tool that can be used to assess the relative reinforcing efficacy or abuse potential of drugs of abuse. This robust source of information can then be provided to governments in order for them to make relevant decisions on the legality of both old and new drugs of abuse (Hursh, 1991). This thesis provided a comprehensive analysis of the reinforcing effects of oral MDMA using both established and novel behavioural economic models.

The multi-analytic approach used in this thesis suggests that oral MDMA represents a relatively weak reinforcer in rats. However, because of notable differences in the way humans take and abuse MDMA that differ from the methods used with animals in this study, the current results may under represent the abuse potential of MDMA in humans. Further research is still necessary to more clearly define the reinforcing effects of oral MDMA and its implications for MDMA's addictive potential in humans.

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