THE ROLE OF DOPAMINE IN THE SENSITISED LOCOMOTOR ACTIVATING EFFECTS OF METHYLENEDIOXYMETHAMPHETAMINE (MDMA) IN RATS.

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This whole thesis thing is a little torturous and you cannot, if you wish in any way to stay sane, do it alone.

I certainly did not.

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Abstract

Under certain regimens of repeated pre-exposure, psychostimulant drugs show an increase in locomotor activity across days of testing and, after abstinence from the drug, a greater responsiveness to a subsequent challenge dose of the drug. This phenomenon, termed behavioural sensitisation, is thought to underlie certain aspects of drug addiction such as drug seeking and relapse. Repeated administration of +/-3, 4-Methylenedioxymethamphetamine (MDMA, ecstasy) produced sensitised hyperactivity in rats suggesting a lasting neurological change. The present studies sought to evaluate some of the parameters around both the induction and expression of behavioural sensitisation to MDMA and to evaluate if the sensitivity of the dopamine (DA) D_1 and D_2 receptors had altered under the current pre-exposure regimen of MDMA. Further, following MDMA pre-exposure that results n behavioural sensitisation, changes in potency to the reinforcing effects of MDMA were investigated through the self administration paradigm. Finally, high performance liquid chromatography (HPLC) was used to evaluate changes in brain amine levels following sensitisation to MDMA locomotor activating effects.

Rats received a pre-treatment regimen consisting of 5 daily injections of MDMA (0.0, 5.0 or 10mg/kg i.p). MDMA-produced locomotor activity was measured after 2, 9 or 28 days of withdrawal. In other groups, hyperactivity following administration the DA D_1 agonist SKF81297 (0.0, 0.5, 1.0, 2.0, 4.0 or 8.0 mg/kg), or the D2-like DA agonist apomorphine (0.0, 0.5, 1.0, 2.0 or 4.0 mg/kg) was measured in groups that received pre-exposure to MDMA (10.0

mg/kg) or vehicle. The effects of the D_1 antagonist SCH23390 (0.0, 0.01, 0.02, or 0.04 mg/kg), the D_2 antagonist eticlopride (0.03, 0.01, 0.003, 0.05, 0.1, or 0.2 mg/kg*)* or the 5-HT_{2C} antagonist RS102221 (0.0, 0.25, 0.5, or 1.0 mg/kg) on MDMA-produced hyperactivity in MDMA or vehicle pre-treated rats was also measured. In Experiment 3, effects of MDMA or vehicle pre-treatment on latency to acquisition of MDMA (0.5 or 1.0 mg/kg/infusion) self-administration was measured. In Experiment 4 effects of pre-treatment on brain tissue levels of DA, its metabolite homovanillic acid (HVA), serotonin (5-HT) and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA) were determined.

The regimen of 5 daily treatments of 10.0mg/kg produced persistent behavioural sensitisation and cross-sensitisation to hyperactivity produced by DA receptor agonists. These effects were not, however, reflected in sensitised responses to the ability of the antagonists to attenuate MDMA-produced hyperactivity. Pre-treatment with MDMA did not decrease latency to acquisition of self-administration. Rather, there was an increased latency to acquisition of self-administration in the MDMA pre-treated rats. MDMA pre-treatment decreased levels of the serotonin metabolite 5-HIAA in the frontal cortex and hippocampus. Following the current pre-treatment regimen, MDMA produced behavioural sensitisation is mediated by neuroadaptations in central dopaminergic substrates. The persistent locomotor sensitisation is similar to that produced by other amphetamine-like stimulants and might underlie use and abuse of this compound.

MDMA

The amphetamine derivative, 3, 4-methylenedioxymethamphetamine (MDMA or ‘ecstasy’) was synthesised around 1912 and a patent was granted in 1914 (Green, Mechan, Elliott, O'Shea, & Colado, 2003; Kalivas, Duffy, & White, 1998). It is chemically similar to amphetamine, methamphetamine, mescaline (see figure 1) and a number of ring-substituted phenethylamines and is both a stimulant and hallucinogenic compound (Battaglia, Brooks, Kulsakdinun, & De Souza, 1988; Baumann, Wang, & Rothman, 2006; Gold, Koob, & Geyer, 1988).

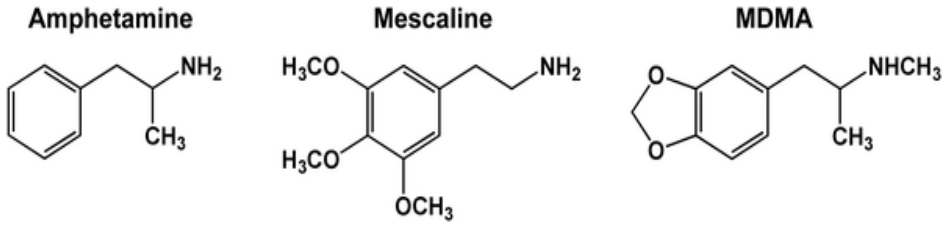

*Figure 1.*chemical structure of Amphetamine, mescaline and MDMA.

Research in the early part of the 20th century is scant but the LD 50 (the Lethal Dose in 50% of animals tested) was investigated by the U.S. military in the 1950s. During the early 60s it was reportedly first used recreationally (Watson & Beck, 1991). In the 1980’s it was used as an adjunct to psychotherapy before the U.S. drug enforcement administration changed its classification to a schedule 1 drug and hence, illegal (Green, et al., 2003).

MDMA was a popular recreational drug in the United Kingdom in the 1980's (Cole and Sumnall, 2003) with popularity of the drug increasing during the 1990's leading to more widespread use. A number of survey studies have suggested increased use throughout the 1990s depending upon the population sampled. For example, in a sample of 158 current drug users 82% said that they had used MDMA in the previous year (Williamson, et al., 1997) while only 4% of medical students surveyed at, or near, the same time reported MDMA use (Webb, Ashton, Kelly, & Kamali, 1998). The same authors reported 13% use by UK university students while U.S. student's use increased from 2.8% in 1997 to 4.7% in 1999 and 10.6% in the final 2000 survey (Strote, Lee, & Wechsler, 2002).

The United Nations Office on Drugs and Crime (UNODC, 2004) presented long-term worldwide trends in production, trafficking and abuse of drugs. The reports revealed that the consumption of certain illicit drugs such as heroin and cocaine were decreasing while during the last previous decade, amphetamine-type stimulants (mainly MDMA) were the second most commonly used illicit drug.

In New Zealand, the Expert Advisory Committee on Drugs reported an increase in overall use. Those who positively responded to the question, "had they ever used MDMA?" increased from 3% - 5.4% between 1998 and 2001. The greatest reported use, as well as greatest increase

in use, came from the 20-24 year age group of survey respondents. In this age group, use of MDMA during the previous 12 months rose from 3%-10% between 1998 and 2001 (The Expert Advisory Committee on Drugs [EACD], 2004). These figures put New Zealand on a roughly equal footing, in terms of use, with other overseas sample results.

Consistent with the reported increases of use have been increases in associated medical complications. MDMA induces a number of serious effects such as cardiac arrhythmias, hypertension, hyperthermia, hyponatremia (disturbance of the salts in the blood), liver complications, seizures and coma (Schifano, 2004). Deaths attributable to ecstasy use are rare but they are increasing. In the U.K., out of all the drug related deaths, ecstasy use in 1997 accounted for 1.2% rising to 4.1% in 2002 (Schifano, Corkery, Deluca, Oyefeso, & Ghodse, 2006).

MDMA Pharmacology

MDMA is a racemic molecule in that it has two enantiomers. The (S)(+)-enantiomer is a more potent dopamine releaser while the (R)(-)-enantiomer shows a higher affinity for serotonin receptors (Johnson, Hoffman, & Nichols, 1986). MDMA is usually formulated and consumed as a racemate, a 1:1 mixture of its enantiomers (Pizarro, et al., 2004) and acts on a number of different neurochemical systems releasing presynaptic serotonin (5-HT), dopamine (DA) and norepinephrine (NE). MDMA induces increases in extracellular

monoamine concentrations through three direct actions (Cole & Sumnall, 2003):

Firstly, MDMA is a substrate for the serotonin (SERT), dopamine (DAT) and norepinephrine (NET) transporters binding to, and blocking the transporter. As presynaptic plasma membrane transporters rapidly remove the released monoamine from the synapse, blockage of this process increases extracellular levels of all the monoamines (Colado, O'Shea, & Green, 2004; Gough, Ali, Slikker, & Holson, 1991; Green, et al., 2003; Lyles & Cadet, 2003; S. R. White, Obradovic, Imel, & Wheaton, 1996). Evidence of the transporter interactions of MDMA can be seen when serotonin selective reuptake inhibitors (SSRIs), such as fluoxetine, when co-administered with MDMA, attenuate increases in 5-HT (Berger, Gu, & Azmitia, 1992; Hekmatpanah & Peroutka, 1990; Rudnick & Wall, 1992). Similarly, the DA reuptake inhibitor, GBR12909, prevented MDMA induced DA release in-vitro (Koch & Galloway, 1997) as well as in-vivo (Nash & Brodkin, 1991) . In addition, extracellular norepinephrine (NE) levels were reduced by co-administration of the NE-uptake blocker, desmethylimipramine (Fitzgerald & Reid, 1990).

Secondly, when MDMA binds to the SERT it also induces a carrier mediated release of neurotransmitter. MDMA enters presynaptic nerve cells through passive diffusion across the membrane wall (Rudnick & Wall, 1992) and through the actions of the SERT into the cell nerve

endings (Crespi, Mennini, & Gobbi, 1997). Once inside the cell, MDMA induces a mechanism of calcium independent release of serotonin into the synapse, by preventing the repackaging of cytosolic 5-HT into vesicles through the reversal of vesicular (Rudnick & Wall, 1992, 1993) and plasma membrane(Iravani, Asari, Patel, Wieczorek, & Kruk, 2000).

Thirdly, MDMA inhibits monoamine oxidase (MAO). MAO is an enzyme with two subtypes; MAO-A is found in the extracellular fluid, and MAO-B is located in the cytosolic fluid (Westlund, Denney, Kochersperger, Rose, & Abell, 1985). Both MAO-A and MAO-B were inhibited by in-vitro application of MDMA although, there was a preferential effect on MAO-A (Gu & Azmitia, 1993; Leonardi & Azmitia, 1994). The consequence of this effect of MDMA resulted in high extracellular levels of 5-HT and a greater level of intracellular 5-HT available for reverse vesicular transport. MAO-A also plays a central role in metabolising serotonin, norepinephrine and dopamine (Kato, Dong, Ishii, & Kinemuchi, 1986). Extracellular increases in the monoamines are thereby also produced by MAO inhibition.

A linear proportional increase in MDMA-produced DA occurred with increasing levels of 5-HT (Jacocks & Cox, 1992; S. R. White, Duffy, & Kalivas, 1994) suggesting that increases in extracellular 5-HT may trigger DA release via interaction with receptors. There are at least 14 distinct 5-HT receptors belonging to seven families [5-HT_1 through 5-

HT_7] (Hoyer, Hannon, & Martin, 2002) with the 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2C}, 5-HT_3 and 5-HT_4 receptors all involved in the modulation of DA release. Of particular interest, activation of the 5-HT_{2A} and 5-HT_{2C} receptors produce opposite effects on DA release. Systemic administration of the 5-HT_{2A} receptor antagonist ketanserin, and the selective 5-HT_{2A} antagonist MDL100,907 attenuated MDMA-induced increases in striatal dopamine efflux (Nash, 1990; Schmidt, Abbate, Black, & Taylor, 1990). In contrast, the selective 5-HT_{2C} receptor agonist, RO 60-0175, decreased DA release (Di Matteo, 2000) while the receptor antagonist, SB 243213, increased DA release (Berg, et al., 2006). These results suggest that in addition to MDMA induced DA release through DAT function, secondary actions of 5-HT also contribute to extracellular DA increases.

The deleterious effects of MDMA

There have been numerous investigations of the long term effects of MDMA in guinea pigs (Battaglia, Brooks, et al., 1988), dogs (Nishisawa, Mzengeza, & Diksic, 1999), non-human primates (Frederick, et al., 1995), chickens (Bronson, Jiang, Clark, & DeRuiter, 1994) and rats (e.g. Commins, et al., 1987; Malpass, White, Irvine, Somogyi, & Bochner, 1999; Marston, Reid, Lawrence, Olverman, & Butcher, 1999).

It has long been noticed that the sensitivity to the neurotoxic effects of amphetamine derivatives such as MDMA differs across mammalian species. Primates are more vulnerable to substituted amphetamines than rats or guinea pigs whereas mice are remarkably tolerant (Stone et al., 1987). In rats, young animals were found to be much more resistant against the long-term neurotoxic effects of these drugs than adult ones (Broening et al., 1994) and different strains of rats have displayed different responses to MDMA. For example, MDMA is demethylenated by the CYP2D1 hepatic cytochrome P450 enzymes in the rat (Kumagai et al., 1994). This enzyme is expressed, differentially in rat strain with subsequent alterations in metabolism of MDMA (Malpass et al., 1999). The Dark Agouti rat for example, exhibits enzymic deficiencies whereas the Sprague Dawley strain has more effective CYP2D1 enzyme capacity.

Mice have also being used in investigations of the long term effects of MDMA, but the pharmacological effects of MDMA appear to differ from those of other species studied (Green, et al., 2003; Lyles & Cadet, 2003). In mice, the acute effects of MDMA are similar to the rat (Logan, Laverty, Sanderson & Yee, 1988) however, repeated large doses of MDMA (3 x 50mg) produced a small prolonged fall in 5-HT and 5-HIAA but marked falls in DA and DOPAC (Logan et al.,1988).

A large number of studies have reported lasting decrements in 5-HT and its major metabolite, 5-hydroxyindoleacetic acid (5-HIAA); reductions in [3H] paroxetine binding that reflect reduced density of SERT; and reduced serotonergic axonal density in brain tissue (Gouzoulis-Mayfrank & Daumann, 2006 ; O'Shea, Granados, Esteban, Colado, & Green, 1998; Ricaurte, McCann, Szabo, & Scheffel, 2000).

Following MDMA exposure there is a biphasic modulation of 5-HT and 5-HIAA. There is an initial rapid increase in extracellular 5-HT (1-4 hours following injection) with levels returning to baseline within 24 hours. Over a period of 3-4 days there is evidence of deficits in 5-HT and 5-HIAA (Battaglia, et al., 1987; Colado, Murray, & Green, 1993; Schmidt, 1987; Stone, Stahl, Hanson, & Gibb, 1986). These deficits were reported following the administration of a single moderately high dose of MDMA (10mg/kg) (Schmidt, 1987) or repeated low (4.0mg/kg twice daily for 4 days) (O'Shea, et al., 1998) or high (10-40mgkg twice daily) (Commins, et al., 1987) doses.

Following a large dose of MDMA (20mg/kg twice daily for 4 days) a marked reduction in the density of uptake sites was observed (Battaglia, et al., 1987). A decrease in 5-HT and 5-HIAA does not, however, necessarily reflect axonal terminal damage. Immunocytochemical evidence supported the finding that neurodegeneration had occurred within terminal, dendritic and cell body regions (Commins, et al., 1987; O'Hearn, 1988).

MDMA induced neurotoxicity in non-human primates has also been reported (Fischer, Hatzidimitriou, Wlos, Katz, & Ricaurte, 1995; Insel, Battaglia, Johannessen, Marra, & De Souza, 1989; Ricaurte, DeLanney, Irwin, & Langston, 1988; Ricaurte, Martello, Katz, & Martello, 1992; Scheffel, Lever, Stathis, & Ricaurte, 1992). There is, however, an important difference between the non-human primate and rodent data. The dose required to induce deficits in non-human primates was less than that required for rodents (De Souza, Battaglia, & Insel, 1990; Ricaurte, 1989) and the deficits were more persistent (Hatzidimitriou, McCann, & Ricaurte, 1999).

It is tempting, given the non-human primate data, to infer that comparable MDMA induced deficits are produced in humans who abuse MDMA. Although there are indications of MDMA induced deficits (McCann, Mertl, Eligulashvili, & Ricaurte, 1999; Ricaurte, DeLanney, Wiener, Irwin, & Langston, 1988), a history of multiple drug use, variables such as questionable drug purity and dosage, make it difficult to draw robust, transferable conclusions from these studies.

Brain reward mechanisms

A number of converging pieces of evidence have implicated mesolimbic DA in brain reward mechanisms. Single cell recordings in monkeys showed increased extracellular DA levels in the ventral

tegmental area (VTA) upon food presentation (Schultz, Apicella, & Ljungberg, 1993). There were extracellular DA increases in the nucleus accumbens (NAc) during sex behaviour in the rat (Pfaus, et al., 1990). Similarly, access to water for water deprived rats, increased nucleus accumbens DA (Young, Joseph, & Gray, 1992). In humans, neuroimaging techniques have provided evidence of increased dopamine activity in ventral striatal areas during reward related tasks (Schott, et al., 2008). Taken together, this strongly suggests that DA is a critical neurotransmitter for the mediation of reinforcement. Animal models are ideally suited for delineating aspects of not only natural rewards such as sex, and food, but also drug induced reinforcement.

The seminal work conducted by Olds and Milner (1954) showed that electrical brain stimulation could be powerfully reinforcing. These 'reward' substrates are within the medial forebrain bundle in what has come to be known as the 'reward pathway'. Subsequent studies have suggested that the reward pathway comprises the midbrain dopaminergic projections from the ventral tegmental area (VTA) into the nucleus accumbens (NAc) shell region and into the medial prefrontal cortex. Excitation and ensuing dopaminergic release from this system (the mesolimbic system) is critical to the acute reinforcing effects of drugs of abuse (Carelli, 2004; Dackis & O'Brien, 2001; Di Chiara, et al., 2004; Kelley & Berridge, 2002; Nestler, 2005; Robinson & Berridge, 1993; Salamone & Correa, 2002; Wise, 1998; Wolf, 2002). Indeed, nearly all drugs of abuse stimulate the release of dopamine at

some point along the mesolimbic pathway and, regardless of the specific and primary mechanism of action, all drugs of abuse activate dopaminergic transmission (directly or indirectly) in the nucleus accumbens (Di Chiara, et al., 2004; Nestler, 2005).

One of the more powerful tools for measuring the reinforcing effect of drugs is the self-administration paradigm that allows drug-taking to be contingent on an operant response. In this procedure, laboratory animals are surgically prepared with an intravenous (IV) catheter and placed in an operant chamber with two response options (two levers, one the active lever, the other inactive). One response is associated with an IV infusion of a drug (active lever) the other response has no consequence (inactive lever). When presented with this choice, a significantly higher level of responding on the active lever suggests positive reinforcement is gained from infusion of the drug (Haney & Spealman, 2008). Virtually all drugs that are abused by humans are reliably self-administered by animals (Fischman & Schuster, 1978; Schuster & Thompson, 1969) and the patterns of use seen in animal I.V. self administration comparable to the pattern of use seen in humans (Gardner, 2000; Spealman & Goldberg, 1978). Thus, the self-administration procedure provides a valid and reliable animal model of drug abuse liability and provides an animal model of human drug-taking and drug-seeking behaviours (Henningfield, Cohen, & Heishman, 1991).

Under baseline conditions, responding under fixed ratio schedules increase as the dose of drug is reduced. It has been suggested that this increase in responding is compensatory and maintains a constant blood level of drug regardless of available dose. Disruptions to the mesolimbic DA system alter this pattern of drug taking. For example, the DA receptor blocker pimozide (Risner & Jones, 1976) or butaclamol (Yokel & Wise, 1976) produced dose-dependent increases in intravenous self administration (IVSA) of amphetamine consistent with a reduction in dose (Pickens & Thompson, 1968). Dialysate samples taken from the nucleus accumbens during IVSA of amphetamine showed elevated DA levels (Ranaldi, Pocock, Zereik, & Wise, 1999).

However, dopamine release alone cannot account for the distinction between occasional drug use and the chronic drug dependent state known as 'addiction'. It has been suggested that drug addiction proceeds as a result of neuroadaptive processes in the brain reward system (Koob, 2006). When drugs of abuse repeatedly activate the reward system of the brain they induce a host of long lasting, complex neural adaptations that are maintained over time ranging from hours to years, and perhaps a lifetime (Kauer & Malenka, 2007; Nestler, 2004). Adaptations of addiction have been modelled in the laboratory using animals (Deroche-Gamonet, Belin, & Piazza, 2004), including a "prominent animal model of addiction", termed behavioural sensitisation (Wolf, 2002 (pg. 147)).

Amphetamine induced Behavioural Sensitisation

Behavioural sensitisation has been studied to investigate neuroadaptations that occur after repeated exposure to drugs of abuse (Wise & Bozarth, 1987). Behavioural sensitisation is a progressive, long lasting increase in the psychomotor stimulating property of drugs of abuse that manifests itself in a number of behaviourally measurable ways. Various behaviours such as, sniffing, rearing and head movements have all been reported although it is usually measured as the enhanced locomotor activity following repeated exposure (Pierce & Kalivas, 1997; Post & Rose, 1976; Robinson, 1984; Robinson & Berridge, 1993; Stewart & Badiani, 1993; Wolf, 1998).

Sensitisation was first reported early in the 1930's (e.g. Downs & Eddy, 1932; Tatum & Seevers, 1931) although it wasn't until the late 1960's and early 70's that investigations into amphetamine induced behavioural sensitisation were pursued in earnest (Robinson & Becker, 1986). Initial studies established that repeated amphetamine administration lead to one of two states, either tolerance or sensitisation. The manifestation of either condition depended upon manipulation of a number of factors such as dose, drug exposure, and time after exposure. For example, continual exposure (for a two or three day period), or repeated multiple high doses of amphetamine, resulted in tolerance to drug produced hyperactivity (Kuczenski & Leith, 1981). On the other hand, repeated intermittent administration

(daily injections), of relatively low doses, induced sensitisation (Robinson & Kolb, 1999). A single exposure to amphetamine also produced sensitisation to a number of drug-produced behaviours, such as stereotypy (Browne & Segal, 1977; Ellison & Morris, 1981) or rotation (Robinson, 1984; Robinson, Becker, & Presty, 1982). Repeated intermittent administration has been reported to produce a more robust and progressive increase in behaviour indicative of behavioural sensitisation (Kalivas & Stewart, 1991).

Typically, single daily injections of 2-4mg/kg amphetamine result in behavioural sensitisation (Robinson & Kolb, 1999). More extreme exposures consisting of daily amphetamine administration in doses ranging from 5 to 32mg/kg (i.p.) produced behavioural tolerance (e.g. Demellweek & Goudie, 1983; Jackson, Bailey, Christie, Crisp, & Skerritt, 1981; Lewander, 1971; Robinson & Becker, 1982). It has been suggested that a critical factor is the interval between treatments rather than the dose (Robinson & Becker, 1986). Thus, relatively infrequent injections spaced up to a week apart may be more efficacious in producing sensitisation than injections given close together (Robinson, 1984).

The observation of sensitisation is also dependant on time since last exposure. Sensitisation was observed 3 days after an amphetamine pre-treatment regime with the magnitude of the response increasing 5 and 30 days post withdrawal (Kolta, Shreve, De Souza, & Uretsky, 1985).

Ideally, more than a day withdrawal following exposure is required as sensitisation has been observed 7, 14 and 28 days after the last dose but not after a single day withdrawal (Hitzemann, Tseng, Hitzemann, Sampath-Khanna, & Loh, 1977).

Behavioural sensitisation is also dependent on the environmental context in which the drug is administered. Conditioned effects elicited by situational and environmental cues associated with the drug come to exert a powerful control over the manifestation of behavioural sensitisation. Typically, when examining environmental and contextual cues, the animals are taken from their home cages and moved to a novel test environment. Half of these, the paired group, are injected with a psychostimulant (drug paired with environment) and half with saline, the unpaired group. When removed from the test environment and placed back in their home cages the saline unpaired group receive the drug, thereby isolating the contextual environment from this group. On the test day, only the previously paired group showed a sensitised response (Pert, Post, & Weiss, 1990).

A number of researchers have used similar methodology to examine the role of context in amphetamine produced sensitisation. Although context dependent sensitisation was blocked (Stewart & Druhan, 1993), and extinguished once gained (Stewart & Vezina, 1991), the majority of reports confirm that environmental contextual cues are extremely important for the manifestation of behavioural sensitisation (Ahmed,

Stinus, Le Moal, & Cador, 1993; Badiani, Anagnostaras, & Robinson, 1995; Badiani, Browman, & Robinson, 1995; Drew & Glick, 1988; Mazurski & Beninger, 1987; Stewart & Druhan, 1993; Vezina, Giovino, Wise, & Stewart, 1989).

Sensitisation and Intravenous Self-Administration

Investigators typically, and successfully, have measured the latency to acquisition of self administration showing drug pre-exposed animals acquiring self administration faster than drug naive animals (Horger, Giles, & Schenk, 1992; Horger, Shelton, & Schenk, 1990; Piazza, Deminiere, Le Moal, & Simon, 1989). Animals pre-exposed to a sensitising regimen of amphetamine were predisposed to-self administer amphetamine (Horger, Giles, & Schenk, 1992; D. Piazza, Le Moal & Simon, 1989) as indicated by decreased latency to acquire an operant response. However, pre-exposure to amphetamine decreased latency to acquisition of only low doses of drug (Piazza, Deminiere, Le Moal, & Simon, 1989) using a low fixed ratio (FR) reinforcement schedule. When high doses of the drug were made available, under the same FR schedule, there was no difference between amphetamine and vehicle pre-exposed animals (Lorrain, Arnold, & Vezina, 2000) suggesting prior exposure may reduce the threshold reinforcing dose.

When high doses of amphetamine were available in the self-administration paradigm, amphetamine break point on a progressive

ratio (PR) schedule of reinforcement were higher for amphetamine pre-exposed rats (Mendrek, et al., 1998; Vezina, Pierre & Lorrain, 1999). In the PR schedule, the number of lever responses required to obtain a reinforcer is increased for each successive reinforcer until a 'break point' that fails to support continued operant responding is reached. It has been suggested that increases in break point from drug pre-exposed animals reflects higher motivation to further seek the drug (Arnold & Roberts, 1997).

Decreases in latency to acquisition and higher break points indicate pre-exposure to amphetamine enhances drug seeking as well as the acquisition rate of self-administration. One suggestion for this alteration has been linked to the sensitisation of mesolimbic dopamine neurons. Higher break points have been associated with increases in NAc DA (Vezina et al., 1999) as well as sensitisation to the locomotor activating effects of amphetamine (Mendrek, et al., 1998; Vezina et al., 1999).

Non-human primates, mice and rats will all self-administer MDMA (Banks, et al., 2007; Braida & Sala, 2002; Cornish, et al., 2003; Daniela, Brennan, Gittings, Hely, & Schenk, 2004; Daniela, Gittings, & Schenk, 2006; Fantegrossi, Ullrich, Rice, Woods, Winger, 2002; Fantegrossi, et al., 2004; Ratzenboeck, Saria, Kriechbaum, & Zernig, 2001; Schenk, Gittings, Johnstone, & Daniela, 2003; Schenk, Hely, Gittings, Lake, & Daniela, 2008; Schenk, et al., 2007). There have

however, been noted differences in latency to acquisition of MDMA self-administration when compared to other self-administered drugs. Acquisition of MDMA self-administration is relatively slow (Schenk et al., 2003) with more variability in the latency to acquisition compared with other self-administered drugs (e.g. Horger, et al., 1990; Horger, et al., 1992).

These differences might be due to the different pharmacology of MDMA. It has been suggested that drugs with a greater effect on DA compared to 5-HT have a higher subjective reinforcement value (Wee, Anderson, Baumann, Rothman, Blough, & Woolverton, 2005) with reinforcing efficacy positively correlated with inhibition of dopamine reuptake (Ritz, Lamb, Goldberg, & Kuhar, 1988; Wilcox, Rowlett, Paul, Ordway, & Woolverton, 2000) and increases in extracellular DA (Self & Nestler, 1995). Moreover, it has been suggested that the ratio of DA to 5-HT is a better indicator of drug abuse potential than solely a positive correlation with DA reuptake inhibition or increased DA release. For example, when different equipotent DA releasers that differed in 5-HT release were self administered by rhesus monkeys, responding was lower when 5-HT potency was higher (Wee, et al., 2005). Further, Ritz and Kuhar (1989) reported a negative correlation between potency as a reinforcer and 5-HT transporter binding affinity. These data suggest that the ratio of 5-HT to DA is an important determinant of self administration.

Because of the predominant effect of MDMA on the serotonin system it has been suggested that the lower DA:5-HT ratio may explain the increased latency to acquisition of self-administration of MDMA (Schenk, et al., 2007). For example, acquisition to MDMA self administration for drug naive rats was produced in about 12 days (Schenk et al., 2003), whereas acquisition to cocaine self-administration has been reported in as few as 5 days (Schenk & Partridge, 1997). With repeated administration of MDMA, there are long-term reductions in brain tissue concentrations of 5-HT and in 5-HT reuptake sites (Ricaurte et al., 2000; Green et al., 2003; Gudelsky andYamamoto, 2003) thereby increasing the DA:5-HT ratio. With continued self-administration studies, further decreases in 5-HT would be produced and this might explain the development of MDMA as an efficacious reinforcer (Schenk, et al., 2007) .

Initiation and Expression of Sensitisation

Behavioural sensitisation is comprised of two distinct components, 1) initiation (also called 'development', 'acquisition' or 'induction) and 2) expression. The initiation of sensitisation is the development of the augmented locomotor behaviour while the expression refers to the manifestation of that behaviour (Kalivas & Stewart, 1991; Pierce & Kalivas, 1997; Robinson & Becker, 1986; Stewart & Badiani, 1993). Effects on different components of the mesolimbic DA system have been attributed to these two processes.

DA in the ventral tegmental area VTA appears to be responsible for the induction of behavioural sensitisation following repeated amphetamine exposure (Nelson, Wetter, Milovanovic, & Wolf, 2007; Pierce & Kalivas, 1997; Vanderschuren & Kalivas, 2000; Vezina, 1996; Wolf & Xue, 1998). Direct infusion of DA antagonists into the VTA completely blocked the acute locomotor activating effects of amphetamine (Vezina & Stewart, 1989). Repeated amphetamine injections into the VTA induced behavioural sensitisation to either systemically, or intra NAc amphetamine (Bjijou, Stinus, Le Moal, & Cador, 1996; Cador, Bjijou, & Stinus, 1995; Hooks, Jones, Liem, & Justice Jr, 1992; Kalivas & Weber, 1988; Vezina & Stewart, 1989). In contrast, injections of amphetamine into other mesolimbic substrates did not result in sensitisation (Hitzemann, Wu, Hom, & Loh, 1980; Kalivas & Weber, 1988; Perugini & Vezina, 1994). It would appear that drug effects within the VTA underlie the induction of behavioural sensitisation.

Acute administration of amphetamine increased DA overflow in the NAc (Carboni, Imperato, Perezzani, & Di Chiara, 1989; Kalivas & Stewart, 1991; Robinson & Berridge, 1993; Sharp, Zetterstrom, Ljungberg, & Ungerstedt, 1987), but repeated intra-NAc administration did not result in further increases in synaptic DA (Dougherty & Ellinwood, 1981). Intra-NAc amphetamine produced locomotor activity (Pierce & Kalivas, 1995), and repeated administration resulted

in sensitisation. However, there was no sensitised response to a further amphetamine challenge (Kalivas & Weber, 1988). Thus the pattern of responding to a sensitising regime of amphetamine differs when the drug is infused into the VTA or the NAc.

A comparison of the effect of amphetamine administered into the VTA and NAc helps to clarify their role in induction and expression of amphetamine sensitisation. (Cador, et al., 1995). Amphetamine injected into the NAc dose-dependently increased locomotor activity but repeated exposure failed to produce behavioural sensitisation. Repeated injections into the VTA did not produce locomotor activity but resulted in locomotor activity following an intra-NAc amphetamine challenge. It was concluded that repeated amphetamine expose to the NAc was not responsible for the dopaminergic adaptations underlying behavioural sensitisation (Cador, et al., 1995)

Role of Dopamine D1 and D2-like Receptors in Amphetamine Sensitisation

In the 1970's it was proposed that there were two classes of dopamine receptor, the D1 and D2 (Cools & Van Rossum, 1976; Kebabian & Calne, 1979). Around the 1990's further heterogeneity revealed at least five subtypes of dopamine receptors (D1-D5) (Civelli, Bunzow, & Grandy, 1993; Sibley & Monsma, 1992). The five subtypes are now divided into two families and these are referred to as the D1-like (D1, D5) and the D2-like (D2, D3, D4) dopamine receptors. The most

notable distinguishing function of the two receptor subtypes is the effect on adenylyl cyclase with the D1-like increasing and the D2-like decreasing adenylyl cyclase activity or having no effect. A further difference is the presence (or otherwise) of an autoreceptor; with pharmacological studies indicating that the DA autoreceptor is of the D2 type (Nisoli, et al., 2009). DA D2 receptors function both as presynaptic autoreceptors and as postsynaptic receptors. Presynaptic autoreceptors modulate dopamine synthesis and release, and inhibit neuronal firing.

Pre-treatment with the D1-like receptor antagonist, SCH23390, blocked the development of sensitisation produced by intra-VTA infusions of amphetamine (Vezina & Stewart, 1989). SCH23390 also blocked the development of sensitisation produced by repeated systemic injections of amphetamine (Stewart & Vezina, 1989). These finding implicated a critical role of D1-like receptors in the VTA in amphetamine sensitisation. However, an alternative explanation for the attenuation of sensitisation was raised. It was suggested that intra-VTA administration of the D1 like antagonist may have diffused into the entire brain (Di Chiara, 1993) explaining why subsequent administration of amphetamine had not produced a sensitised response. As a test of this hypothesis, intra-VTA amphetamine was co-administered with SCH23390, sulpiride (a selective D2 antagonist) or kitanserin (5-HT_2 antagonist). SCH23390, but not ketanserin or sulpiride, dose dependently blocked the induction of behavioural sensitisation. (Bjijou,

et al., 1996). Systemic administration of the D2 receptor antagonist, Ro22-2586, also failed to effect the development of sensitisation (Vezina & Stewart, 1989). As was found with sulpiride, intra-VTA administration of other D2 antagonists, spiperone or eticlopride, also failed to alter the induction of sensitisation to the locomotor activating effect of amphetamine (Bjijou, et al., 1996; Vezina, 1996). Thus DA D1-like, but not D2-like, receptors within the VTA appear critical to the initiation of sensitisation to amphetamine.

Repeated amphetamine administration induced a subsensitivity of D_2 autoreceptors (Wolf, White, Nassar, Brooderson, & Khansa, 1993) which may, in part, explain the failure of the D_2 antagonists to block the induction of sensitisation. These autoreceptors are impulse regulating on the pre-synaptic neuron and prolonged exposure to amphetamine reduced the responsiveness of the receptors thereby increasing DA synthesis and release. White and Wang (1984) showed that a relatively high daily dose of amphetamine (1 or 2 x 5mg/kg i.p. for 5 days) significantly reduced the ability of intravenous apomorphine (a non-selective DA agonist, having a slightly higher affinity for D_2-like dopamine receptors) to suppress dopamine firing in the VTA suggesting a subsensitivity of the D_2 autoreceptor. With increases in dopamine release being induced through D_2 autoreceptor subsensitivity, blocking the D_2 receptor, as Vezina and Stewart (1989) and Bjijou and colleagues (1996) had done, would not be expected to block the process

of induction to amphetamine but rather augment the process (Vanderschuren, Schoffelmeer, Mulder, & De Vries, 1999).

DA VTA autoreceptor subsensitivity is a transient alteration which does not persist during withdrawal from repeated amphetamine. Following repeated exposure to a low dose of amphetamine, and 3 days withdrawal, intra-VTA application of the D_2 agonist, quinpirole, failed to reduce firing rates suggesting a subsensitivity of the autoreceptor. This decreased response, however, was no longer evident 14 days following treatment, even though behavioural sensitisation persisted (Wolf et al., 1993).

MDMA and Locomotor Activity

MDMA increases the release and prevents reuptake of DA. Following MDMA administration, increases in extracellular DA have been reported in striatum (Gudelsky & Yamamoto, 2008; Schmidt, Levin, & Lovenberg, 1987; Steele, Nichols, & Yim, 1987), nucleus accumbens (Bankson & Yamamoto, 2004; Cadoni, et al., 2005), prefrontal cortex (Nair & Gudelsky, 2004) and hippocampus (Shankaran & Gudelsky, 1998).

The mechanism of DA release is both transporter and impulse dependent with DAT inhibitors (Nash & Brodkin, 1991; Shankaran, Yamamoto, & Gudelsky, 1999) since the sodium channel blocker,

tertrodotoxin, (Yamamoto, Nash, & Gudelsky, 1995) attenuated release. MDMA induced locomotor activity was also attenuated by systemic administration of the D_1 like antagonist, SCH23390, (Daniela et al., 2004) and the D_2 antagonist, eticlopride (Ball, Budreau & Rebec, 2003).

Although MDMA primarily promotes DA release via the transporter, the serotonin selective reuptake inhibitor (SSRI), fluoxetine, suppressed MDMA stimulated DA release suggesting a role of serotonergic mechanisms (Callaway, Wing, & Geyer, 1990). Further, 5-HT_2 agonists potentiated (Gudelsky et al., 1994) and 5-HT_2 antagonists suppressed (Nash, 1990; Schmidt et al., 1994; Yamamoto et al., 1995) the MDMA-induced DA increase. More specifically, the 5-HT_{2A} receptors may modulate DA through increasing regulation of either DA synthesis or DA neuron firing rate (Schmidt et al. 1992; Gudelsky et al. 1994). Indeed, basal firing rate of DA neurons were increased by 5-HT_{2C} receptor antagonists and inhibited by 5-HT_{2C} receptor agonists (Di Matteo et al. 2000; Gobert et al. 2000) through a tonic inhibitory influence on release (Ball & Rebec, 2005).

Because of the well documented role of DA in locomotor activity it is not surprising that both peripheral and central administration of MDMA increased locomotor activity. Gold and Koob (1988) were one of the first to demonstrate MDMA produced locomotor activity in rats and since then there have been a number of other reports (e.g. Bubar,

Pack, Frankel, & Cunningham, 2004; Yamamoto & Spanos, 1988). MDMA induced locomotor activity was decreased by systemic administration of the D_1 like antagonist, SCH23390 (Daniela, et al., 2004) and the D_2 like antagonist, eticlopride (Ball, Budreau, & Rebec, 2003). Moreover, pharmacological blockade of the DAT inhibited MDMA induced locomotor activity (Callaway et al., 1990). The selective 5-HT_{2A} antagonist MDL 100,907, also suppressed MDMA produced hyperactivity. Other less selective 5-HT_{2A} antagonists (ritanserin, methiothepin, MDL 28,133A, SR46349 and clozapan) also reduced MDMA stimulated activity (Ball & Rebec, 2005; Kehne, et al., 1996) but the 5-HT_{2C} antagonist, SB242084, potentiated the locomotor stimulant effects of MDMA (Fletcher, Sinyard, & Higgins, 2006). It has been suggested that the 5-HT antagonists altered MDMA produced hyperactivity by modulating DA release (Ball & Rebec, 2005).

Of interest, chronic treatment with DOI, although a 5-HT_{2A} agonist, produced an inhibitory regulation of the 5-HT_{2C} receptor, which increased MDMA produced locomotor activity. This was attributed to 5-HT_{2A} mechanisms because the response to the 5-HT_{2C} receptor agonist, MK212, remained unaffected by repeated DOI pre-treatment. (Ross, Herin, Frankel, Thomas, & Cunningham, 2006). Indeed, repeated DOI treatment decreased 5-HT_{2A} receptor protein expression in the PFC and shell of the NAc. Because 5-HT_{2A} receptor activation increased DA release (Ball & Rebec, 2005), these findings suggest that

5-HT_{2A} receptors in the DA terminal areas of the PFC and NAc might be critical to MDMA produced hyperactivity.

MDMA and behavioural sensitisation

Few studies have examined behavioural sensitisation to MDMA and fewer still have examined the mechanisms underlying the development or expression of sensitisation. Repeated intermittent administration of MDMA produced a progressive and enduring increase in the behavioural response to the drug. One of the early investigations into MDMA behavioural sensitisation was conducted by Spanos and Yamamoto (1989) who studied both the acute and chronic behavioural effects of MDMA. Acute effects showed a dose related increase in MDMA produced locomotor activity. Chronic exposure, consisting of alternate-day injections with locomotor challenge doses after the sixth and twelfth injections, produced a dose dependent increase in all behavioural measures. Furthermore, authors reported correlated extracellular in-vivo voltammetery measures of DA release paralleling the time course of MDMA induced hyperlocomotion. When examining the chronic behavioural effects of MDMA exposure Kalivas, Duffy and White (1998) concurred with Spanos and Yamamoto (1989), finding an augmented behavioural response to the drug. In addition, and paralleling amphetamine induced DA release, they found a dose dependent increase in extracellular DA in the nucleus accumbens suggesting there may be patterns of MDMA produced neuroadaptations that overlap with other commonly abused amphetamines. In corroboration

with this finding, MDMA induced increases in cell firing in the dorsal striatum were increased in sensitised rats (Ball et al., 2006). Serotonergic mechanisms might also be involved since the 5-$HT_{1B/1D}$ antagonist, GR127935, attenuated the development of sensitisation (McCreary et al., 1999). However, Modi, Yang, Swann, & Dafn (2006) failed to find cross sensitisation to amphetamine and methylphenidate after repeated MDMA administration. Chronic administration produced sensitisation of only a transient nature evident on challenge day 13 but not on day 38. Chronic dosage of 5 mg/kg persisted for a longer period of time with motor indices of sensitisation still evident on day 38. The administration of 10.0 mg/kg MDMA however, produced increases in locomotor activity. This does not rule out the possibility of overlapping neural adaptations but may suggest those adaptations to be more akin to cocaine rather than amphetamine or methylphenidate.

Intra-NAc core administration of the D_1-like receptor antagonist, SCH23390, prevented the expression, but not the development, of behavioural sensitisation (Ramos, Goni-Allo, & Aguirre, 2004). Although Ramos et al. (2004) did not observe sensitised responding after SCH23390 D_1 blockade through pre treatment with MDMA, D_1 receptor activation must still have occurred. The longer half life of MDMA versus SCH23390 would ensure D_1 receptor activation past any evident time analysis of the 60 minute test for locomotion or post activity test chamber when the rats were placed back in their home cage. Indeed, the obvious interpretation is that made by the authors in

that the D_1 receptor is not involved in the induction of sensitised responding. It was argued that projections from the prefrontal cortex (PFC) might mediate behavioural sensitisation to MDMA because ibotenic acid lesions of the dorsal medial PFC, that destroyed cell bodies, blocked both the induction and expression of sensitisation (Ramos, Goni-Allo, & Aguirre, 2005b). Dopaminergic mechanisms were implicated since administration of the D_1 like receptor agonist, SCH23390, into the medial PFC blocked the expression of sensitisation. Because SCH23390 is also a 5-HT_{2C} receptor agonist, this mechanism in the medial PFC might play a key role in behavioural sensitisation to MDMA. This idea was supported by the finding that attenuation of sensitisation produced by SCH23390 was reversed by administration of the 5-HT_{2C} receptor antagonist, RS 102221 and sensitisation was produced by the 5-HT_{2C} agonist MK212 (Ramos, Goni-Allo, & Aguirre, 2005a). These data support the idea that MDMA induced sensitisation was mediated by 5-HT_{2C} receptor stimulation in the medial PFC and not by the blockade of medial PFC D_1 receptors.

The current investigation

MDMA induces locomotor, as well as sensitised locomotor responding that can be attenuated through dopaminergic antagonists. Despite a plethora of evidence suggesting a crucial role of dopamine in behavioural sensitisation to psychostimulants, its role in MDMA sensitisation is yet to be fully investigated. It has been postulated that

activation of DA D_1 receptors initiate the neural adaptations underlying amphetamine sensitisation (e.g. Stewart and Vezina 1989; Kalivas and Stewart 1991; Bjijou et al. 1996; Vezina 1996). DA D_2 pre-synaptic receptors show evidence of transient sub-sensitivity (White and Wang (1984) while DA D_2 post-synaptic receptors may become sensitised (Wolf et al., 1993). However, the role in MDMA induced sensitisation is less clear. One way to address this is through behavioural pharmacology.

The following set of experiments aims to firstly determine parameters for the development of sensitisation to the locomotor activating effects of MDMA and it is hypothesised that repeated intermittent administration will produce an augmented locomotor activity in response to a further challenge dose of the drug. Secondly, the thesis will determine whether cross-sensitisation is produced to the locomotor activating effects of D_1-like and/or D_2-like receptor agonists and antagonists. Thirdly, the relevance of sensitisation to drug self administration will be evaluated. Finally, because deficits in 5-HT have been reported following exposure to MDMA, HPLC analysis will be used. Tissue levels of 5-HT, DA and their primary metabolites will be measured to ascertain what changes in neurochemical levels result from repeated administration of MDMA.

Experiment 1: Induction and expression of behavioural sensitised responding to MDMA locomotor activating effects

General methodology

Subjects

The subjects were male Sprague-Dawley rats, weighing between 250-350g (approximately 60 days old). The animals were bred at Victoria University in Wellington, New Zealand and were initially housed in pairs and then housed singly in a temperature- (21˚C) and humidity- (55%) controlled room. The colony was maintained on a 12-hr light/dark cycle with lights on at 0700. Food and water were available *ad libitum* except during testing periods. Laboratory animal care principles of the Victoria University of Wellington Animal Breeding Facility were followed, and the Victoria University of Wellington Animal Ethics Committee approved all protocols.

Apparatus for locomotion studies

Eight open field chambers (450mm x 450mm; Med Associates (ENV-515) Vermont, USA) equipped with four banks of 16 photocells on each of the internal walls of the chamber were used to measure horizontal locomotion. Photocells were set at 25mm above the floor of the chamber and spaced evenly at 25mm centres around the periphery.

The open field boxes were interfaced with a computer and data were obtained using Med Associates software. Each activity chamber was enclosed in sound attenuating boxes (Med associates; Vermont USA). A beam 'box' was pre-set encompassing a 3 x 3 beam square (50mm x 50mm). Movement outside of this 'box' broke the beams and constituted one locomotor count.

All testing was conducted during the light cycle. A red house light was illuminated during testing and white noise was also continually present to mask extraneous disturbances. Prior to and after each locomotor activity test, the chamber interiors were cleaned and wiped with Virkon 'S' disinfectant (Southern Veterinary Supplies, NZ).

Drugs:

- Racemic MDMA hydrochloride, (ESR Ltd, Porirua, New Zealand).
- *d*-Amphetamine, (SIGMA; Australia).
- SCH23390 Hydrochloride, *[R(+)-7-Chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride]*, (Tocris Bioscience, Natick, Massachusetts).
- SKF81297 hydrobromide, *[R-(+)-6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1-H-3-benzazepine hydrochloride]*, (Tocris Bioscience, Natick, Massachusetts).
- Apomorphine hydrochloride, *[R-5,6,6a,7-tetrahydro-6-methyl-4H-dibenzo[de,g]quinoline-10,11-diol hydrochloride]*, (Tocris Bioscience, Natick, Massachusetts).

- Eticlopride, *[S(-)-3-Chloro-5-ethyl-N-[(1-ethyl-2-pyrrolidinyl)methyl]-6-hydroxy-2-methoxybenzamide hydrochloride]*, (SIGMA; Australia).

All the above drugs were dissolved in sterile saline (0.9%NACL).

- RS102221, *[8-[5-(2,4-Dimethoxy-5-(4-trifluromethylphenylsulfonamido)phenyl-5-oxopentyl]-1,3,8-triazaspiro[4,5]decane-2,4-dione hydrochloride]*, (Tocris Bioscience, Natick, Massachusetts).

All the above drugs were dissolved in sterile saline (0.9%NACL) apart from RS102221 which was suspended in a solution of 1% polysorbate 80 (Tween® 80).
Subcutaneous (SC) or Intraperitoneal (IP) injections were in a volume of 1 ml/kg. All drug doses refer to the salt.

General Sensitisation Protocol

Rats were housed individually and were weighed and handled daily, one week prior to the commencement of all experiments.

Days 1-5:
Rats were transported daily from their home cages to the locomotor activity room and placed into the middle of the open field chambers. Locomotor activity was recorded for 15 or 30 minutes, recording was then paused while rats were administered drug or saline and activity was recorded for an additional 60 minutes. Activity data were collected at 5

min intervals during the 30 min pretreatment and 60 min post-treatment periods.

Days 6 & 7:

For the majority of experiments, there was a two day withdrawal period during which the rats remained in the home cages. A number of experiments had extended withdrawal periods (see table 1) in which the rats remained in their home cages.

Day 8:

Rats were transported from their home cages to the locomotor activity room and placed into individual activity chambers. Activity was recorded during a 15 or 30 minute pretreatment and 60 minute post-treatment period.

Data Analysis

Data analyses (unless otherwise specified) were conducted on the activity counts during the post injection interval. Locomotor responses were analysed using a 1, 2 or 3-way (as specified in each results section) repeated measures Analysis of Variance (ANOVA) with the repeated measure of time.

Overall layout for experiment 1A and 1B

Table 1.

	Pre-treatment					
	Exp. 1A		Exp. 1B			
	Amphetamine (2.0mg/kg)	Vehicle	MDMA (5.0mg/kg)	Vehicle	MDMA (10.0mg/kg)	Vehicle
Day 1 vs. day 5	Yes	Yes	Yes	Yes	Yes	Yes
2-day Withdrawal	Yes	Yes	Yes	Yes	Yes	Yes
9-day withdrawal	No	No	No	No	Yes	Yes
28-day withdrawal	Yes	Yes	No	No	Yes	Yes

Table 1. Within experiment 1, drug pre-treatment and withdrawal periods vary. The table identifies the drug pre-treatment administered and withdrawal period used.

Experiment 1a Amphetamine-produced sensitisation

Background

Repeated intermittent exposure to a number of stimulant drugs produces a sensitised locomotor response. The sensitised behavioural responses reflect a host of neuroadaptations. These complex changes are greatly impacted upon by a number of parameters in the drug administration regimen including withdrawal time from the last drug administration (Kolta, et al., 1985) dose (Kalivas & Duffy, 1993; Paulson, Camp, & Robinson, 1991; Paulson & Robinson, 1995), drug exposure duration (Robinson & Becker,1986), and the context in which the drug is delivered (Badiani, Browman, et al., 1995; Badiani, Camp, & Robinson, 1997;

Robinson, Browman, Crombag, & Badiani, 1998; Stewart & Badiani, 1993).

To this end Experiment 1 as a whole was designed to determine the protocols required to induce reliable sensitisation to the locomotor activating effects of MDMA. To start, experiment 1 reproduced amphetamine sensitisation and then used the delivery and withdrawal protocols to attempt to induce MDMA sensitisation. Different doses of MDMA were used in the pre-treatment regimen, and following drug abstinence, varying challenge doses were administered in order to determine whether there were changes in the dose response function. Because MDMA is an amphetamine derivative, it was hypothesised that the induction and expression protocols for sensitisation to the behavioural effects of amphetamine and MDMA would be similar.

Experiment 1a Procedure

On days 1-5, rats (numbers vary and are reported in each experiment) were given a single daily administration of amphetamine (0.0 or 2.0 mg/kg, IP). This dose and injection regimen has been shown to produce persistent sensitisation to the locomotor stimulant effects of amphetamine (Vanderschuren, Schmidt, et al., 1999) and has been used in a number of previous investigations (Laudrup & Wallace, 1999; McNamara, Davidson, & Schenk, 1993; Nordquist, et al., 2008).

After either 2 or 28 days of withdrawal, during which all rats were left in home cages, the locomotor activating effects of amphetamine (0.0 or 0.5 mg/kg, IP) were measured as described above.

Experiment 1a Results.

Amphetamine pre-treatment day 1 vs. day 5

Figure 1.1 shows locomotor activity as a function of time on Days 1 and 5 of the pre-treatment regimen. A mixed 3-way ANOVA *[Day (1 & 5) x Drug (amph or vehicle) X Time (12 five min bins)*] on the counts post injection ('time 0') revealed a significant main effect of Day ($F(1,22) = 21.65$, $p < 0.05$), Drug ($F(1,22) = 219.74$, $p < 0.05$) and a significant interaction between Day and Drug ($F(1,22) = 14.98$, $p < 0.05$). There was also a 3 way interaction for Day x Drug x Time ($F(11,242) = 2.46$, $p < 0.05$). A post hoc analysis of the amphetamine data *(Day x Time)* showed that locomotor activity counts were higher on day 5 compared to day 1 of testing ($F(1,22) = 18.95$, $p < 0.05$).

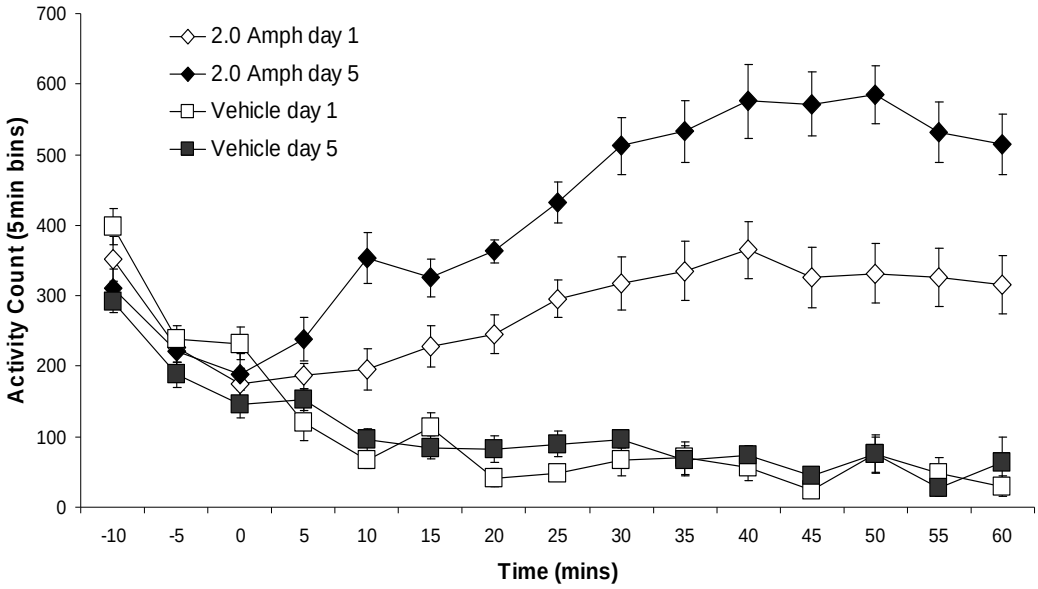

Figure 1.1. Locomotor counts for the 75 minutes of testing across days. Rats were administered either amphetamine (2.0mg/kg i.p.) *(n=12)* or vehicle *(n=12)* each day in the testing chamber. Locomotor counts are summed into bins of 5 minute intervals with time '0' being the time of drug injection.

Amphetamine pre-treatment 2-day withdrawal

Figure 1.2 shows locomotor activity as a function of time on challenge day (day 8). A mixed 3-way ANOVA *[pre-treatment (amph or vehicle) x Dose (0.0 or 0.5mg/kg) x time (12 five min bins)]* revealed a significant effect of pre-treatment ($F(1,82) = 3.99$, $p < 0.05$), and Dose ($F(1,82) = 11.56$, $p < 0.05$), but no interaction between pre-treatment and Dose ($F(1,82) = 1.04$, ns).

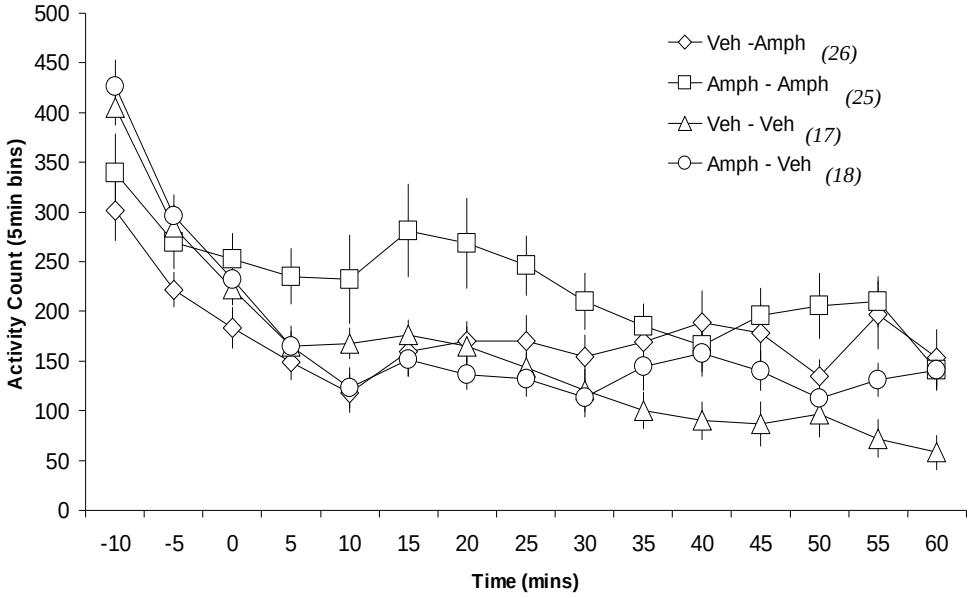

Figure 1.2. Locomotor counts for the 75 minutes of testing following injection of either amphetamine (0.5mg/kg i.p.) or vehicle. Data were collected following 2 days withdrawal. The first listing in the legend identifies the group's pre-treatment drug while the second indicates the challenge drug. Sample sizes are in brackets beside the listings. Locomotor counts are summed into bins of 5 minute intervals with time '0' being the time of drug injection.

Group differences were examined with a 1-way ANOVA *(Veh-Amph, Amph-Amph,)* on post injection totals. An overall difference between the groups was found ($F(1,49) = 4.18$, $p < 0.05$). Whereas the low dose of amphetamine failed to produce significant locomotor activation in the vehicle pre-treated rats, increased activity was produced in the amphetamine pre-treated rats during the initial 30 minute post injection period. When total locomotion for just the initial post injection 30 minute period was compared activity in the Amph-Amph group was significantly higher than activity of all other groups ($p<0.05$).

Amphetamine pre-treatment 28-day withdrawal

A further group of vehicle *(n=7)* and amphetamine *(n=7)* pre-treated rats were administered 0.5 mg/kg i.p. amphetamine 28 days following the sensitisation regimen. Figure 1.3 shows the time course for the total 75 minutes following amphetamine (0.5mg/kg i.p.) administration.

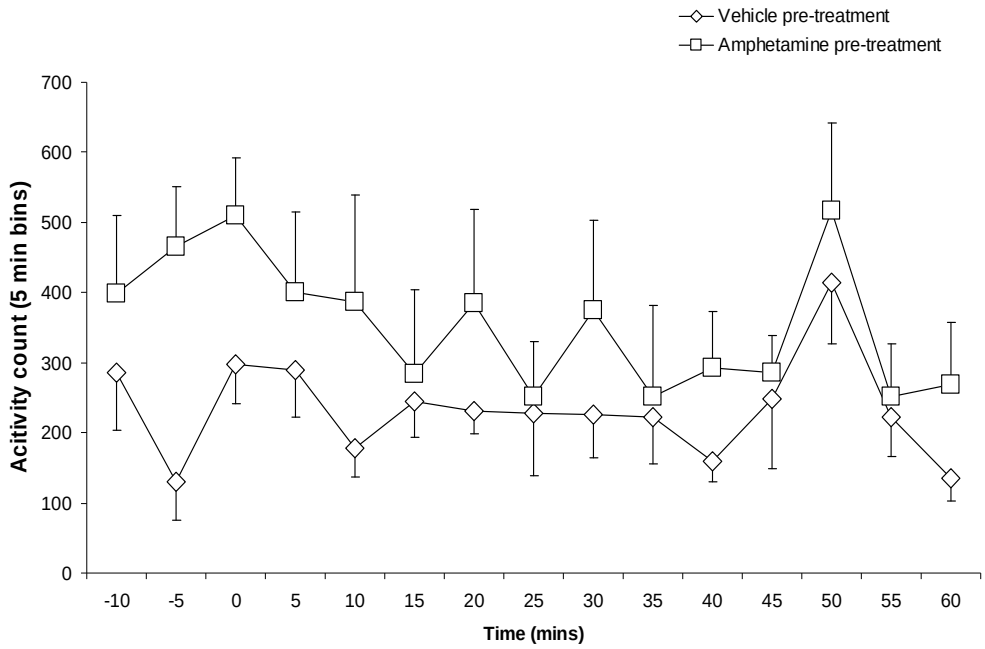

Figure 1.3. Locomotor counts for the 75 minutes of testing following 28 days withdrawal. All animals were challenged with amphetamine (0.5mg/kg i.p.) with the legend indicating vehicle and amphetamine pre-treated rats. Locomotor counts are summed into bins of 5 minute intervals with time '0' being the time of drug injection
.

The amphetamine pre-treated rats tended to be more responsive to the effect of amphetamine but the effects were of much smaller magnitude than when testing was conducted following 2 days withdrawal (Figure 1.2). Additionally, amphetamine pre-treated rats tended to have higher activity scores during the pre-treatment phase of testing although

variability throughout testing was high. A repeated measures ANOVA *(pre-treatment x time)* failed to reveal any significant differences between the two groups over the 30 minutes post injection ($F(1,6) = 1.085$, $p > 0.05$) or the 60 min post injection period ($F(1,12) = 1.247$, $p > 0.05$).

Experiment 1b, MDMA-Produced Sensitisation

Procedure

On days 1-5, rats were given a single daily administration of MDMA (0.0, 5.0 or 10.0 mg/kg i.p.) and activity was measured. These doses were chosen based on previous literature that has demonstrated sensitisation (Ramos, et al., 2005a; Spanos & Yamamoto, 1989). After 2, 9 or 28 days of withdrawal, during which all animals remained in their home cages, locomotor activating effects of MDMA (0.0, 2.5, 5.0 & 10.0 mg/kg i.p.) were measured as above. (28 day withdrawal animals were housed in pairs during the 28 day drug abstinence period).

Experiment 1b Results.

5.0 mg/kg MDMA pre-treatment day 1 vs. day 5

During initial tests, a large sample of rats were administered repeated intermittent administration of the lower (5.0 mg/kg, IP) dose of MDMA. The locomotor activating effects of MDMA as a function of time on Days 1 and 5 of treatment are presented in Figure 1.4. MDMA produced hyperactivity ($F(1,84) = 83.24$, $p < 0.05$) was not substantially altered by repeated exposure and the ANOVA failed to reveal significant effects of Day ($F(1, 84) = 2.2$, ns) or an interaction between Day and Drug ($F(1, 84) = 3.21$, ns).

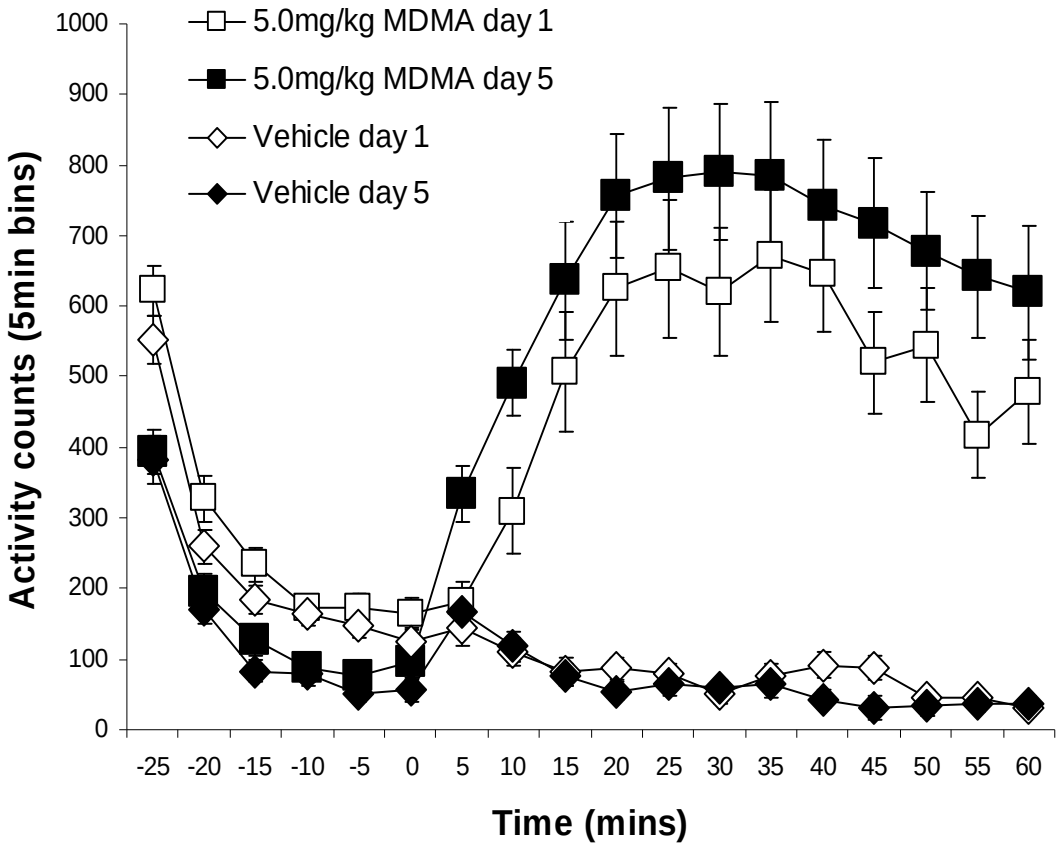

Figure 1.4. Average locomotor counts for the 90 minutes of testing on day 1 and 5 following administration of MDMA (5.0mg/kg i.p.) *(n=43)* or vehicle *(n=43)* in the test boxes. Locomotor counts are summed into bins of 5 minute intervals with time '0' being the time of drug injection.

5.0 mg/kg MDMA pre-treatment 2-day withdrawal

Following a two day withdrawal the motor activating effects of various doses of MDMA (0.0, 2.5, 5.0 10.0 mg/kg) were measured. Figure 1.5 shows the time course data.

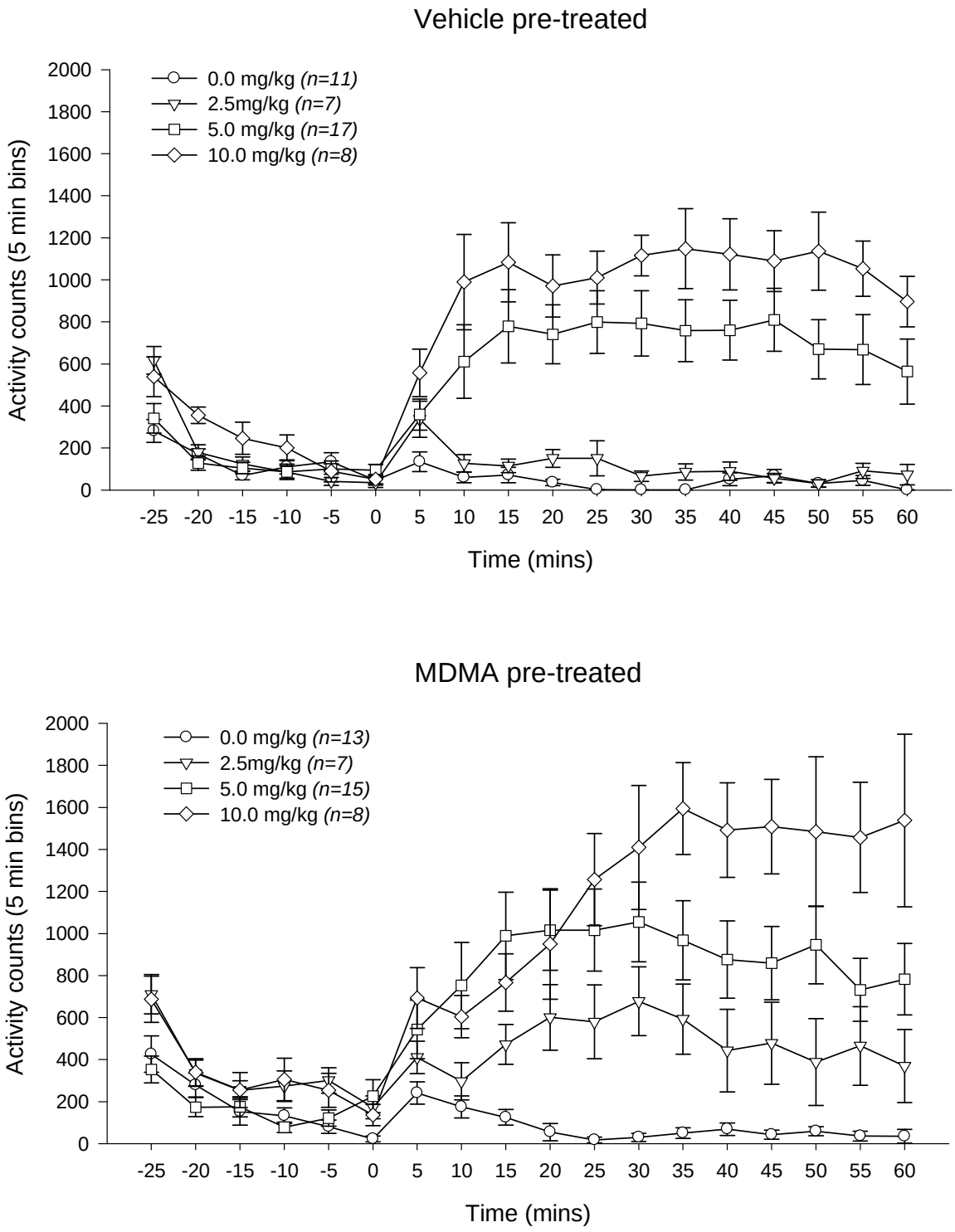

Figure 1.5. Locomotor activating effects of various doses of MDMA following a 2-day withdrawal from daily administration of MDMA (5.0mg/kg i.p.) or vehicle. Top panel is the time course of locomotor activity for vehicle pre-treated rats. Bottom panel is the time course of locomotor activity for MDMA pre-treated rats. Locomotor counts are summed into bins of 5 minute intervals with time '0' being the time of drug injection

A mixed 3-way ANOVA *[Pre-treatment (MDMA or vehicle) x Dose (0.0, 2.5, 5.0, 10.0) x time (12 five min bins)]* revealed a main effect of Pre-treatment ($F(1,79) = 4.08$, $p<0.05$) and Dose ($F(3,79) = 23.73$, $p<0.05$) but no significant interaction between Pre-treatment and Dose ($F(3, 79) = 0.44$, ns).

Figure 1.6 presents the total activity data collapsed across time following each dose of MDMA for the MDMA and vehicle pre-treatment groups.

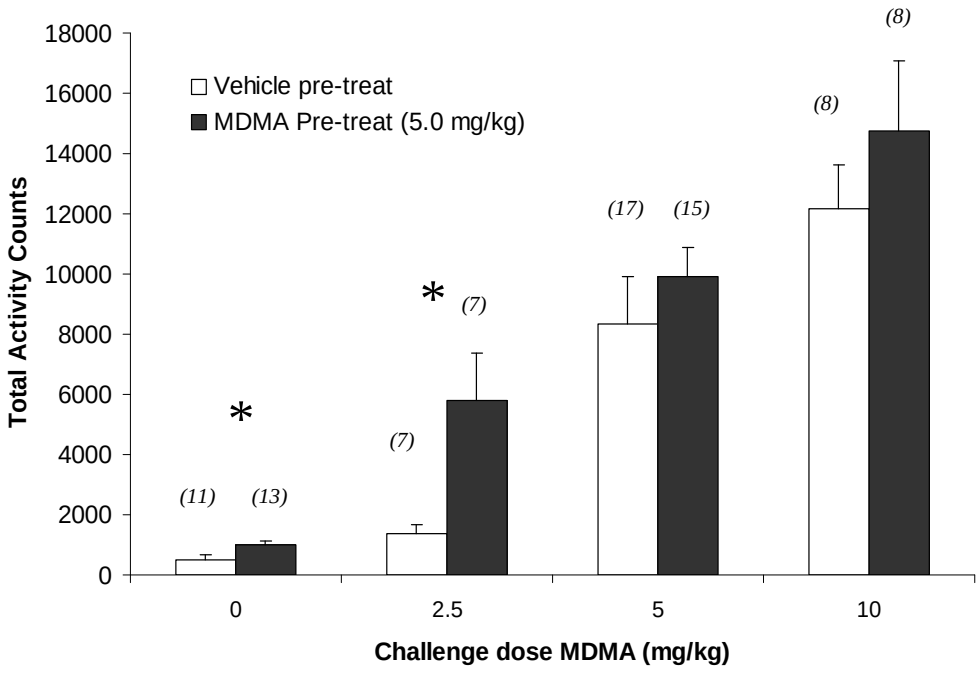

Figure 1.6. Total locomotor counts on challenge day for the 60 minutes post injection with vehicle and MDMA (5.0 mg/kg i.p.) pre-treated rats. After two days of withdrawal rats were challenged with MDMA (0.0, 2.5, 5.0 or 10.0mg/kg i.p.). * difference from vehicle pre-treated group.

Analysis on total locomotor activity counts during the 60 minutes post-injection period was conducted using a 2-way ANOVA *(Pre-treatment x Dose).* Main effects were echoed from the repeated measures analysis

above [a main effect for Pre-treatment ($F(1,79) = 4.08$, $p<0.05$) and Dose ($F(3,79)=23.73$, $p<0.05$)]. Independent samples t-tests revealed MDMA pre-treated rats were more responsive to the 0.0 mg/kg ($t(22) = -2.04$, $p< 0.05$) and the 2.5 mg/kg ($t(6.396) = -2.57$, $p< 0.05$) doses (Levene's test for equal variances violated, adjusted df reported).

<u>10.0 mg/kg MDMA pre-treatment day 1 vs. day 5</u>

Figure 1.7 shows MDMA-produced hyperactivity on Days 1 and 5 of the higher dose pre-treatment regimen (0.0 *(n=19)* or 10.0 *(n=21)* mg/kg i.p.) A mixed 3-way ANOVA *[Day (1 & 5) x Drug (MDMA or vehicle) X Time (12 five min bins)*] on the post injection ('time 0') data revealed a main effect of Drug ($F(1,38) = 284.57$, $p< 0.05$), Day ($F(1,38) = 4.87$, $p<0.05$) and an interaction ($F(1,38) = 7.86$, $p<0.05$). There was also a 3 way interaction for Day x Drug x Time ($F(11,418) = 4.43$, $p< 0.05$). Post hoc analysis on the MDMA data *(Day x Time)* showed that MDMA-produced hyperactivity was greater on Day 5 compared to Day 1 of treatment ($F(1,36) = 5.16$, $p< 0.05$).

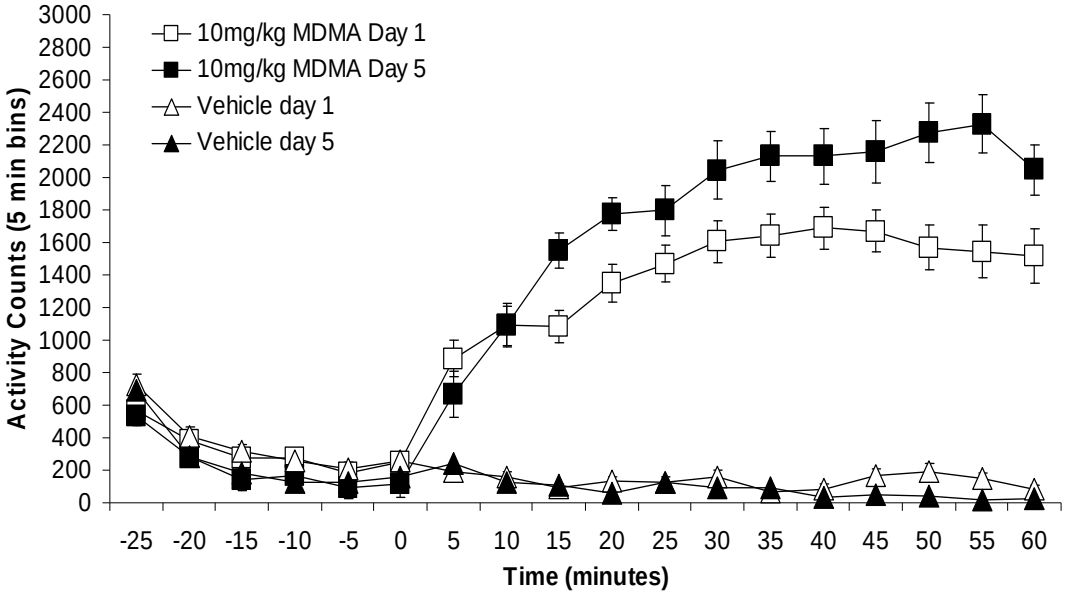

Figure 1.7 Average locomotor counts for the 90 minutes of testing across days. Rats were administered either MDMA (10.0mg/kg i.p.) *(n=21)* or vehicle *(n=19)* each day in the test chambers. Locomotor counts are summed into bins of 5 minute intervals with time '0' being the time of drug injection.

<u>10.0 mg/kg MDMA pre-treatment 2-day withdrawal</u>

Following a two day withdrawal locomotor activating effects of various doses of MDMA (0.0, 2.5, 5.0 10.0 mg/kg) were measured. Figure 1.8 below presents the time course data.

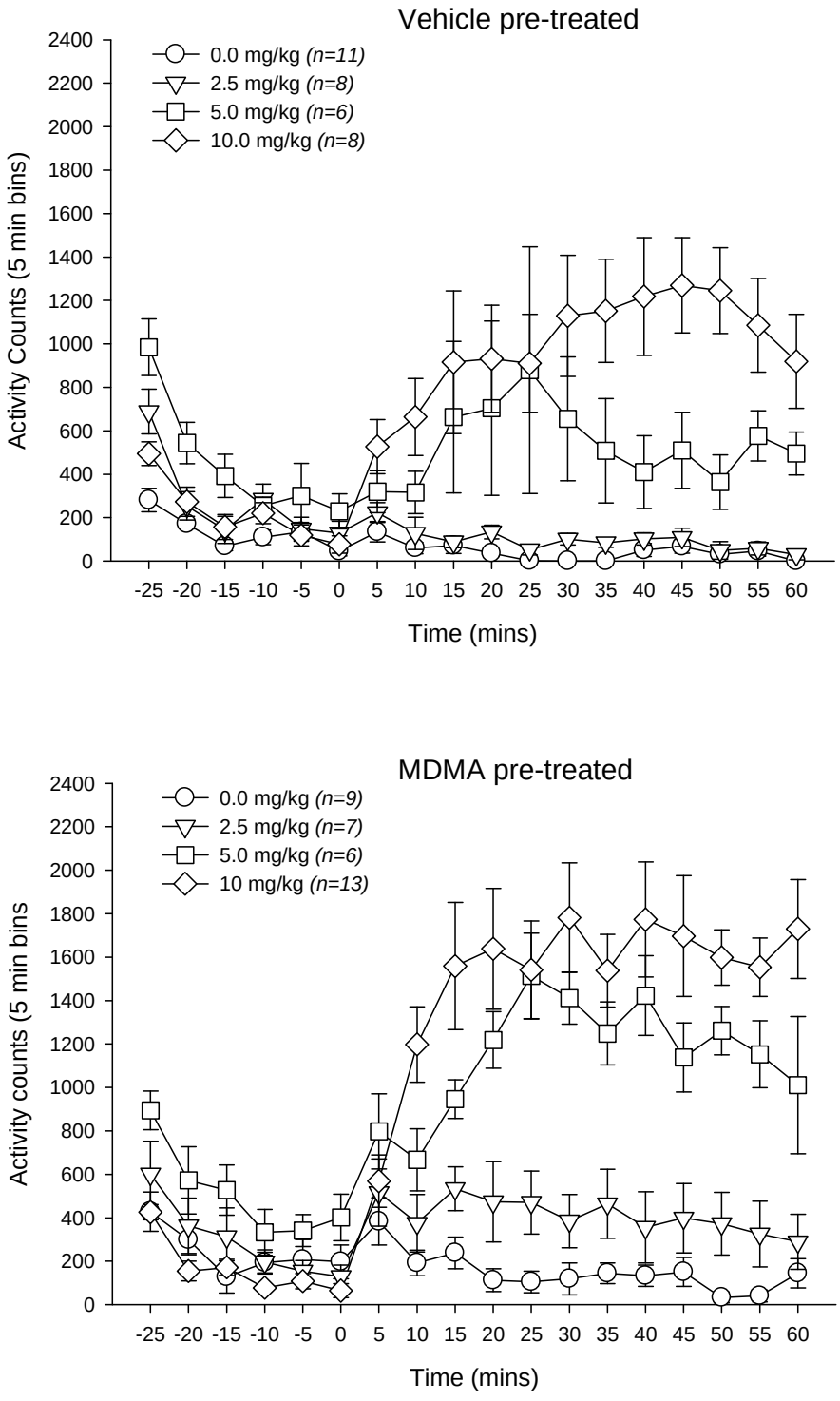

Figure 1.8. Locomotor activating effects of various doses of MDMA following a 2-day withdrawal from daily administration of MDMA (10.0mg/kg i.p.) or vehicle. Top panel is the time course of locomotor activity for vehicle pre-treated rats. Bottom panel is the time course of locomotor activity for MDMA pre-treated rats. Locomotor counts are summed into bins of 5 minute intervals with time '0' being the time of drug injection

A repeated measures ANOVA *[pre-treatment (MDMA or vehicle) x Dose (0.0, 2.5, 5.0, 10.0) x time (12 five min bins)]* revealed a main effect of Pre-treatment ($F(1,60)=15.841$, $p<0.05$) and Dose ($F(3,60)=35.71$, $p<0.05$) but no significant interaction between Pre-treatment and Dose ($F(3, 60) = 1.46$, ns).

Figure 1.9 presents the total activity data collapsed across time following each dose of MDMA for the MDMA and vehicle pre-treatment groups.

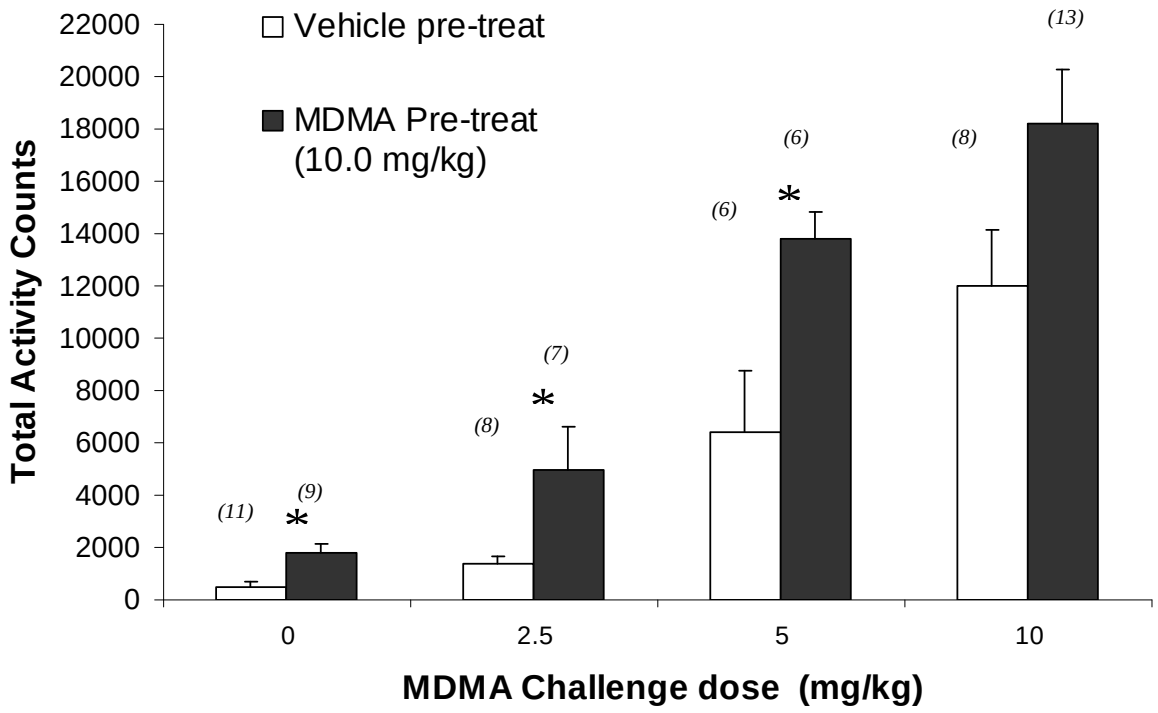

Figure 1.9. Total locomotor counts on challenge day for the 60 minutes post injection with vehicle and MDMA (10.0 mg/kg i.p.) pre-treated rats. After two days of withdrawal rats were challenged with MDMA (0.0, 2.5, 5.0 or 10.0mg/kg i.p.). Numbers in brackets above each column is the sample size. * difference from vehicle pre-treated group

Analysis on total locomotor activity counts was conducted by using a 2-way ANOVA *(Pre-treatment x Dose)*. Post hoc t-tests revealed an increase in the activating effect of all doses of MDMA ($p<0.05$) accepting the 10.0 mg/kg challenge dose.

10.0 mg/kg MDMA pre-treatment and 9 day withdrawal

Figure 1.20 shows the time course of MDMA-produced hyperactivity (0.0 or 5.0 mg/kg, IP) for rats that had been pre-treated with the higher dose of MDMA (0.0 or 10.0 mg/kg, IP) 9 days earlier.

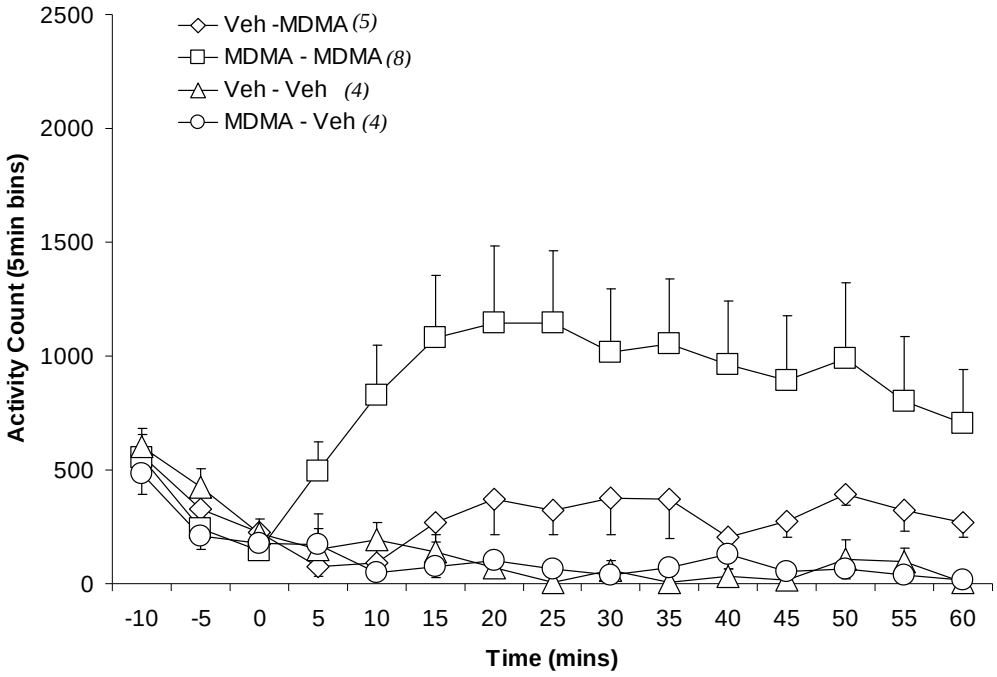

Figure 1.20. Locomotor activating effects of MDMA (0.0 or 5.0mg/kg i.p.) following a 9-day withdrawal from daily administration of MDMA (10.0mg/kg i.p.) or vehicle. The first listing in the legend identifies the group's pre-treatment drug while the second indicates the challenge drug. Sample sizes are in brackets beside the listings. Locomotor counts are summed into bins of 5 minute intervals with time '0' being the time of drug injection.

A repeated measures 3-way ANOVA *[pre-treatment (MDMA or vehicle) x challenge (MDMA or vehicle) x time (12 five min bins)]* failed to reveal a significant effect of pre-treatment ($F(1,17) = 2.59$, ns), or, perhaps due to the small sample sizes , an interaction between pre-treatment and challenge ($F(1,17) = 2.60$, ns). However, there was a main effect of Challenge ($F(1,17) = 5.66$, $p<0.05$).

Figure 1.21 shows the time course of MDMA-produced hyperactivity (0.0 or 10.0 mg/kg, IP) for rats that had been pre-treated with MDMA (0.0 or 10.0 mg/kg, IP) 9 days earlier.

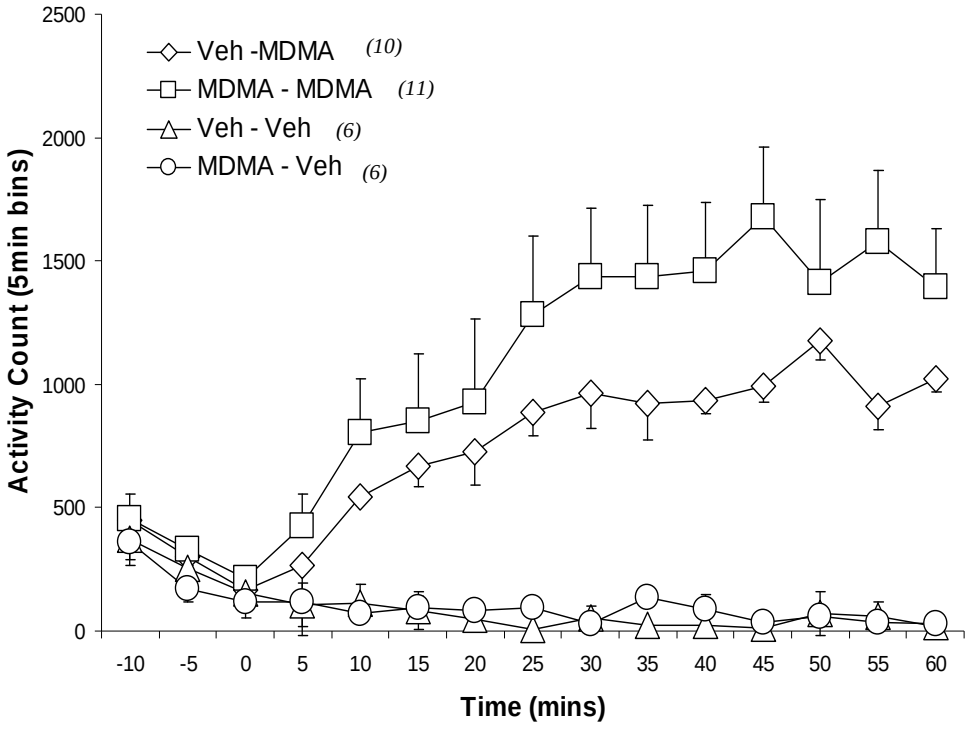

Figure 1.21. Locomotor activating effects of MDMA (0.0 or 10.0mg/kg i.p.) following a 9-day withdrawal from daily administration of MDMA (10.0mg/kg i.p.) or vehicle. The first listing in the legend identifies the group's pre-treatment drug while the second indicates the challenge drug. Sample sizes are in brackets beside the listings. Locomotor counts are summed into bins of 5 minute intervals with time '0' being the time of drug injection.

A repeated measures 3-way ANOVA *[pre-treatment (MDMA or vehicle) x challenge (MDMA or vehicle) x time (12 five min bins)]* failed to reveal a significant effect of pre-treatment ($F(1,29) = 2.097$, ns), or an interaction between pre-treatment and challenge ($F(1,29) = 1.26$, ns), but a main effect for challenge ($F(1,29) = 93.39$, $p < 0.05$).

Figure 1.22 presents the total post-injection activity data collapsed across time following 9 days withdrawal for the MDMA and vehicle pre-treatment groups.

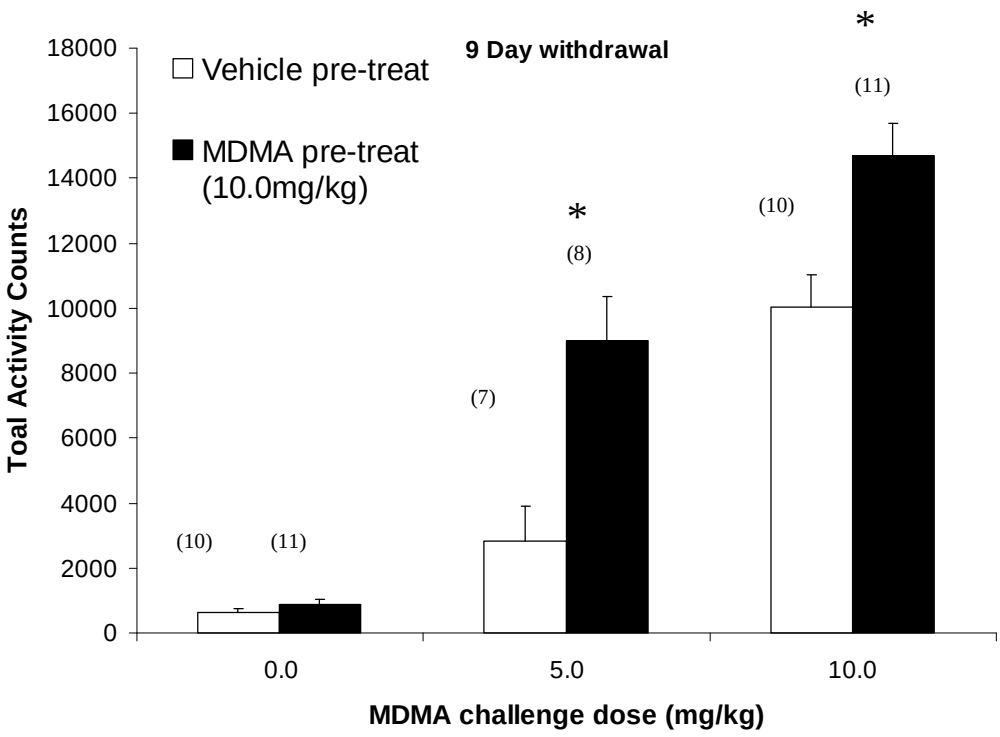

Figure 1.22. Total post injection locomotor counts on challenge day for the 60 minutes post injection with vehicle and MDMA (10.0 mg/kg i.p.) pre-treated rats. After nine days of withdrawal rats were challenged with MDMA (0.0, 5.0 or 10.0mg/kg i.p.). Numbers in brackets above each column is the sample size. * difference from vehicle pre-treated group

Analysis on total locomotor activity counts was conducted by using a 2-way ANOVA *(Pre-treatment x Dose)*. There were main effects for both pre-treatment ($F(1,49) = 5.39$, $p < 0.05$) and dose ($F(2,49) = 32.48$, $p < 0.05$). Post hoc contrasts revealed an increase in the activating effect of both the 5.0mg/kg MDMA and 10.0 mg/kg MDMA challenge dose ($p<0.05$).

10.0 mg/kg MDMA pre-treatment and 28 day withdrawal

Figure 1.23 shows the time course of rats challenged with MDMA (5.0 & 10.0mg/kg i.p.) following the 28 day withdrawal period from the MDMA (10.0mg/kg) pre-treatment

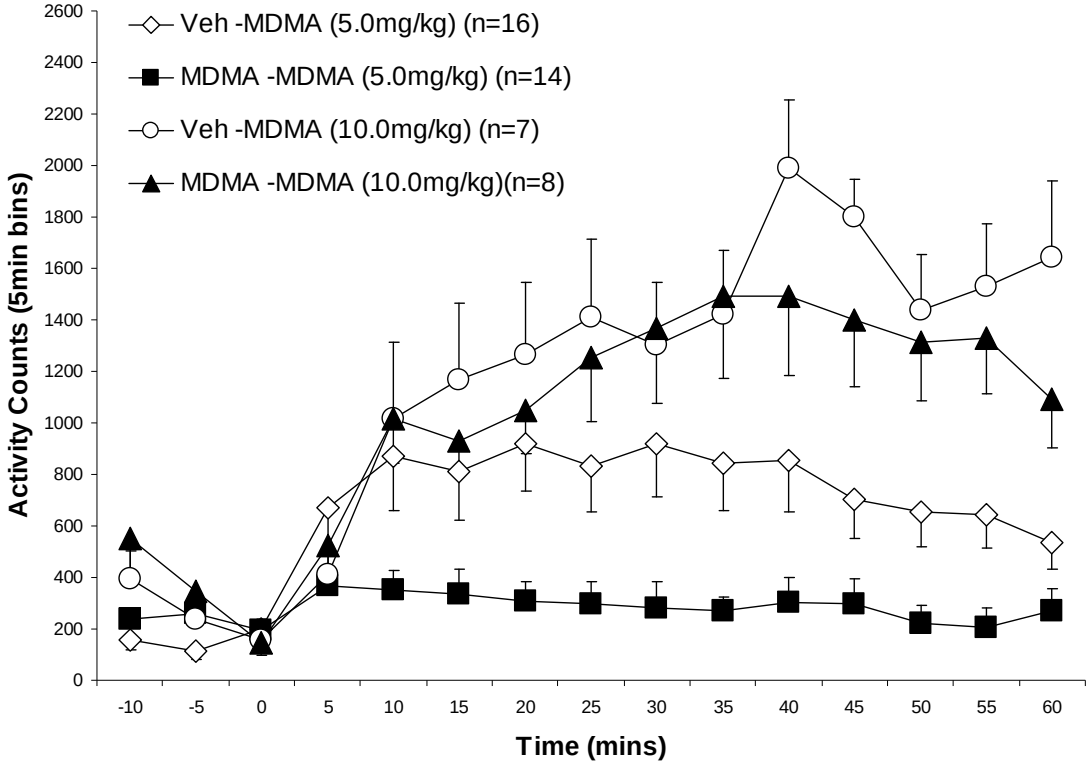

Figure 1.23. Locomotor activating effects of MDMA (5.0 or 10.0mg/kg i.p.) following a 28-day withdrawal from daily administration of MDMA (10.0mg/kg i.p.) or vehicle. The first listing in the legend identifies the group's pre-treatment drug while the second indicates the challenge drug. Sample sizes are in brackets beside the listings. Locomotor counts are summed into bins of 5 minute intervals with time '0' being the time of drug injection.

A repeated measures 3-way ANOVA *[pre-treatment (MDMA or vehicle) x Dose (5.0 & 10.0 MDMA) x time (12 five min bins)]* revealed a significant effect of pre-treatment ($F(1,41) = 4.97$, $p < 0.05$), in addition to a main effect of Dose ($F(1,41) = 25.53$, $p < 0.05$), but no interaction between pre-treatment and Dose ($F(1,41) = 1.04$, ns).

Figure 1.24 presents the total post-injection activity data collapsed across time following 28 days withdrawal for the MDMA and vehicle pre-treatment groups.

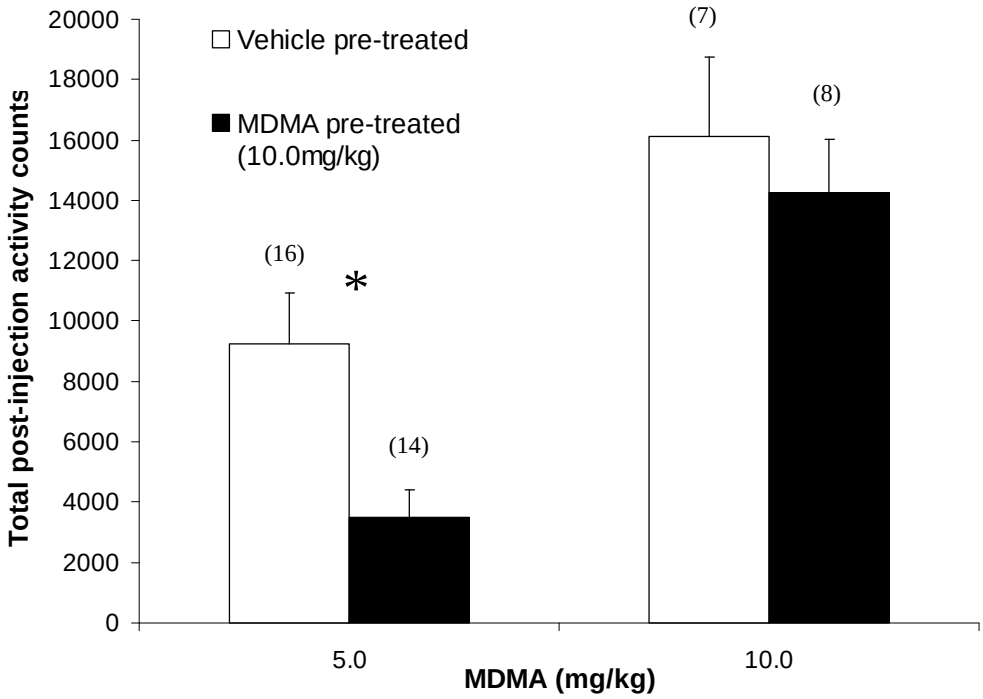

Figure 1.24. Total post injection locomotor counts on challenge day for the 60 minutes post injection with vehicle and MDMA (10.0 mg/kg i.p.) pre-treated rats. After 28 days of withdrawal rats were challenged with MDMA (5.0 or 10.0mg/kg i.p.). Numbers in brackets above each column are the sample sizes. * difference from vehicle pre-treated group

Analysis on total locomotor activity counts was conducted by using a 2-way ANOVA *(Pre-treatment x Dose)* revealing both a Dose ($F(1,41) = 4.97$, $p < 0.05$) and Pre-treatment ($F(1,41) = 4.97$, $p < 0.05$) main effect. Post hoc contrasts revealed an increase in the activating effect of the 5.0 mg/kg MDMA challenge dose ($p<0.05$) but no difference in the 10.0mg/kg MDMA challenge dose.

Experiment 1 Discussion

This experiment was designed to develop protocols for sensitisation to the locomotor activating effects of MDMA. Repeated intermittent administration of both amphetamine and MDMA produced sensitised hyperactivity. This sensitised response was apparent during the pre-treatment regimen and also following a 2 and 9-day withdrawal period for the MDMA pre-treated rats.

Amphetamine-induced locomotor activity increased markedly from day 1 to day 5. Following a 2-day withdrawal period, the response to a low dose of amphetamine was also enhanced. Additional tests were conducted to examine the persistence of the sensitised response however an amphetamine-produced sensitised response was no longer apparent following a 28 day withdrawal period. Although, 4 of the 7 amphetamine pre-treated animals had total post-injection locomotor counts approaching twice that of the average vehicle pre-treated group but variability was large and, as a group, it was not statistically reliable

This finding is in contrast to other studies that have demonstrated an increase in the sensitised response following an extended withdrawal period of 28 days (Hitzemann , Tseng, Hitzemann, Sampath-Khanna & Loh, 1977; Paulson & Robinson,1995, 1996). It is possible that a different pre-treatment regimen may have resulted in a more persistent sensitised response in the above studies. However, under the current

conditions, sensitisation to the locomotor activating effects of amphetamine was observed 3, but not 28 days, following exposure.

In contrast to the effects of repeated exposure to amphetamine, MDMA-produced hyperactivity following the low dose (5mg/kg) failed to increase between days 1 and 5 of exposure. This result concurs with other findings (Ball, Budreau, & Rebec, 2006). Higher dose exposures however, showed an increase in MDMA produced activity from days 1-5 of exposure.

Following exposure to repeated doses of 5.0 mg/kg MDMA, a sensitised response to the effects of the lower dose of 2.5 mg/kg was observed. Repeated exposure to the higher dose of 10.0mg/kg MDMA increased locomotor activity between days 1 and 5 and following two days of withdrawal a sensitised response was observed to two lower doses (2.5 and 5.0 mg/kg) of MDMA. Thus, the dose-effect curve for MDMA-produced hyperactivity was shifted leftwards following both pre-treatment regimens. Two other investigations have shown sensitisation following a similar MDMA pre-treatment regimen. In one, rats were treated for six consecutive days with 10.0 mg/kg MDMA, and tested with the same dose of MDMA following a 5-day withdrawal period (Modi, Yang, Swann, & Dafny, 2006). In the other, rats were treated for 5 days with a single dose of 10.0mg/kg per day followed by a 2 day withdrawal period (Colussi-Mas & Schenk, 2008). In both investigations 10.0 mg/kg pre-treatment produced a robust increase in MDMA-produced

hyperactivity across days during the pre-treatment period. The current investigation, in conjunction with the above data, suggests that a single, daily administration of the 10.0mg/kg pre-treatment dose compared to that of the 5mg/kg is the more effective pre-treatment dose during a 5-day exposure period to observe sensitisation.

The present results suggest that a short withdrawal period enhances the manifestation of MDMA- induced behavioural sensitisation. The majority of investigations into MDMA sensitisation have also imposed a relatively short withdrawal period ranging from 48 hours to 12 days (Kalivas, et al., 1998; McCreary, Bankson, & Cunningham, 1999; Ramos, et al., 2004; Spanos & Yamamoto, 1989). Consistent with these findings is the current data that show after 9 days withdrawal form repeated exposure to 10.0mg/kg MDMA there was a sensitised response.

Following longer withdrawal period of 28 days, however, sensitisation was no longer apparent. Instead, when compared to controls, the hyperactive response to 5.0 mg/kg MDMA - was decreased. This finding contrasts with a study that demonstrated sensitisation when testing was conducted following a 38 day withdrawal period (Modi, et al., 2006). The current results however, should be interpreted cautiously as the total locomotor activity counts in the saline control rats were extremely high. However, the locomotor counts for the MDMA pre-treated animals are comparable to the locomotor counts observed after 2 days (Figure 1.9) and 9 days (figure 1.22) withdrawal. One possible reason for the

increased response in vehicle rats is that during the 28 day withdrawal period rats were housed in pairs rather than singly as in the other groups. Housing environment is known to alter subsequent drug responses and animals housed in groups were more sensitive to amphetamine induced locomotor activity (Schaefer & Michael, 1991). If this is also true for MDMA produced hyperactivity the data suggest a differential influence of prior drug exposure.

Of interest, there was a greater response in locomotor activity following administration of vehicle to the MDMA pre-treated rats. This conditioned effect has also been demonstrated following repeated administration of amphetamine (Anagnostaras & Robinson, 1996; Drew & Glick, 1988; Mazurski & Beninger, 1987; Vezina, et al., 1989). Although not specifically tested for, the current results suggest a role of context in the expression of MDMA-induced locomotor sensitisation.

Summary experiment 1

A sensitisation protocol was developed to induce sensitised responding to MDMA. An augmented locomotor response was evident across days of pre-treatment (day 1 vs. 5) and also evident with withdrawal periods of up to 9 days after the last exposure. Repeated administrations of either 10.0 or 5.0 mg/kg for 5 days showed sensitised locomotor responding to a lower dose of the drug, however, the regimen of 5 daily treatments of 10.0mg/kg produces persistent sensitisation.

Experiment 2: Changes in sensitivity of the D_1 and D_2 receptor to repeated intermittent exposure of MDMA

Background

An important mechanism underlying amphetamine produced hyperactivity is an increase in extracellular levels of DA in cell bodies and terminal regions of the mesolimbic system. Repeated intermittent administration of amphetamine increases the dopamine response and this sensitised neurochemical response is believed to result in behavioural sensitisation. The expression of sensitisation is typically measured as an increase in drug produced hyperactivity that can be explored with pharmacological manipulation of dopamine release, through for example, receptor activation or suppression.

The role of the D_1 and D_2 receptors in amphetamine sensitisation has previously been investigated. Sensitisation was blocked by the D_1-like antagonist, SCH23390 (Drew & Glick, 1990; Vezina & Stewart, 1989; Vezina, 1996) and sensitised locomotor activation was observed in response to the selective D_1 dopamine receptor agonist, SKF81297, in amphetamine pre-treated rats (Chen et al., 2003). Intra VTA pre-exposure to the D_2 antagonist, eticlopride, blocked amphetamine produced locomotor activity (Tanabe, Suto, Creekmore, Steinmiller, Vezina, 2004) but effects of the mixed D_1/D_2 agonist, apomorphine, has been equivocal. Following amphetamine-produced sensitisation, sensitisation to apomorphine-produced

stereotypy was observed (Kuczenski & Segal, 1999) but this pre-treatment regimen failed to increase apomorphine-produced horizontal activity (Vanderschuren et al., 1999).

Blockade of dopamine D_1 like and D_2 –like receptors significantly attenuated MDMA-induced locomotor activity in rats (Ball et al., 2003; Daniela et al., 2004). A role of dopamine receptors in both the initiation and expression of sensitisation has been suggested (Ramos et al., 2004; 2005a; 2005b). Cross sensitisation to amphetamine has also been demonstrated suggesting common neural adaptations mediating behavioural sensitisation (Modi et al., 2006).

To date, the role of the D_1 and D_2 receptors in the expression of sensitisation to the behavioural effects of MDMA has not been comprehensively examined. The current investigation aims to identify potential changes in D_1 and D_2 receptor sensitivity as a result of repeated intermittent exposure to MDMA. It is hypothesised that the DA D_1 and D_2 receptors will be sensitised following a regimen of repeated intermittent MDMA administration. The response to the selective D_1-like agonist, SKF81297, and the D_1/D_2 agonist, apomorphine will be determined in MDMA sensitised rats. Additionally, the potency of selective antagonists to attenuate MDMA produced hyperactivity will also be measured.

Experiment 2a method

Experiment 2 follows the previously described general sensitisation methodology of five, single daily injections of MDMA (10.0mg/kg) followed by 2 days withdrawal.

The ranges of doses chosen for SCH23390 have been shown to attenuate MDMA produced hyperlocomotion (Daniela et al., 2004). The dose range of eticlopride was based on Ball et al. (2003) demonstration of the attenuation of MDMA produced hyperactivity. Doses of the D_1 selective agonist, SKF81297, were based on Reavill, Bond, Overend, & Hunter, (1993) and doses of apomorphine were chosen based on previous amphetamine sensitisation studies (Kuczenski & Segal, 1999; Vanderschuren, Beemster, & Schoffelmeer, 2003; Võikar, et al., 1999). The doses for the selective 5-HT_{2C} antagonist, RS102221, were based on Bonhaus, et al., (1997).

Experiment 2a Results.

Effects of SKF81297 in MDMA sensitised rats

Figure 2.1 shows the time course of the effects of SKF81297 in vehicle and MDMA pre-treated rats. A three-way ANOVA *[Pre-treatment (10.0 MDMA or vehicle) X Dose (0.0, 0.5, 1.0, 2.0, 4.0 and 8.0) X Time (12 five minute bins)]* was conducted. There was a main effect of Pre-treatment ($F(1,72) = 20.48$, $p < 0.05$), and of Dose ($F(5,72) = 16.36$, $p < 0.05$), but no interaction between Pre-treatment and Dose ($F(5,72) = 1.04$, ns).

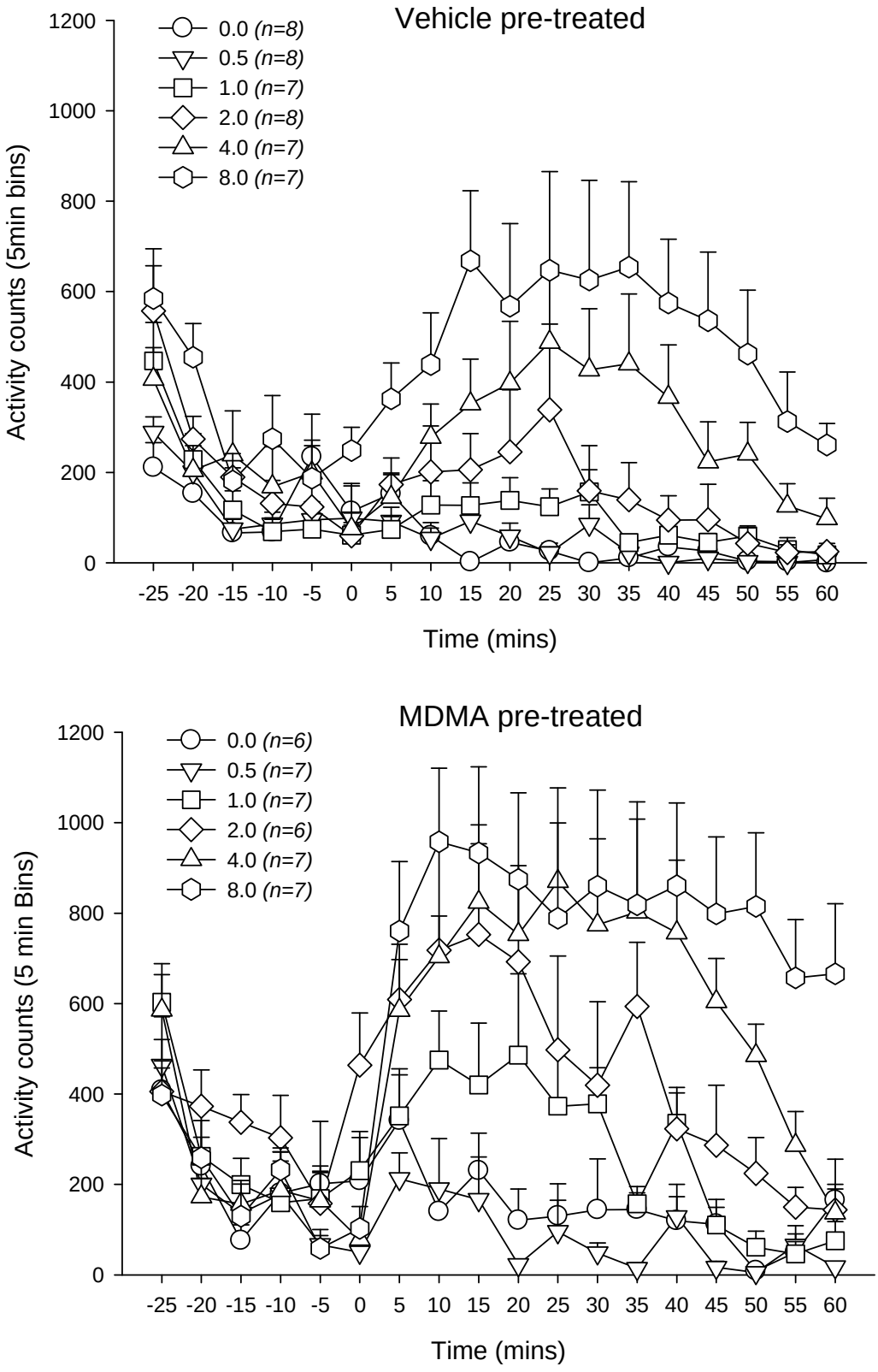

Figure 2.1. Locomotor activating effects of SKF81297 in MDMA and vehicle pre-treated rats. Symbols represent the mean (+SEM) number of activity counts. Locomotor counts are summed into bins of 5 minute intervals with time '0' being the time of drug injection.

Figure 2.2 below presents the above data as total locomotor counts following the injection of various doses of SKF81297. Analysis on total locomotor activity counts was conducted by using a 2-way ANOVA *(Pre-treatment x Dose)* with post hoc contrasts. Post hoc contrasts revealed an effect of pre-treatment for the 0.0 mg/kg groups ($F(1,12)= 14.64$, $p< 0.05$), the 1.0 mg/kg groups ($F(1,12)=14.0$, $p< 0.05$), the 2.0 mg/kg groups ($F(1,12)= 5.68$, $p< 0.05$) and the 4.0 mg/kg groups ($F(1,12)= 5.66$, $p< 0.05$).

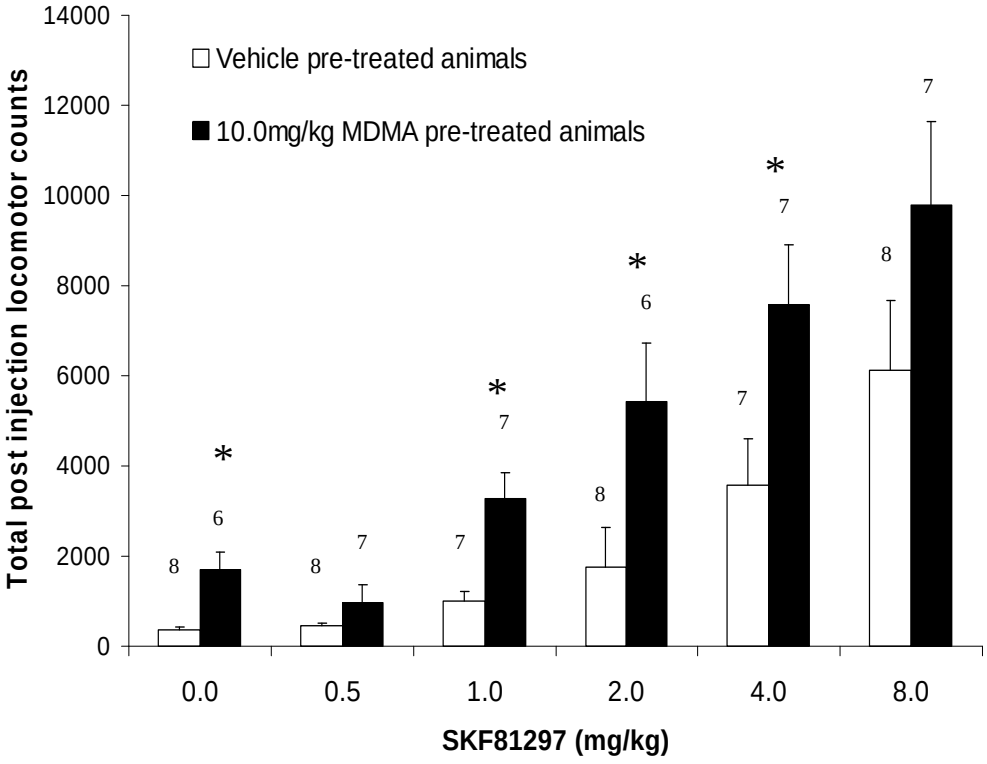

Figure 2.2. Mean total locomotor counts (+SEM) on challenge day during the 60 minute post injection period with for vehicle and MDMA (10.0 mg/kg i.p.) pre-treated rats. Numbers above each column represent the sample size used. * difference from vehicle pre-treated group

Effects of SCH23390 in MDMA sensitised rats

Following the sensitisation regimen of 5 daily injections of MDMA (0.0 or 10.0mg/kg) and 2-day withdrawal effects of the D_1–like antagonist, SCH23390, on the locomotor activating effect of 5.0 mg/kg MDMA was measured. Figure 2.3 shows locomotor activity as a function of time on test day (day 8). A three-way ANOVA *[Pre-treatment (10.0 MDMA or vehicle) X Dose (0.0, 0.01, 0.02, and 0.04) X Time (12 five minute bins)]* revealed a main effect of Pre-treatment ($F(1,54) = 13.40$, $p < 0.05$), and Dose ($F(3,54) = 4.74$, $p < 0.05$), but no interaction between Pre-treatment and Dose ($F(3,54) = 1.29$, ns).

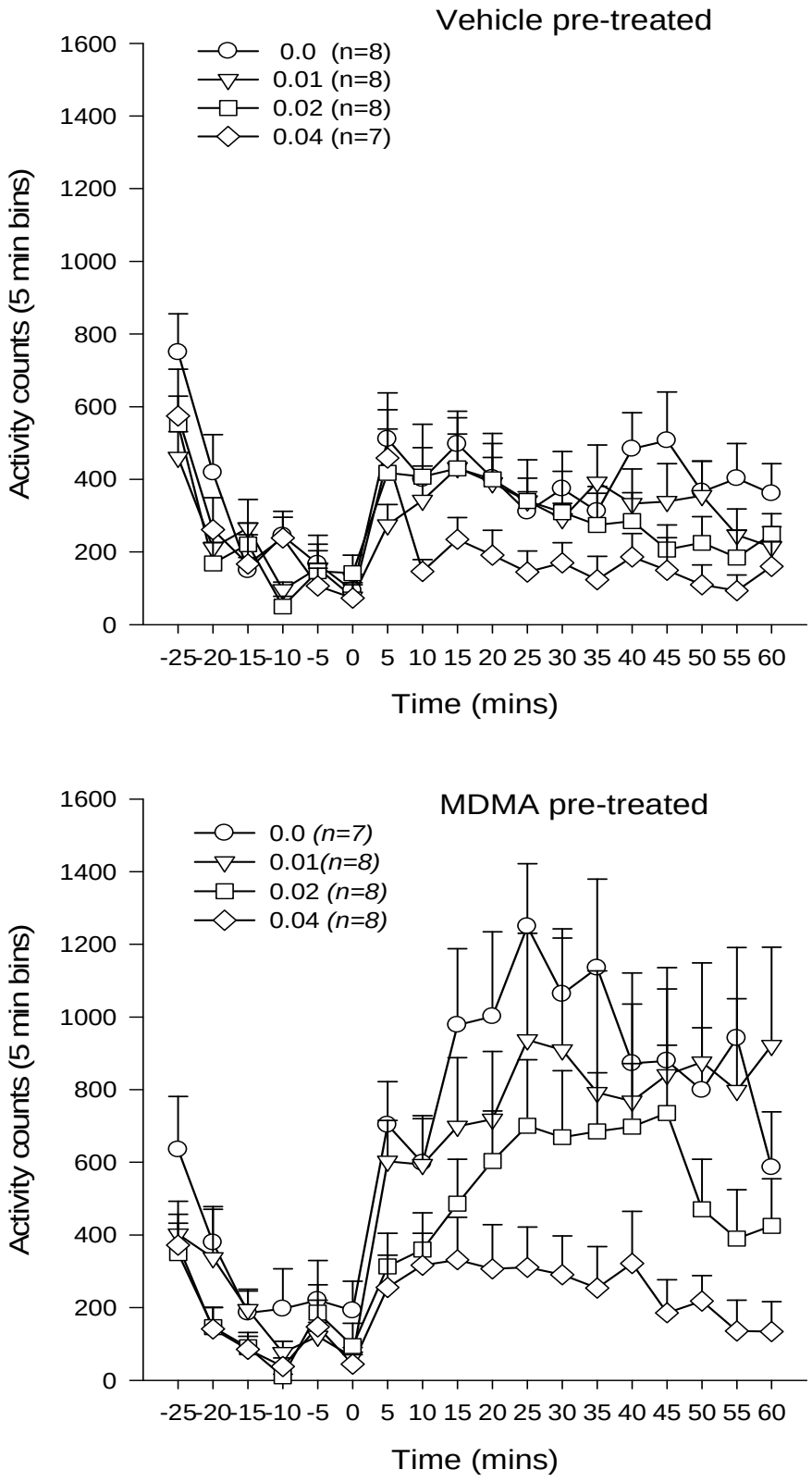

Figure 2.3. Locomotor activating effects of SCH23390 in MDMA and vehicle pre-treated rats. Symbols represent the mean (+SEM) number of activity counts. Locomotor counts are summed into bins of 5 minute intervals with time '0' being the time of drug injection.

Figure 2.4 shows the above data presented as total locomotor counts following the injection of MDMA. A one-way ANOVA on data from vehicle pre-treated animals failed to reveal a main effect of Dose ($F(3,27) = 1.79$, ns). There was however a main effect of dose in the MDMA pre-treated rats ($F(3,27) = 3.30$, $p<0.05$). In the MDMA pre-treated group, post hoc analysis showed the 0.04 SCH23390 dose significantly decreased MDMA produced hyperactivity ($F(1,13) = 13.80$, $p<0.05$).

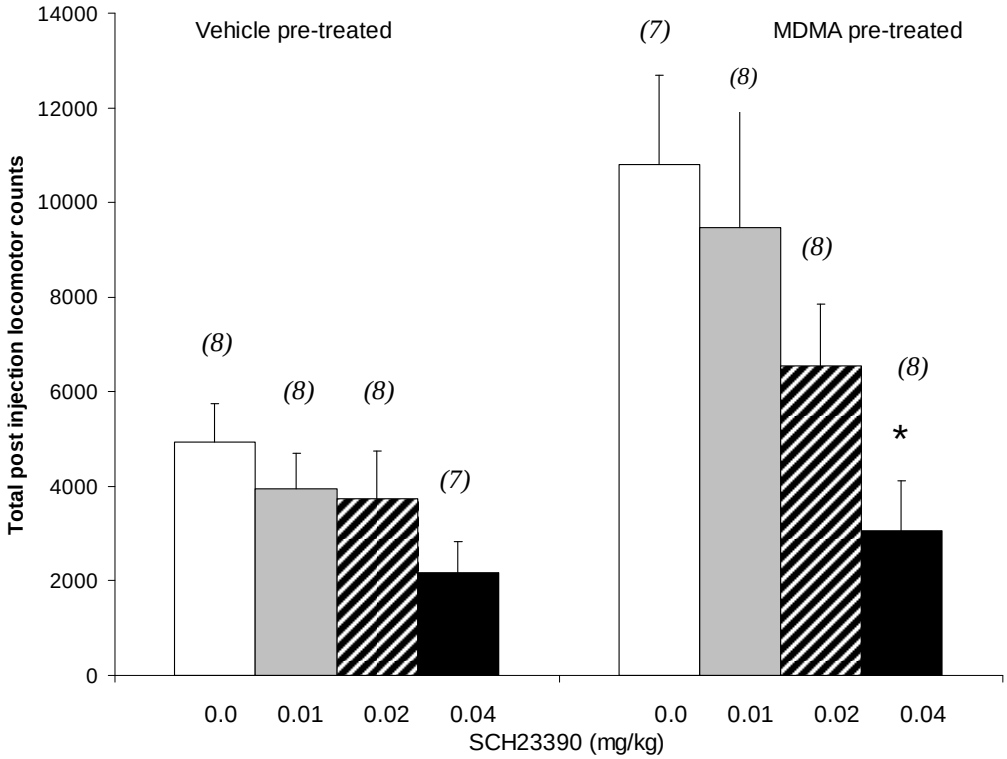

Figure 2.4. Effects of SCH 23390 on MDMA-(5.0 mg/kg) produced hyperactivity in vehicle and MDMA pre-treated rats. Symbols represent the mean number of activity counts (+SEM). Numbers in brackets above each column is the sample size. * difference from vehicle pre-treated group

Because there was a sensitised response to MDMA, the data from vehicle and MDMA pre-treated rats were rescored as a percentage change from 'baseline' responding in Figure 2.5 below. A two-way ANOVA *[Pre-*

treatment (10.0 MDMA or vehicle) X Dose (0.0, 0.01, 0.02, and 0.04)] failed to reveal a significant effect of Pre-treatment ($F(1,54) = 0.24$, ns) or a significant interaction between Pre-treatment and Dose ($F(3,54) = 0.22$, ns) but a significant main effect of Dose was obtained ($F(3,54) = 4.88$, $p<0.05$). Subsequent post hoc analysis with a one-way ANOVA on vehicle pre-treated rats revealed that the 0.04 dose significantly reduced MDMA-produced hyperactivity ($F(1,14)$ 6.50, $p <0.05$). In the MDMA pre-treated group the 0.04 dose of SCH 23390 also significantly decreased MDMA-produced hyperactivity ($F(1,14)$ 13.80, $p <0.05$).

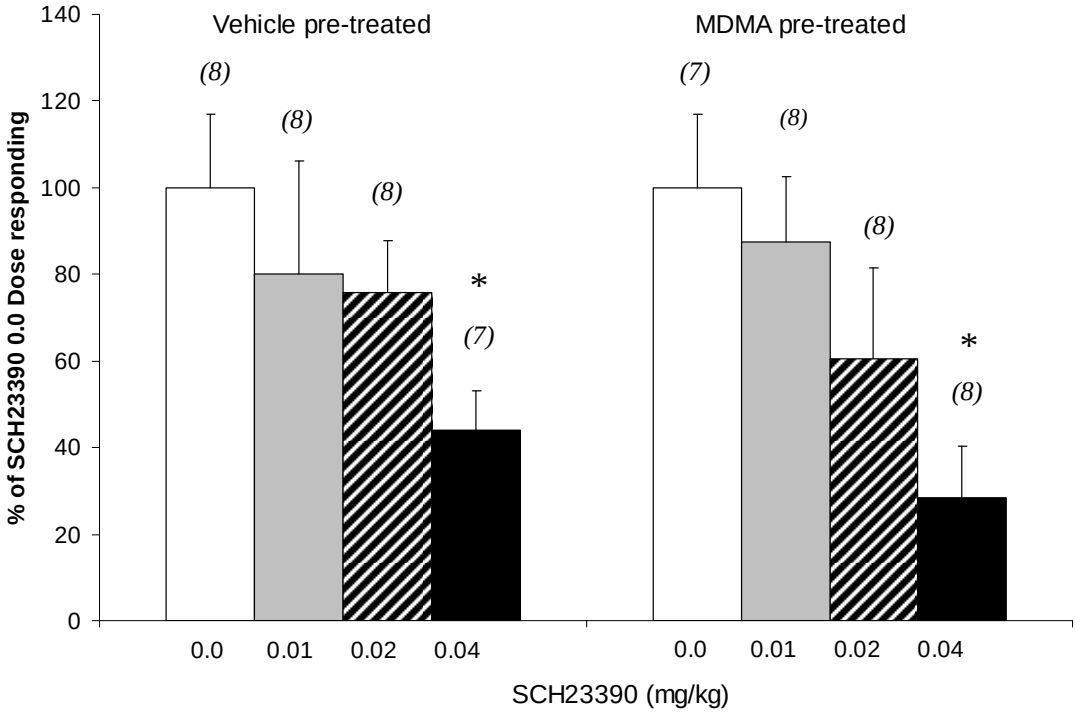

Figure 2.5. Effect of SCH 23390 on MDMA (5.0 mg/kg) produced hyperactivity in vehicle and MDMA-pre-treated rats. Data are expressed as mean percent change from vehicle (+SEM). * difference from vehicle pre-treated group.

Experiment 2b Results.

Effects of Apomorphine in MDMA sensitised rats

Figure 2.6 shows the effect of apomorphine in vehicle and MDMA pre-treated rats. A three-way ANOVA *[Pre-treatment (10.0 MDMA or vehicle) X Dose (0.0, 0.5, 1.0, 2.0 and 4.0) X Time (12 five minute bins)]* revealed a main effect of Pre-treatment ($F(1,72) = 20.93$, $p < 0.05$), and of Dose ($F(4,72) = 11.97$, $p < 0.05$). There was also a significant interaction between Pre-treatment and Dose ($F(4,72) = 3.76$, $p < 0.05$).

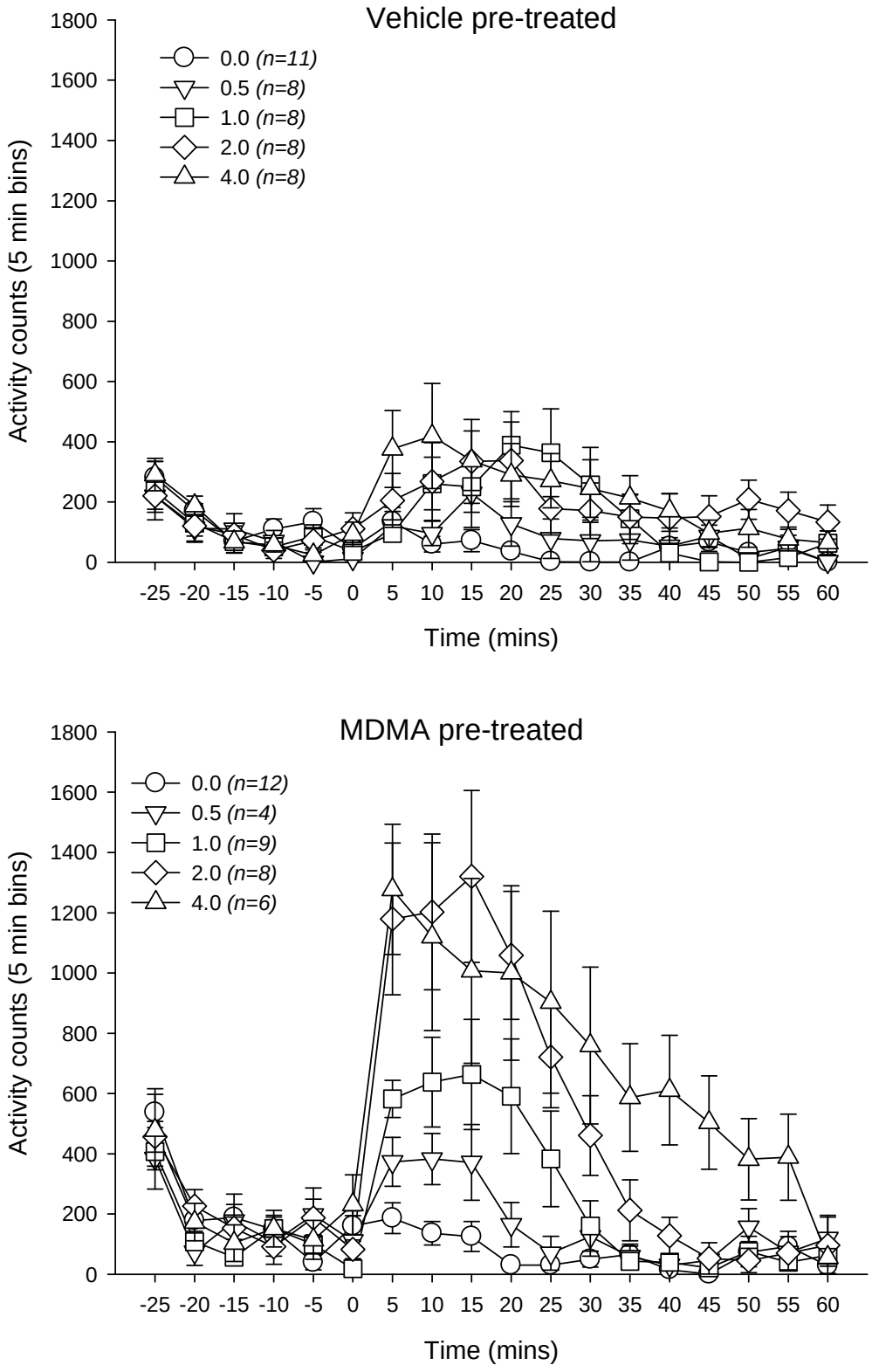

Figure 2.6. Locomotor activating effects of apomorphine in MDMA and vehicle pre-treated rats. Symbols represent the mean (+SEM) number of activity counts. Locomotor counts are summed into bins of 5 minute intervals with time '0' being the time of drug injection.

Figure 2.7 below presents the above data expressed as total post injection locomotor counts. Analysis on total locomotor activity counts was conducted by using a 2-way ANOVA *(Pre-treatment x Dose)* with post hoc contrasts. Post hoc results revealed that MDMA pre-treated rats were more responsive to the 2.0mg/kg ($F(1,14)= 6.76$, $p< 0.05$), and the 4.0 mg/kg ($F(1,12)= 6.97$, $p< 0.05$) doses.

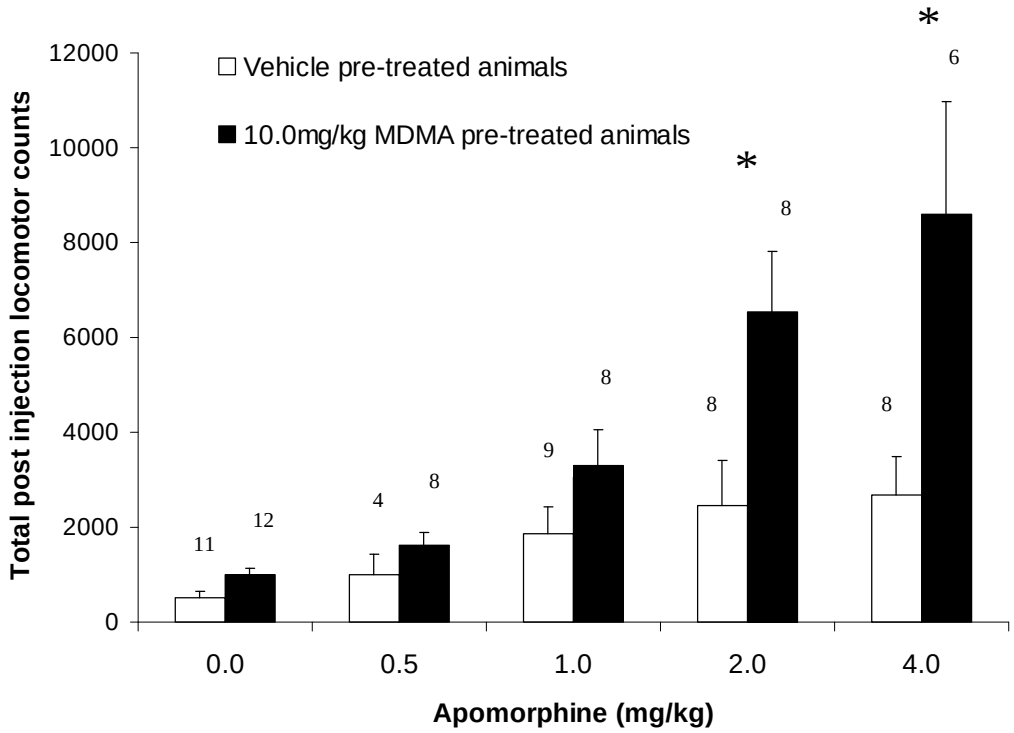

Figure 2.7. Mean total locomotor counts (+SEM) on challenge day during the 60 minute post injection period with for vehicle and MDMA (10.0 mg/kg i.p.) pre-treated rats. Numbers above each column represent the sample size used. * difference from vehicle pre-treated group

Effects of eticlopride in MDMA sensitised rats

Figure 2.8 shows the effect of eticlopride on MDMA-produced locomotor activity in the vehicle and MDMA pre-treated rats. A three-way ANOVA *[Pre-treatment (10.0 MDMA or vehicle) X Dose (0.0, 0.05, 0.1, and 0.2) X Time (12 five minute bins)]* was conducted. There was no main effect of Pre-treatment ($F(1,50) = 2.29$, ns), or interaction between Pre-treatment and Dose ($F(3,50) = 1.21$, ns) but a main effect of Dose ($F(3,50) = 11.58$, $p < 0.05$) was produced

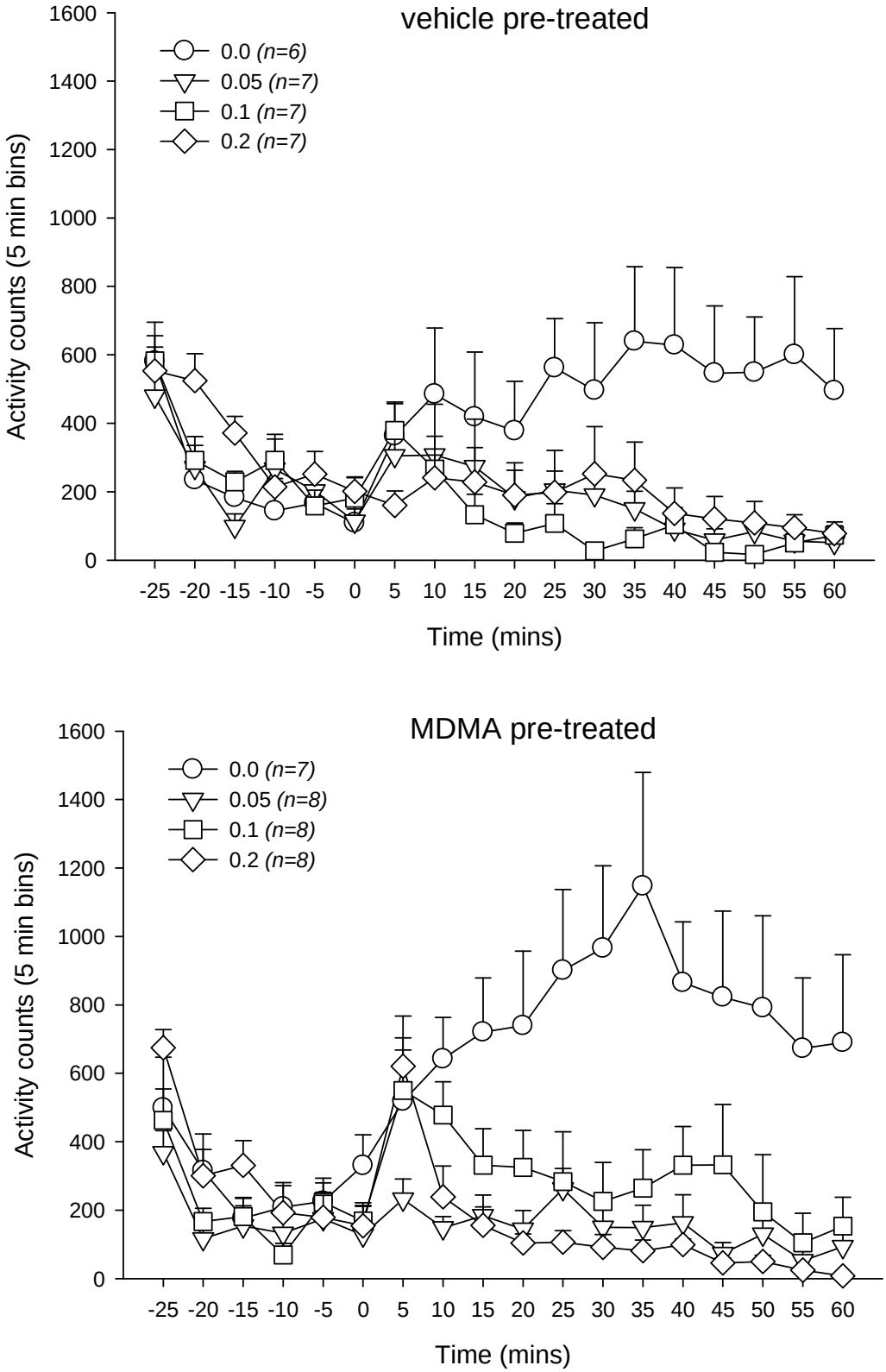

Figure 2.8. Effects of eticlopride on MDMA-(5.0 mg/kg) produced hyperactivity in vehicle and MDMA pre-treated rats. Symbols represent the mean number of activity counts (+SEM). Numbers in brackets above each column is the sample size. Locomotor counts are summed into bins of 5 minute intervals with time '0' being the time of drug injection.

Figure 2.9 shows the data presented as total post injection locomotor counts. There was a main effect of Dose for both the vehicle ($F(3,23) = 4.35$, $P<0.05$) and MDMA ($F(3,27) = 8.23$, $p<0.05$) pre-treated rats. A one-way ANOVA on the data from the vehicle and MDMA-pre-treated rats revealed that all doses of eticlopride reduced MDMA-produced hyperactivity ($p <0.05$).

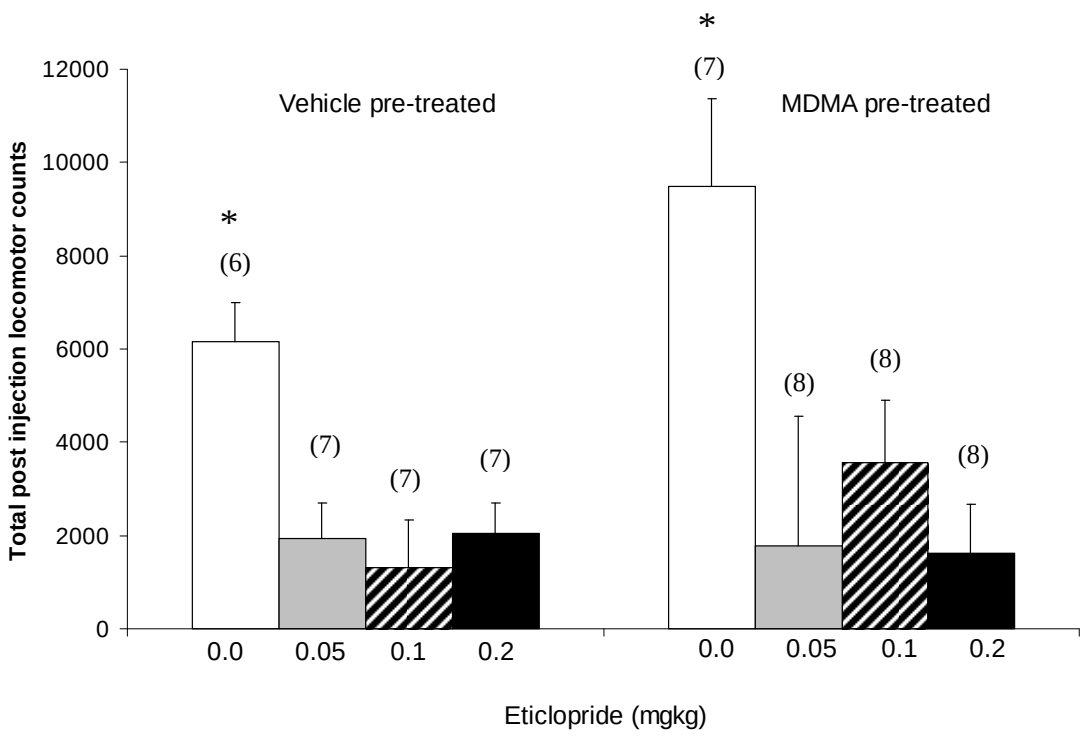

Figure 2.9. Effects of eticlopride on MDMA (5.0 mg/kg) produced hyperactivity in vehicle and MDMA pre-treated rats. Symbols represent the mean number of activity counts (+SEM). Numbers in brackets above each column is the sample size. * difference from vehicle pre-treated group.

Figure 2.10 presents the above data as a percentage change from baseline. A two-way ANOVA *[Pre-treatment (10.0 MDMA or vehicle) X Dose (0.0, 0.01, 0.02, and 0.04)]* was conducted. There was a significant effect of Dose ($F(3,50) = 11.48$, $p<0.05$) but the effect of Pre-treatment ($F(1,50) = 0.09$, ns) or the interaction between Pre-treatment and Dose ($F(3,50) = 0.51$, ns) were not significant.

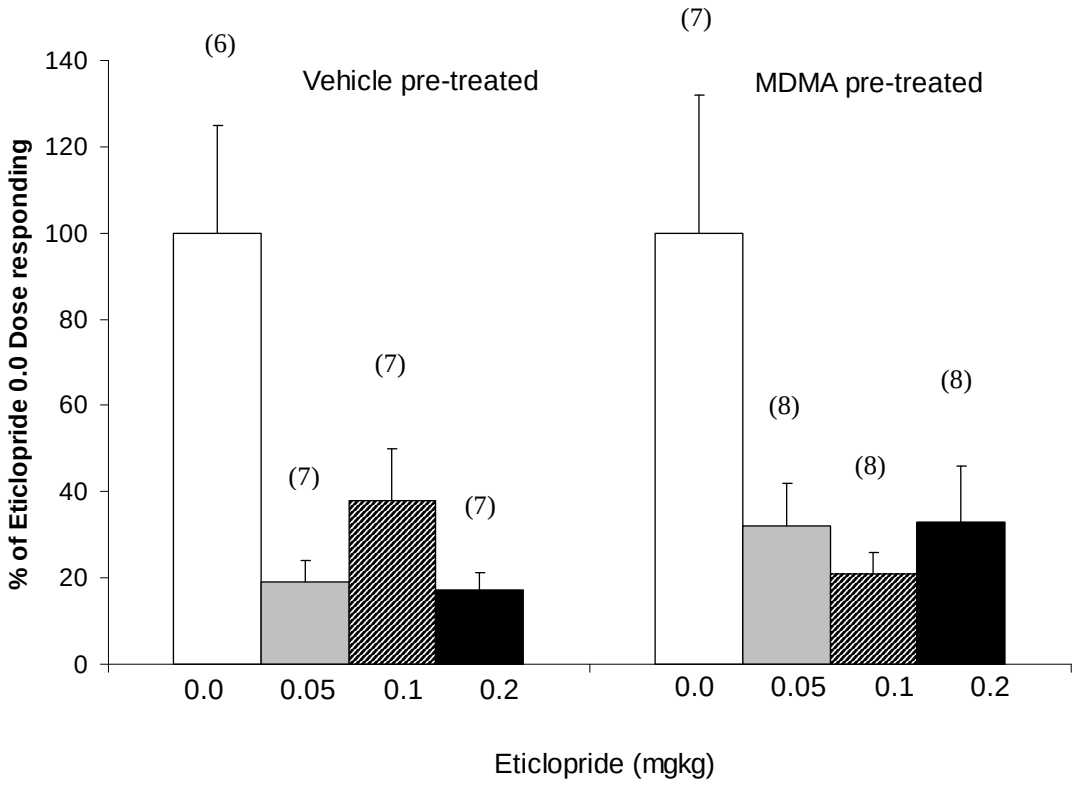

Figure 2.10. Effect of eticlopride on MDMA (5.0 mg/kg) produced hyperactivity in vehicle and MDMA-pre-treated rats. Data are expressed as mean percent change from vehicle (+SEM).

Effects of Lower Doses of Eticlopride in MDMA sensitised rats

In the previous groups, the lowest dose of eticlopride produced a large suppression of MDMA-produced activity in both groups. Therefore, several groups were subsequently tested with a lower dose range. Figure 2.11 shows the time course of MDMA (5.0 mg/kg) produced hyperactivity following this lower dose pre-treatment. A three-way ANOVA *[Pre-treatment (10.0 MDMA or vehicle) X Dose (0.03, 0.01, 0.003 and 0.0) X Time (12 five minute bins)]* was conducted. There was a significant main effect of Dose ($F(3,41) = 3.15$, $p<0.05$) but no significant main effect of Pre-treatment ($F(1,41) = 1.49$, ns) or a significant interaction between Pre-treatment and Dose ($F(3,41) = 2.06$, ns).

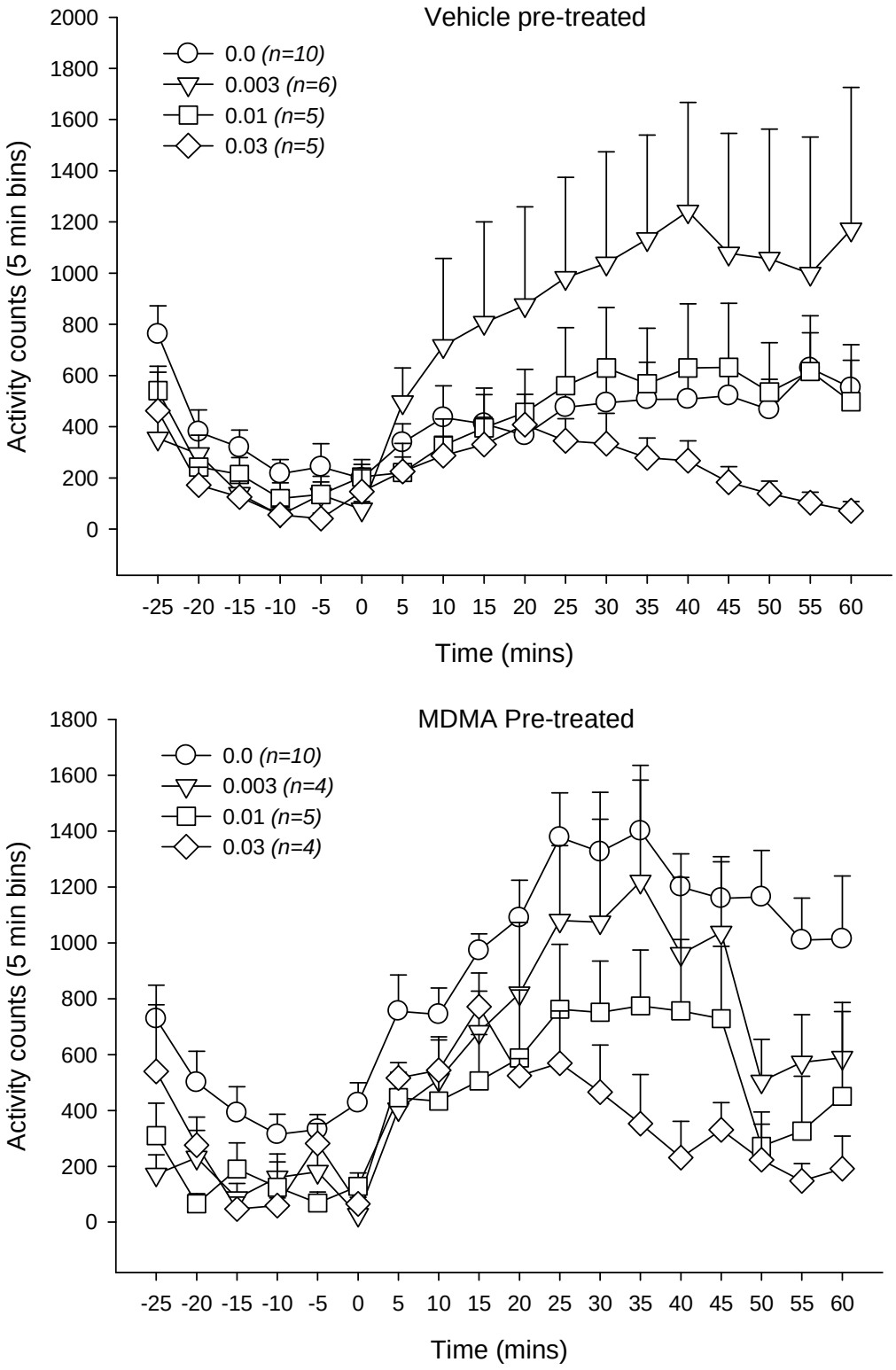

Figure 2.11. Effects of low doses of eticlopride on MDMA (5.0 mg/kg) produced hyperactivity in vehicle and MDMA pre-treated rats. Symbols represent the mean number of activity counts (+SEM).Numbers in brackets above each column is the sample size. Locomotor counts are summed into bins of 5 minute intervals with time '0' being the time of drug injection.

Figure 2.12 shows the data above presented as total locomotor counts following the injection of MDMA. A two way ANOVA revealed a significant main effect of Dose ($F(3,41) = 3.15$, $p<0.05$). A one-way ANOVA showed a significant main effect of Dose in the MDMA ($F(3,22) = 5.86$, $p<0.05$) pre-treated groups but not in the vehicle pre-treated groups ($F(3,22) = 1.78$, ns). A one-way ANOVA on MDMA pre-treated rats showed all but the 0.003 dose to be significantly reduced compared to that of the 0.0 dose ($p <0.05$).

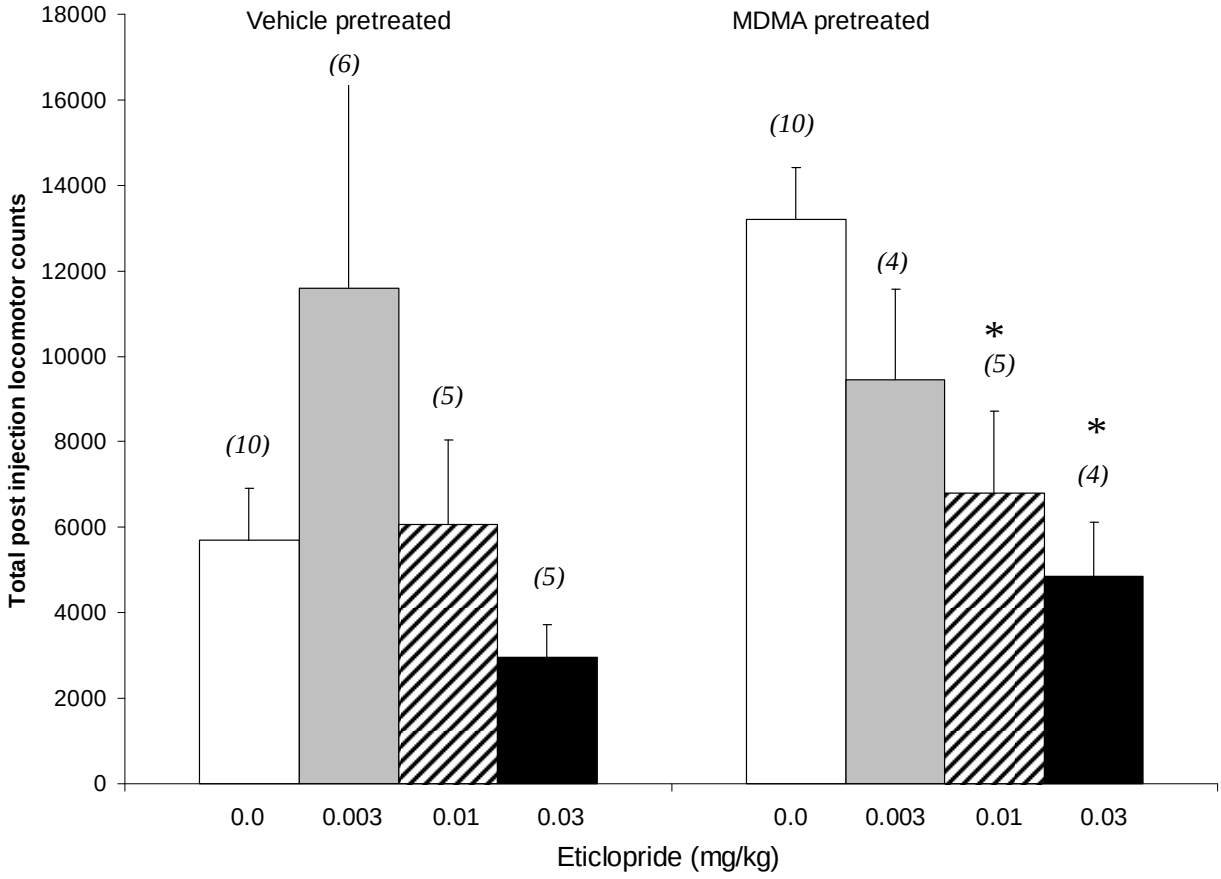

Figure 2.12. Effects of eticlopride on MDMA (5.0 mg/kg) produced hyperactivity in vehicle and MDMA pre-treated rats. Symbols represent the mean total number of activity counts (+SEM). Numbers in brackets above each column is the sample size.

Experiment 2c Results.

Effects of RS102221 in MDMA sensitised rats

Figure 2.13 shows the effect of RS102221 in vehicle and MDMA pre-treated rats. A three-way ANOVA *[Pre-treatment (10.0 MDMA or vehicle) X Dose (0.0, 0.25, 0.5, and 1.0) X Time (12 five minute bins)]* revealed a significant main effect of Pre-treatment ($F(1,50) = 54.26$, $p<0.05$) and a significant interaction between Pre-treatment and Dose ($F(3,50) = 3.99$, $p<0.05$) , but the effect of Dose was not significant ($F(3,50) = 1.06$ ns).

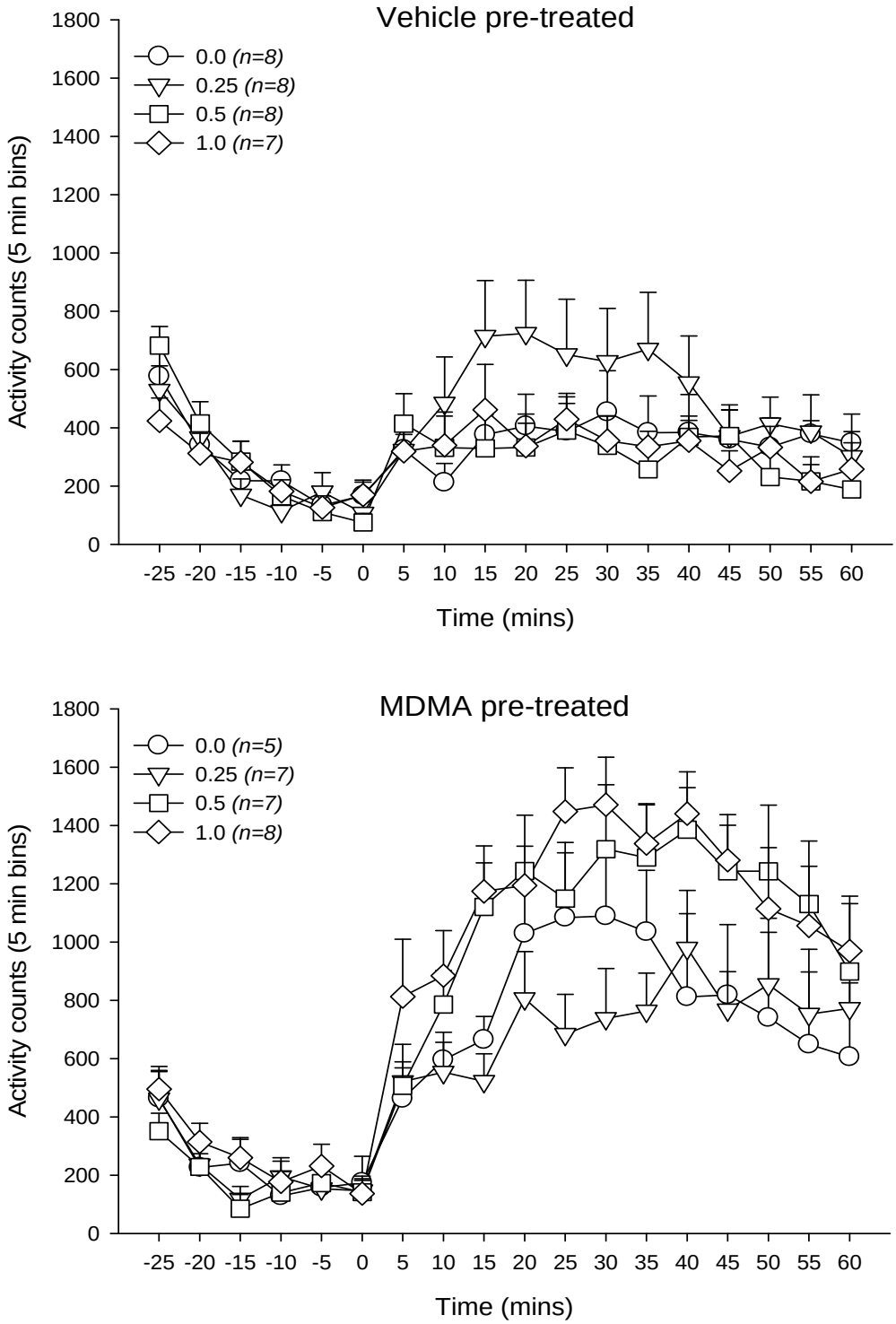

Figure 2.13. Effects of RS102221 on MDMA (5.0 mg/kg) produced hyperactivity in vehicle and MDMA pre-treated rats. Symbols represent the mean number of activity counts (+SEM). Numbers in brackets above each column is the sample size. Locomotor counts are summed into bins of 5 minute intervals with time '0' being the time of drug injection.

Figure 2.14 shows the data above presented as total number of locomotor counts following the injection of 5.0mg/kg MDMA. A one-way ANOVA on vehicle data showed no effect of Dose ($F(3,27) = 1.11$, ns) but a one-way ANOVA on the data from the MDMA pre-treated groups revealed a main effect ($F(3,23) = 3.10$, $p<0.05$). Post hoc analysis revealed that the doses of 0.5 and 1.0 mg/kg RS102220 increased activity relative to the 0.25 mg/kg dose ($p<0.05$).

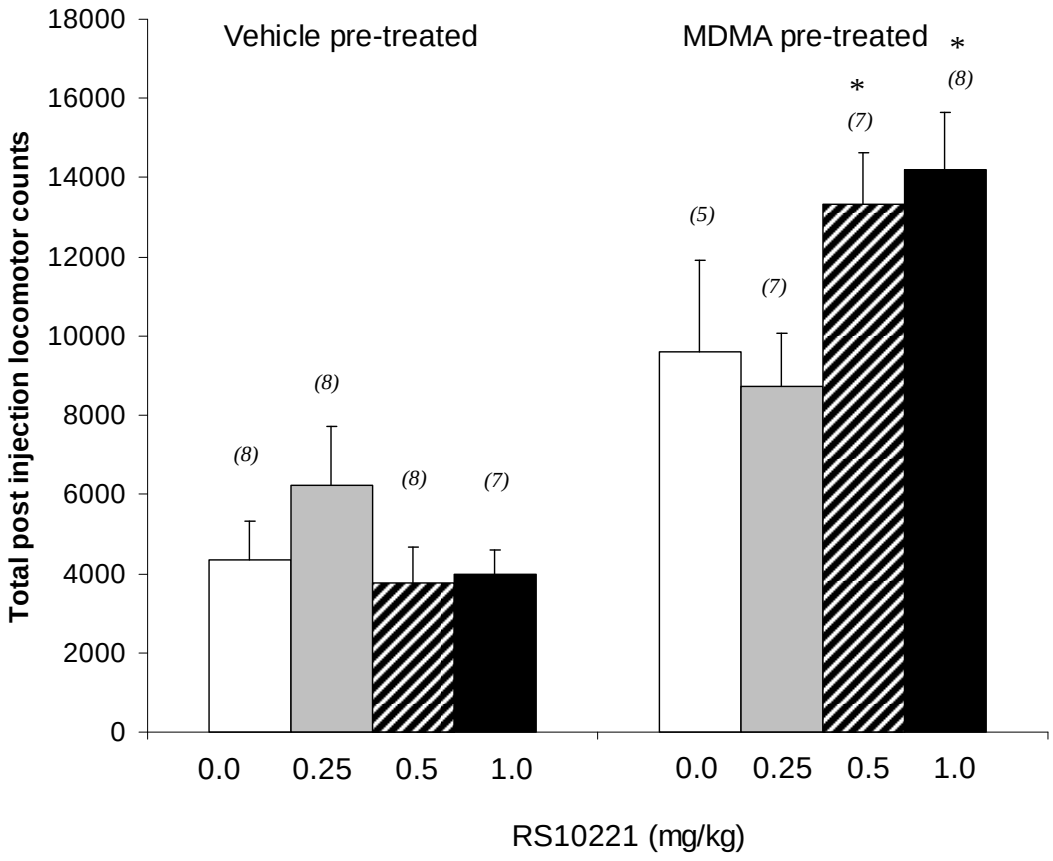

Figure 2.14. Effects of RS102221 on MDMA (5.0 mg/kg) produced hyperactivity in vehicle and MDMA pre-treated rats. Symbols represent the mean total number of activity counts (+SEM). Numbers in brackets above each column is the sample size. * difference from vehicle pre-treated group

Figure 2.15 presents the above data as a percentage change from 'baseline' (0.0 RS102221 dose) responding. A two-way ANOVA *Pre-treatment (10.0 MDMA or vehicle) X Dose (0.0, 0.25, 0.5 and 1.0mg/kg)]* showed no significant effect of Pre-treatment ($F(1,51) = 0.87$, ns), no main effect of Dose ($F(3,51) = 0.31$, ns) but an interaction between Pre-treatment and Dose ($F(3,51) = 2.99$, $p<0.05$). A one-way ANOVA showed a difference in MDMA pre-treated data ($F(3,26) = 3.10$, $p<0.05$) with the difference between the 0.25 and 1.0 dose $F(1,14) = 7.39$, $p<0.05$).

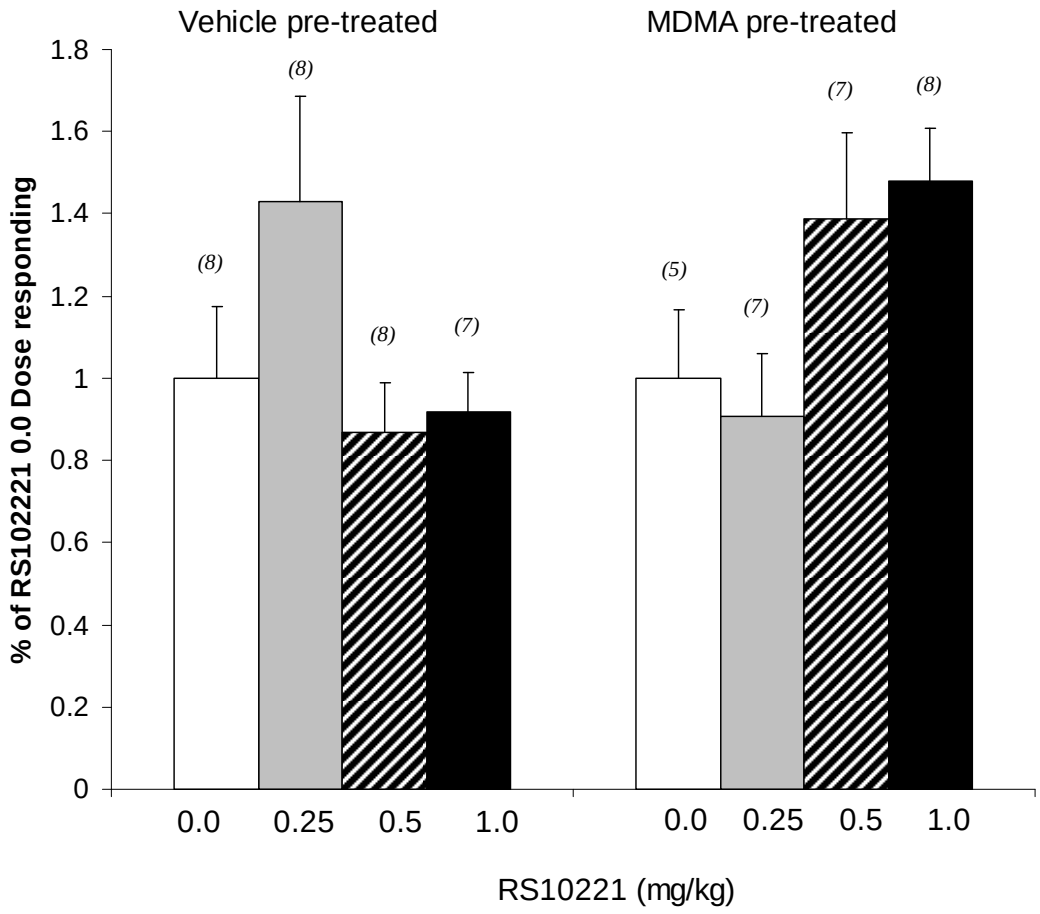

Figure 2.15. Effect of RS102221 on MDMA (5.0 mg/kg) produced hyperactivity in vehicle and MDMA pre-treated rats. Data are expressed as mean percent change from vehicle (+SEM).

Experiment 2 Discussion

In accordance with experiment 1, and previous literature, the present study demonstrated that pre-exposure to MDMA produces sensitisation to its locomotor producing effects. The locomotor activating effects of amphetamine are widely agreed to be mediated by DA (Kalivas & Stewart, 1991; Nestler, 1992; Robinson & Berridge, 1993: Segal, 1989) and the present investigation demonstrated hyperlocomotor producing effects of repeated MDMA might also reflect sensitisation in underlying dopaminergic substrates.

Following repeated exposure to MDMA the D_1 agonist, SKF81297, produced a greater locomotor response when compared to vehicle pre-treated animals. The leftward shift in the dose response curve suggests a sensitisation of the D_1 receptor. Repeated exposure to amphetamine also produced D_1 receptor sensitivity in sub-cortical structures such as the NAc (Henry & White, 1991) and VTA (Vezina, 1996). Further, pre-treatment with the D_1–like receptor antagonist, SCH 23390, blocked both the development and expression of amphetamine produced behavioural sensitisation (Vezina, 1996; Vezina and Stewart 1989). In contrast, the development of MDMA-produced sensitisation was unaffected by pre-treatment with SCH23390 while but SCH23390 dose dependently blocked the expression of sensitisation (Ramos et al., 2004).

In the current investigation, the administration of the D_1–like receptor antagonist, SCH23390, decreased MDMA produced locomotor activity to a comparable degree in both MDMA and vehicle pre-treated animals. This reduction in the response to MDMA supports the idea that the D_1 receptor plays a role in the expression of MDMA-produced hyperactivity, as has been previously suggested (Daniela et al., Ball et al., Ramos et al.,). The failure to observe an increased potency of SCH 23390 in the MDMA pre-treated rats is not consistent with the idea that the D_1 receptor became supersensitive as a result of pre-exposure.

One possibility is that the effects of SCH 23390 are due to activity at alternate receptor sites. Thus, although SCH23390 has been extensively used as a dopamine D_1 receptor antagonist (Bourne, 2001) it also binds with high affinity to 5-HT_{2C} receptors functioning as an antagonist (Millan, Newman-Tancredi, Quentric, & Cussac, 2001). Administration of 5-HT_{2C} receptor antagonists increased DA firing rates and 5-HT_{2C} receptor agonists decreased firing rates (Di Matteo et al. 2000; Gobert et al. 2000). Therefore, it is possible that these two effects of SCH 23390 counteracted each other. That is, the DA blocking effects would be expected to result in an antagonism of MDMA-produced hyperactivity whereas the effects on the 5HT2c receptor would be expected to enhance MDMA-produced hyperactivity. Indeed, the current results support this hypothesis as the selective 5-HT_{2C} antagonist, RS102221, potentiated MDMA-produced hyperactivity in the MDMA pre-treated rats.

The locomotor activating effects of the mixed D_1/D_2 agonist, apomorphine, were increased across a range of doses in MDMA sensitised animals. Apomorphine is non-selective but it has greater affinity for the D_2 receptor (Li, et al., 2006). The K_i for the D_2 receptor was 5 nanomolars (nM) and for the D_1 receptor it was 500 nM (Missale, Nash, Robinson, Jaber, & Caron, 1998). Apomorphine therefore is a preferential DA D_2 agonist.

Following amphetamine pre-treatment, there have been reports of either no change or down regulation of D_2 receptors (Muller and Seeman, 1979; Robinson & Becker, 1986) although DA D_2 receptor agonists have been shown to induce augmented behavioural responses in sensitised rats (Levy et al., 1988; Ujike et al., 1990). It has been suggested, however, that sensitisation is accompanied by an increased sensitivity of the high-affinity D_2 post synaptic receptors (Seeman, Tallerico, Ko, Tenn, & Kapur, 2002; Seeman, McCormick & Kapur, 2007). This might explain the increased apomorphine-produced hyperactivity observed in the present study.

A transient decrease in the sensitivity of D_2 auto receptors has also been reported following repeated amphetamine administration (Wolf et al., 1993). To ascertain whether this could explain the supersensitive response to apomorphine, effects of a low dose of the D_2 antagonist, eticlopride, which would have preferentially blocked D_2 autoreceptors (Salmi, Malmgren, Svensson, Ahlenius, 1998), were determined. Under these circumstances, an increase in MDMA -produced hyperactivity might have been expected. This was not observed, however, even when extremely low doses of eticlopride

were administered. These data are, therefore, not consistent with the idea that the autoreceptor became desensitised. Rather, the data suggest that MDMA pre-treatment resulted in an up-regulation of the postsynaptic D_2 mechanisms.

Sustained receptor activation by DA agonists may alter the D_2 coupling of the G-protein without alteration of receptor densities (Rudissaar, Harro, Pruus, Rinken, & Allikmets, 2008). Extracellular signals produce increases or decreases of second messengers such as cyclic adenosine monophosphate (cAMP) resulting in a number of biological responses (Gelowitz & Berger, 2001). For example, when cocaine is chronically administered there is increased adenylyl cyclase and cAMP dependent protein kinase in neurons in the NAc (Miserendino, & Nestler, 1995). Further, administration of cholera toxin in the NAc, activating adenylyl cyclase, enhances the acute locomotor activating effects of amphetamine (Cunningham & Kelley, 1993). The uncoupling of the G-protein had been reported following chronic administration of the D_2 agonist, apomorphine, without altering locomotor activity (Rudissaar, et al., 2008). However, supersensitivity of the D_2 receptors following unilateral lesions of the striatal system, enhanced G_i coupling and subsequent locomotor responses (Cai, Wang, & Friedman, 2002; Cai, Zhen, Uryu, & Friedman, 2000). These data suggest that in neurons expressing D_2 receptors an enhanced G_i coupling may be produced following amphetamine sensitisation however, the exact mechanisms of action are, as yet, still unclear (Rudissaar et al., 2008; Schwendt, & McGinty, 2007; Traynor & Neubig, 2005). It is seemingly likely however,

that during the MDMA sensitising regimen changes in coupling to the adenylyl cyclase inhibiting D_2 G-protein occurred. This may explain the differential locomotor producing effects of the D_2 agonist and antagonist.

Experiment 2 summary

The current results are consistent with the hypothesis that locomotor sensitisation to MDMA is mediated by DA receptor mechanisms. There was cross-sensitisation between the effects of MDMA and both the D_1-like agonist, SKF81297, and the preferential DA D_2-like agonist, apomorphine. These effects were not, however, reflected in sensitised responses to the antagonists. Whereas the failure for the response to SCH 23390 to be increased in MDMA sensitised rats might be due to non selective effects, the failure to observe differential effects of the D_2-like antagonist, eticlopride, suggests that there is an alteration in coupling of the D_2 receptor mechanisms under conditions of D_2 blockade.

Experiment 3: Changes in potency of reinforcement of MDMA as measured in the self-administration paradigm following repeated intermittent exposure to MDMA

Background

There is now strong evidence supporting the relationship between drug induced changes in mesolimbic dopamine and the rewarding effects and abuse liability of drugs as modelled by self administration. For example, neurotoxic lesions (Roberts, Koob, Klonoff, & Fibiger, 1980) or administration of D_1-like and D_2-like receptors antagonists (Caine & Koob 1994; Pierre & Vezina, 1998; Woolverton & Virus, 1989) produced a rightward shift in the dose effect curve. Conversely, administration of direct or indirect (Schenk et al, 2003; Spealman, Barrett-Larimore, Rowlett, Platt, & Khroyan, 1999) dopamine agonists produced a leftward shift (Caine & Koob, 1994) supporting the idea that dopaminergic mechanisms underlie the reinforcing effects of drugs of abuse.

The magnitude of the initial reinforcing effects of drugs was inversely related to drug dose. Thus, self-administration was acquired more rapidly when higher doses were available (Schenk et al, 1993; Carroll & Lac, 1997). Following systemic pre-treatment with amphetamine or cocaine, latency to acquisition of self-administration was reduced, suggesting an enhancement of the initial reinforcing effects (Horger et al., 1990; 1992; Schenk & Partridge, 2000; Vezina et al., 1999). Pre-treatment with drugs other than the self-

administered drug has also been shown to decrease the latency to acquisition of self-administration (Schenk et al, 1993; Schenk & Izenwasser, 2002). These findings suggest that pre-exposure to psychostimulants may increase their reinforcing effects and may produce neuroadaptations that are common and might underlie the abuse liability of some drugs. Given the role of dopamine in self administration it further suggests that these neuroadaptations are within the dopamine system.

Latency to acquisition of self-administration of other drugs of abuse was inversely related to drug dose; higher doses led to more rapid acquisition (Schenk et al., 1991, 1993; Schenk & Partridge, 2000). In the case of MDMA, only one report has provided data on the relationship between acquisition rate and available dose for self-administration. In that study (Schenk et al., 2007), two doses of MDMA (0.25 and 1.0 mg/kg/infusion) were available. Latency to acquisition did not differ as a function of dose and, in both cases, latency to acquire self-administration was more variable and longer than the latency to acquire cocaine (0.5 mg/kg/infusion) self-administration.

Pre-exposure to MDMA facilitated cocaine self-administration (Fletcher, Robinson, & Slippoy, 2001), suggesting a sensitised response but, to my knowledge, there have not been studies that have examined the effects of prior exposure to MDMA on latency to acquisition of MDMA self-administration.

Experiment two demonstrated an augmented locomotor response to both D_1 and D_2 agonists following repeated MDMA administration. It is therefore hypothesised that a sensitised dopamine response would facilitate the acquisition of self-administration. In the current experiment two doses of MDMA were chosen for this acquisition study (0.5 and 1.0 mg/kg/infusion). Both doses were based on previous research showing acquisition of MDMA within approximately 12 days (Schenk, et al., 2003; 2007).

Materials and methods

[1]Subjects

Subjects were male Sprague-Dawley rats bred in the vivarium at Victoria University of Wellington. They were initially housed in hanging polycarbonate cages in groups of four to six per cage, but once they reached weights of 250–275 g, they were individually housed. The humidity (74%) and temperature (21C) controlled animal colony was maintained on a 12:12-h light/dark cycle with lights on at 0700 hours. Food and water were freely available except during the short duration (2 hour) self-administration tests described below.

Surgery

Rats were implanted with a silastic catheter in the right jugular vein. The rats were deeply anesthetized with ketamine (60.0 mg/kg, IP) and pentobarbital

[1] The Materials and methods sub-sections named ‘subjects’, ‘surgery’ and ‘apparatus’ have previously been described in Schenk S, Gittings D, Johnstone M, Daniela, E. (2003).

(20.0 mg/kg, IP), the external jugular vein was isolated, a catheter inserted and the distal end (22 ga stainless steel tubing) was passed subcutaneously to an exposed portion of the skull, where it was fixed to embedded jeweller's screws with dental acrylic. Each day, the catheters were infused with 0.1 ml of a sterile saline solution containing heparin (30.0 IU/ ml) and ampicillin (250,000 IU/ml) to prevent infection and the formation of clots. The rats were allowed 5 days post-surgery for recovery prior to behavioural testing.

Apparatus

Self-administration training and testing were conducted in test chambers (Med Associates, ENV 001) enclosed in sound attenuating closets. The testing room containing the 31 test chambers was humidity (55%) and temperature (21ºC) controlled. Each chamber was equipped with two levers and a stimulus light. Depression of one lever (the active lever) resulted in an infusion of drug. Depression of the other lever (the inactive lever) was without programmed consequence. Infusions were in a volume of 0.1 ml delivered over 12.0 sec via Razel pumps equipped with 1.0 rpm motors and 20.0 ml syringes.

Procedure

Rats received a pre-treatment consisting of MDMA (0.0 *(n = 17)* or 10.0mg/kg *(n = 18)*), i.p) as per the 5 day sensitising regimen described in the general procedure section of Experiment 1. This was followed by a two

day withdrawal period. Catheters were implanted the following day and animals were allowed to recover for a further 5 days. Therefore, self administration testing began 9 days following the pre-treatment phase. This withdrawal period was chosen because a sensitised response to MDMA was produced 9 days following repeated MDMA administration in Experiment 1b.

Self-administration tests were conducted during daily 2 hour sessions. Every session began with an experimenter delivered infusion of MDMA. Thereafter, each depression of the active lever (FR1 reinforcement schedule) resulted in an automatic infusion of MDMA [0.5 or 1.0 mg/kg/infusion] paired with the illumination of a stimulus light located directly above the active lever.

Testing continued for 14 days or until the number of active lever responses was greater than 10, and a preference for the active lever was demonstrated, as per Daniela et al., (2006) and Schenk et al., (2007). The number of days required to meet this criterion was determined for each rat.

Results

Figure 3.1 shows the cumulative percentage of rats in each pre-treatment group that acquired MDMA self-administration as a function of days and MDMA dose. For all rats, regardless of pre-treatment and subsequent self-administration dose, a total of 46% reached the self-administration criterion.

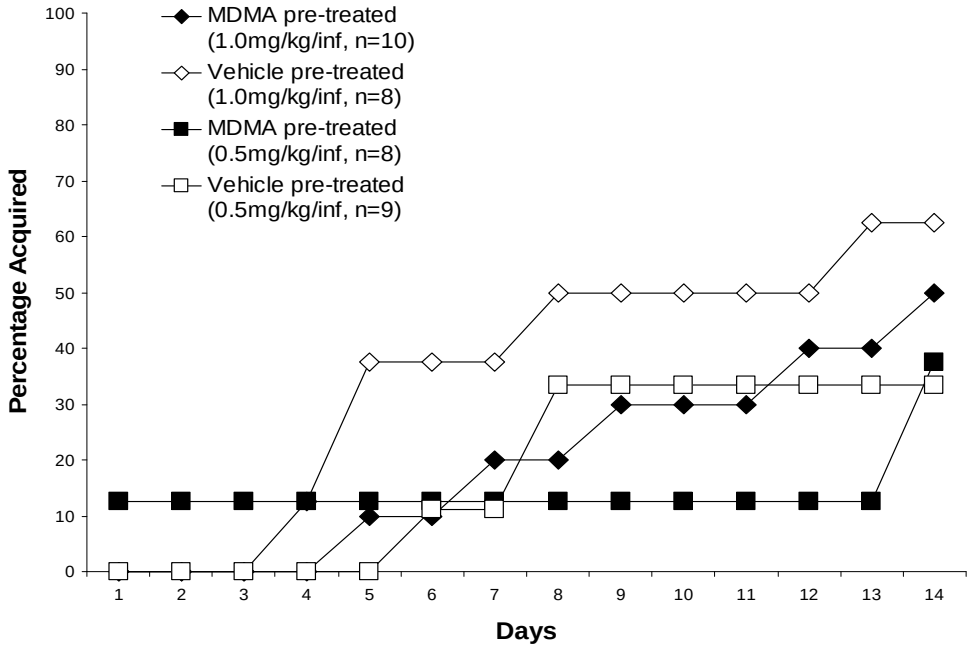

Figure 3.1. Cumulative percentage of rats that acquired MDMA self-administration as a function of days of testing, pre-treatment condition and MDMA dose.

Of the vehicle pre-treated group that self-administered 1.0mg/kg/infusion nearly 40% met the criteria for acquisition by day 5 and in this group the highest percentage of rats that acquired self-administration during the 14 day test period. There was an increase in the latency to acquisition for the vehicle pre-treated group that self-administered the lower dose of 0.5 mg/kg/infusion MDMA. The MDMA pre-treated rats were slower to acquire self-administration and a lower percentage met the criteria within the test period when compared to their vehicle pre-treated counterparts.

Rats pre-exposed to 10.0mg/kg MDMA for five days and tested with 1.0 mg/kg/infusion MDMA had an average daily intake of 9.80mg/kg. Rats pre-exposed to vehicle for five days and tested 1.0 mg/kg/infusion MDMA had an average daily intake of 8.51mg/kg. There was no difference in average

daily intake between MDMA and vehicle pre-exposed groups in the 1.0mg/kg/inf condition [t(6) =1.45, p= 0.196].

Rats pre-exposed to 10.0 MDMA for five days and tested with 0.5 mg/kg/infusion MDMA had an average daily intake of 3.59mg/kg. Rats pre-exposed to vehicle for five days and tested 0.5 mg/kg/infusion MDMA had an average daily intake of 6.90mg/kg. There was a significant difference in average daily intake between MDMA and vehicle pre-exposed groups in the 0.5mg/kg/infusion condition [t(4) =-3.396, p= 0.027] with vehicle animals having a higher daily average intake.

Figure two shows the range of responses on the active and inactive lever for all rats that failed to reach criterion. The failure for these rats to acquire was related to the criterion of 10 responses rather than lack of active lever preference.

Table 2.

		Range of responses	
Pre-treatment	MDMA (mg/kg/infusion)	Inactive lever	Active lever
Vehicle *(n=6)*	0.5	0.3 – 3.1	1.6- 3.6
Vehicle *(n=3)*	1.0	0.0 - 5.0	0.6 - 5.3
MDMA *(n=5)*	0.5	0.2 - 3.6	1.4 – 4.6
MDMA *(n=5)*	1.0	0 - 3.2	0.8 – 4.6

Table 2. Range of responses on the active and inactive lever for rats that failed to reach criterion.

Discussion

The present study was designed to determine whether pre-exposure to MDMA, under conditions that produced sensitisation to the locomotor activating effects of MDMA, decreased the latency to acquisition of MDMA self-administration. Somewhat surprisingly, pre-treatment with MDMA did not decrease latency to acquisition. Indeed, in comparison to vehicle pre-treated animals there was an increased latency to acquisition for the MDMA pre-treated rats.

An inverse relationship between drug dose and latency to acquisition of self-administration of other drugs of abuse has been demonstrated (Schenk et al., 1991, 1993; Schenk & Partridge, 2000). Consistent with these findings latency to acquisition of MDMA self-administration was shorter for the group that self-administered the higher dose and a higher percentage of these rats met the criterion for acquisition within the temporal parameters of this experiment.

In contrast to results of other studies, however, MDMA pre-treatment failed to decrease the latency to acquisition of self-administration. One possibility is that the doses of MDMA tested were too high to allow reliable decreases to be observed. Indeed, pre-exposure to amphetamine decreased the latency to acquisition of self-administration of low doses (Piazza, et al., 1989; Pierre & Vezina, 1997) but failed to alter acquisition of self-administration when

higher doses were available (Lorrain, Arnold, & Vezina, 2000; Mendrek, Blaha, & Phillips, 1998).

Another possibility is that pre-treatment with MDMA as in the present study sensitised the rats to the aversive effects of MDMA and hence delayed the acquisition of self administration. Indeed, there have been several reports of MDMA-produced aversion. A conditioned taste aversion was produced by MDMA (Lin, Atrens, Christie, Jackson, & McGregor, 1993). MDMA also increased the latency to emerge from a darkened hide box (McGregor, et al., 2003) and reduced activity on the elevated plus maze (Bull, Hutson, & Fone, 2004), suggesting an anxiogenic effect (Navarro & Maldonado 2002). These initial aversive properties of MDMA might explain the gradual acquisition of self-administration in vehicle and also in MDMA-pre-treated rats.

A final possibility for the failure of MDMA pre-treatment to enhance the reinforcing effects of MDMA relates to pharmacological effects of repeated exposure. It has been suggested that reinforcing efficacy of drugs is related to the relative effects on DA and 5HT neurotransmission. More specifically, it has been suggested that increased serotonergic effects are associated with decreased potency as a reinforcer. This idea is based on a number of empirical findings. Firstly, administration of the 5-HT precursor tryptophan, which increases brain 5-HT synthesis, decreased self-administration of cocaine (McGregor, Lacosta, & Roberts, 1993) and amphetamine (Smith, Yu, Smith, Leccese, & Lyness, 1986). Secondly, pre-treatment with 5-HT reuptake inhibitors reduced cocaine self-administration (Carroll, Lac,

Asencio, & Kragh, 1990). Thirdly, the motivation to self-administer amphetamine analogues with greater 5-HT potency, but equipotent DA potency, was reduced as measured by progressive ratio responding (Wee, et al., 2005).

A number of studies have shown that MDMA increased 5-HT levels (Kalivas et al., 1998) and that chronic or long term exposure compromised 5-HT neurotransmission (Battaglia, Yeh, & De Souza, 1988). These pretreatment regimens, however, were more stringent than those used in this study. Therefore the effects of the current regimen of MDMA on tissue levels of DA, 5-HT and their major metabolites were measured in Experiment 4.

Experiment 4: Alterations of brain amine levels following repeated intermittent administration of MDMA

Background

Amphetamine administration increased DA levels and synaptic dopamine overflow throughout various neural substrates such as the striatum (Kolta, et al., 1985), prefrontal cortex (Ichikawa, Chung, Li, Dai, & Meltzer, 2002),VTA (Wolf, et al., 1993), NAc (Kalivas & Stewart, 1991; Robinson & Berridge, 1993) and all DA terminal fields (Vezina, 2004). Because local application of amphetamine into the VTA, but not into the NAc, produced a sensitised locomotor response (Cador, et al., 1995; Kalivas & Weber, 1988),

it has been suggested that neuroadaptations initiated by the acute increase in synaptic DA in the VTA mediate sensitised hyperactivity (Vezina, 2004).

The relationship between augmented DA release and the observation of locomotor sensitisation has been equivocal. In some studies, concordance between sensitised behavioural and neurochemical responses has been reported. For example, 7 days after repeated amphetamine administration there were increases in amphetamine-produced DA in the NAc of rats which coincided with sensitised hyperlocomotion (Scholl, Feng, Watt, Renner, & Forster, 2009). Other studies, however, have failed to replicate these effects. In one, sensitised hyperlocomotion was observed 7 days after withdrawal but there were no differences in amphetamine induced dopamine overflow in nucleus accumbens or striatal tissue (Weinstein, Narayanan, Byrnes, Uretsky, & Wallace, 1997). In another, sensitised locomotor activity observed 2 days following withdrawal that was not associated with an augmented DA response; a sensitised DA response was not observed until 10 days later (Kuczenski, Segal, & Todd, 1997; Wolf, et al., 1993). Finally, after repeated high doses of amphetamine, amphetamine-produced stereotypical behaviour was observed but there were no changes in amphetamine-produced increases in NAc DA levels (Segal, & Kuczenski 1999). These data suggest that, under certain regimens of administration, the behavioural and neurochemical indices of sensitisation to amphetamine may be dissociated.

Following acute administration, MDMA increased extracellular synaptic 5-HT and produced a moderate increase in dopamine levels (Schmidt, 1987).

Following chronic or high dose administration, however, MDMA reduced brain levels of 5-HT, tryptophan hydroxylase and the 5-HT metabolite, 5-HIAA, (see Capela et al., 2009 for a review). Under some conditions, repeated administration of MDMA also produced behavioural and neurochemical sensitisation (Bubar, et al. 2004; Kalivas, et al, 1998; Yamamoto & Spanos, 1988). Behavioural sensitisation was accompanied by a moderate decrease in ventral striatal 5-HT (Ludwig, Mihov, & Schwarting, 2007).

In order to determine whether sensitisation produced in the present investigations was accompanied by changes in overall levels of 5-HT or DA, rats were pre-treated with 10.0 mg/kg MDMA in a manner that induced sensitisation and sacrificed two days after their final drug administration. Using HPLC tissue levels of DA, its metabolite HVA, 5-HT and its metabolite 5-HIAA were measured. It is hypothesised that, in accordance with previous literature, there will be a reduction in 5-HT levels of brain neurotransmitter along with its metabolite 5-HIAA. It is not predicted that there will be any change in DA or HVA.

General Procedure

Rats were pre-treated once daily with MDMA (0.0, 10.0 mg/kg) or, as a comparison, amphetamine (2.0 mg/kg I.P) for five days in as described in the general sensitisation protocols, experiment 1. Following two days withdrawal

rats were rendered unconscious with CO_2 in an air tight chamber, decapitated and the brains rapidly removed.

Brain Dissection

Whole brains were placed in a stainless steel block for dissection into 1.0 mm coronal slices (Heffner, Hartman, & Seiden, 1980). Slices were placed onto an inverted petri dish chilled by ice. The dorsal striatum, NAc, frontal cortex and amygdala and were dissected, placed in vials, weighed and stored at -80º C until analysed.

HPLC analysis

Tissue samples were homogenised in 0.1 N perchloric acid and centrifuged at 10 000g for 30 min at 4º C. The supernatant was filtered and injected onto a high-performance liquid chromatography system (Agilent 1100 series) with electrochemical detection. The injection volume was 10 µL for the striatum and 20 µl for the other regions. 5-HT, 5-HIAA, DA and HVA were separated using a C18 reversed phase column (150 × 4.6 mm, 5 µm particle size; Eclipse XDB-C18, Agilent, USA). The mobile phase consisted of NaH2PO4 (75 mM), octane-1-sulphonic acid (1.7 mM), EDTA (0.25 mM), triethylamine (100 µL/L) and methanol (10%), and was adjusted to pH 3 with phosphoric acid. The flow rate was 1 ml/min. Detection was performed using a coulometric detector (Coulochem III, ESA, USA). The guard cell potential was set at 450 mV and the analytical cell potential at 400 mV.

Chromatograms were acquired with ChemStation software. Concentrations are expressed as ng per mg of tissue.

Peak areas corresponding to the DA, 5-HT, HVA and 5-HIAA were measured and concentrations were determined from the regression curve obtained with external standards. Working external standards (500 – 15.125 ng/ml in 0.1N perchloric acid) were prepared daily from 1 mg/ml stock solutions and kept at -80°C.

Results

Figure 4.1 below shows a chromatogram of 5-HT, 5-HIAA, DA and HVA standards separated using a C18 reversed phase column.

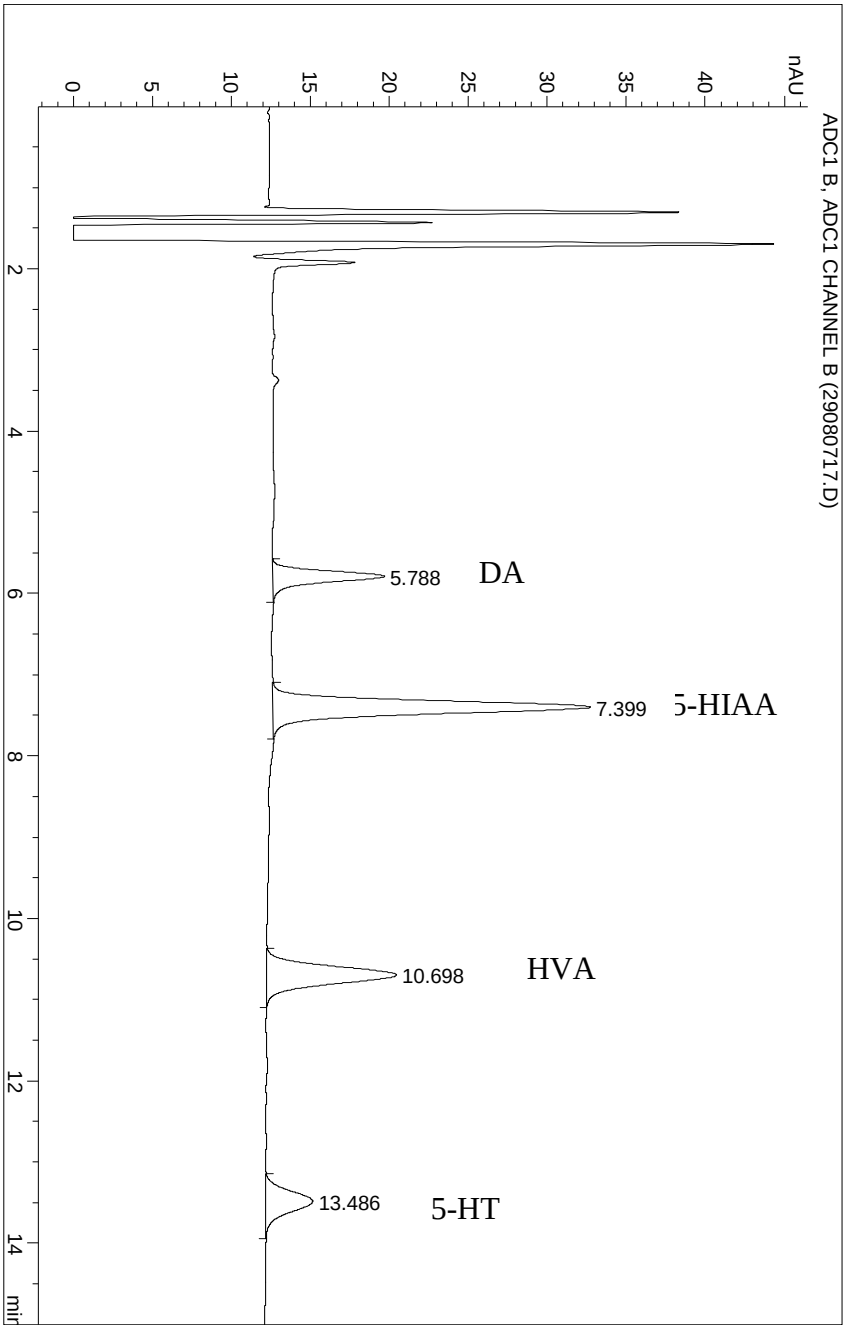

Figure 4.1. Chromatogram of amine standards injected onto a C18 reversed phase column

Table 4.1 presents results of the analyses of each of five brain regions from control rats and those that had undergone a sensitising regimen of MDMA (10.0 mg/kg I.P) or amphetamine (2.0mg/kg. I.P). Amphetamine and MDMA were compared to vehicle groups with a Bonferonni correction applied.

Table 4.1. Neurochemical concentrations in tissue of rats that received either vehicle, MDMA (10.0 mg/kg I.P) or amphetamine (2.0mg/kg. I.P) during a 5-day pre-treatment regimen. ** = difference from vehicle pre-treated group ($p < 0.01$), n=6 except for the 'Amygdala MDMA' group where n= 5.

Structure		*Concentration ng/mg tissue*			
		DA	5-HIAA	HVA	5-HT
Striatum					
	Vehicle	31.14 ± 3.69	0.55 ± 0.03	1.65 ± 0.14	0.79 ± 0.11
	Amph	30.26 ± 2.93	0.52 ± 0.04	1.44 ± 0.08	0.79 ± 0.11
	MDMA	30.76 ± 3.05	0.46 ± 0.03	1.48 ± 0.13	0.74 ± 0.08
Nucleus Acc					
	Vehicle	12.08 ± 2.68	0.79 ± 0.04	1.37 ± 0.11	0.87 ± 0.13
	Amph	13.07 ± 2.53	0.68 ± 0.06	1.10 ± 0.10	0.83 ± 0.06
	MDMA	13.91 ± 3.49	0.63 ± 0.04	1.09 ± 0.11	0.86 ± 0.14
Frontal Cortex					
	Vehicle	0.20 ± 0.04	0.54 ± 0.03	0.08 ± 0.01	0.98 ± 0.14
	Amph	0.20 ± 0.01	0.43 ± 0.03**	0.08 ± 0.01	0.94 ± 0.08
	MDMA	0.22 ± 0.03	0.38 ± 0.02**	0.07 ± 0.01	0.92 ± 0.16
Amygdala					
	Vehicle	0.88 ± 0.16	0.62 ± 0.06	0.11 ± 0.01	1.12 ± 0.14
	Amph	0.88 ± 0.16	0.52 ± 0.07	0.07 ± 0.01	1.18 ± 0.18
	MDMA	0.86 ± 0.09	0.42 ± 0.05	0.07 ± 0.01	0.94 ± 0.17
Hippocampus					
	Vehicle	0.07 ± 0.01	0.54 ± 0.02	0.005 ± 0.003	0.68 ± 0.09
	Amph	0.06 ± 0.00	0.44 ± 0.02**	0.000 ± 0.000	0.75 ± 0.07
	MDMA	0.07 ± 0.01	0.35 ± 0.02**	0.003 ± 0.002	0.75 ± 0.07

Discussion

A large number of studies have reported neurochemical effects of MDMA pre-treatment. In most studies, the MDMA pre-treatment regimen was substantive with animals exposed to doses of 20-40 mg/kg/day. These exposures typically resulted in behavioural tolerance rather than sensitisation to the behavioural effects of MDMA. Tolerance was accompanied by substantial decreases in both 5-HT and 5-HIAA (Marston et al., 1999; McNamara et al. 1995). The regimen in the current study was less extreme and produced a different profile of behaviour; i.e. sensitisation rather than tolerance. Differences in the neurochemical consequences were also observed in the present study.

There were no significant effects of the daily exposures on 5-HT although there was a significant decrease in the primary metabolite, 5-HIAA, in two of the 5 sites measured. This finding is consistent with the decrease in 5-HIAA in the frontal cortex previously reported (Ludwig, Mihov, & Schwarting, 2008). Comparable effects were produced by repeated exposure to a sensitising regimen of amphetamine.

A number of studies have also failed to observe changes in basal DA and 5-HT levels, synthesis or metabolism following repeated exposure to amphetamine that resulted in sensitisation (Bonhomme, Cador, Stinus, Le Moal, & Spampinato, 1995; Paulson, et al., 1991). Other studies, however, have suggested that following more stringent exposure regimens alterations

in tissue levels were produced. For example, a reduction in levels of 5-HT and 5-HIAA in the striatum was observed 24 hrs after the last dose of a daily administration regimen of 5.0 mg/kg amphetamine (McMillen, Scott, & Williams, 1991). Additionally, chronic amphetamine administration in cats (twice daily increasing doses of 5-15mg/kg amphetamine for ten days) reduced levels of 5-HT and 5-HIAA up to50% in the cortex, hippocampus, striatum, brain stem and spinal chord when measured 9 days but not 14 days post withdrawal (Trulson & Jacobs, 1979).

In the current results it is noticeable that the 5-HT metabolite 5-HIAA was reduced while levels of the parent molecule remained unchanged. Release and reuptake of 5-HT is the main source of extracellular 5-HIAA. Once released, the SERT moves the 5-HT molecules back into the presynaptic cell where it is bound into storage vesicles or deaminated to 5-HIAA. This metabolite is not stored but is passed back through to the extracellular area (Stenfors & Ross, 2004). Without 5-HT release there is a reduction in 5-HIAA levels. A reduction in release could explain why there is no difference in absolute tissue levels of 5-HT but, at the same time, there is a reduction in 5-HIAA levels. If there was a reduction in firing rates of serotonin neurons, differences in levels of 5-HIAA would be seen in all 5-HT terminal regions. Although only effects of MDMA or amphetamine were significant in two regions, for all regions there was a trend for reduced 5-HIAA.

5-HT release is regulated by the 5-HT_{1A} and 5-HT_{1B} receptors with selective antagonists blocking both the feedback mechanism and increasing

extracellular 5-HT while reducing absolute 5-HIAA levels (Hjorth, et al., 2000). *(Note that this is an absolute decrease in 5-HIAA and as there is 1000 times more extracellular 5-HIAA than 5-HT there remains a larger proportional amount of 5-HIAA).* However, there is a differential role of the 5-HT_{1A} and the 5-HT_{1B} receptor in mediating changes in 5-HIAA levels as a function of 5-HT. Administration of the 5-HT_{1A} receptor antagonist, robalzotan, directly into the frontal cortex, increased citalopram-produced extracellular 5-HT and decreased 5-HIAA levels (Hjorth, 1998). Administration of the 5-HT_{1B} receptor antagonist, GR127935, also increased citalopram induced increases in extracellular 5-HT levels, but no change in 5-HIAA levels was produced. Although antagonism of both the 5-HT_{1A} and the 5-HT_{1B} autoreceptors increased 5-HT levels, only antagonism of the 5-HT_{1B} disrupted the normal inverse relationship between 5-HT and5-HIAA. Stenfors and Ross (2004) interpreted this as due to stimulation of 5-HT_{1B} autoreceptors by the elevated synaptic 5-HT concentration resulting in decreased 5-HT release. Applied to the current results it may suggest that intermittent MDMA administration produces an altered 5-HT_{1B} auto receptor state, reducing 5-HT release (but not overall storage levels) and subsequent decrease of 5-HIAA levels.

Experiment 4 Summary

In previous studies, pre-exposure to amphetamine or MDMA under conditions that produced sensitised behavioural responses did not alter tissue levels of either DA or 5-HT (Bonhomme, et al. 1995; Paulson, et al., 1991).

The current results are consistent with this except that decreased levels of the serotonin metabolite 5-HIAA was produced in the frontal cortex and hippocampus. The reduction in 5-HIAA levels may be due to a disruption to the 5-HT_{1B} autoreceptor. Further evidence for this could be obtained through a microdialysis assay of terminal regions of serotonin cells which would be hypothesised to show a reduction in extracellular levels of 5-HT. Electrophysiology studies would be required to confirm a reduction in tonic firing of 5-HT cells.

General Discussion

The aim of the current thesis was to determine (1) some of the parameters for induction and expression of locomotor sensitisation following repeated MDMA exposure (2) Changes in sensitivity of the dopamine receptor mechanisms in sensitisation, and (3) if sensitisation was linked to reductions in the potency of the reinforcing effects of MDMA as measured by self administration and (4) what, if any, long term alterations in brain tissue levels of amines resulted from repeated exposure to MDMA. A group of four experiments were used to evaluate the above questions and results can be briefly summarised in the following manner.

1) A single dose of 10.0mg/kg MDMA administered daily for five days produces reliable behavioural sensitisation.
2) Following the sensitisation regimen, there is cross sensitisation to D_1 and D_2 agonists. However, even though a dose dependent

reduction in MDMA produced responding is attenuated by D_1 and D_2 antagonists, the potency of these antagonists is not altered by MDMA pre-treatment. This suggests that there are underlying mechanisms other than just receptor hypersensitivity responsible for the augmented locomotor activating effect of MDMA following a sensitisation dosing regimen.

3) The protocol that induces locomotor sensitisation does not decrease latency to acquisition of MDMA in the self- administration paradigm suggesting there are no changes in the potency of MDMA as a reinforcer after the current pre-treatment regimen
4) Following five single daily injections of 10.0mg/kg MDMA there are only minor reductions in the 5-HT metabolite 5-HIAA and no change in other brain amines tested.

Experiment one demonstrated that of the two pre-exposure regimens, pre-treatment with 10.0 mg/kg/day MDMA was the more effective and that behavioural sensitisation, unlike amphetamine sensitisation, was relatively short-lived. A sensitising regimen of amphetamine has shown increases in DA overflow up to three months following drug exposure (Hamamura, et al., 1991). Further, sensitised locomotor activity was apparent one year following amphetamine exposure (Paulson, et al., 1991). This may suggest that persistent sensitisation does not occur under the current pre-treatment regimen of MDMA however, a clear sensitised locomotor response was evident in all of the behavioural assays. These sensitised behavioural responses may nevertheless underlie motivational aspects of drug seeking and contribute to compulsive drug-taking that characterises abuse (Robinson & Berridge, 1993, 2003).

It has been suggested that escalating dose administrations produce a different behavioural profile to that of repeated intermittent administration (Segal & Kuczenski, 1999). Animals treated previously with an intermittent or escalating dose of amphetamine have exhibited different patterns of FosB and c-Fos expression in mesolimbic dopaminergic cell bodies (Murphy, Pezze, Russig and Feldo, 2001). Furthermore, following different escalating dose administrations of amphetamine Russig, Murphy and Feldon (2005) found a reduction in amphetamine produced locomotor activity in day 1 of the withdrawal phase but an increase (behavioural sensitisation) after 5 or 38 days. Given different administration schedules of amphetamine lead to different behavioural consequences, future research could establish if the long term expression of MDMA is evident following an escalating pre-treatment dosing regimen compared to that of the current intermittent exposure.

The current investigation demonstrated cross sensitisation to other dopamine agonists suggesting an underlying dopamine mechanism that is common in locomotor activating effects of psychostimulants. It has been suggested that the action of amphetamine in midbrain DA cell bodies is necessary for the induction of behavioural sensitisation. For example, repeated amphetamine injections into the ventral tegmental area, but not into the DA terminal field in the nucleus accumbens, produce an enhanced locomotor response to subsequent peripheral administration of amphetamine (Kalivas and Weber 1988; Vezina and Stewart 1990). Also, the local microinjection of a DA D_1 receptor antagonist into the ventral tegmental area is sufficient to prevent the

development of behavioural sensitisation to systemic amphetamine treatment (Stewart and Vezina 1989). Ramos and colleagues (2004, 2005) investigated the action of D_1 antagonist, SCH23390, in the prevention of development and expression of MDMA produced hyperlocomotion. It was argued that projections from the prefrontal cortex (PFC) might mediate behavioural sensitisation to MDMA and that dopaminergic mechanisms were implicated the expression of sensitisation. With the current investigation strongly implicating a sensitised D_1 receptor and an augmented D_2 mechanism in response to the current regimen of MDMA administration, it would be advantageous for future investigations to examine if these changes translate into increases in extracellular dopamine release using a more specific D_1 antagonist as SCH23390 activates 5-HT modulating effects of DA.

Microdialysis analysis in the ventral tegmental area, nucleus accumbens and frontal cortex may aid in answering the questions as to what, if any, common underlying dopamine mechanisms exist with MDMA and amphetamine sensitisation. There have been consistent reports of amphetamine induced sensitisation related adaptations in DA neurotransmission in striatal (Kalivas and Stewart, 1991; Robinson, 1991; Robinson and Berridge, 1993; White and Wolf, 1991) caudate or nucleus accumbens (Kolta et al., 1985, Robinson and Becker, 1982; Robinson et al., 1982; Wilcox et al., 1986; Yamada et al., 1988) DA release. To date however, few studies have investigated neuroadaptations following repeated MDMA administrations that produce behavioural sensitisation. Fos expression, which measures protein changes in the expression of the immediate early gene c-fos, has been positively correlated

with the behavioural consequences of repeated drug exposure in the NAc shell (Colussi-Mas & Schenk, 2008) suggesting DA mediated changes from repeated intermittent exposure. As experiment two demonstrated alterations in receptor mechanism sensitivity and experiment four failed to show any changes in gross storage pools of brain amine levels, microdialysis techniques may help in the future. Recording relative extracellular levels of brain amines in specific areas of the mesolimbic DA pathway may indicate downstream alterations in DA overflow responsible for MDMA produced locomotor sensitisation.

Changes in dopamine sensitivity may have been expected to increase the reinforcing effects of MDMA. When this was tested by examining latency to acquisition of self-administration, acquisition was delayed in MDMA pre-treated animals. Of interest, pre-treatment with MDMA in rats sensitises cocaine-induced behavioural responses and increases cocaine-stimulated place preference (Horan et al., 2000; Kalivas et al., 1998). As the current pre-treatment regimen produced sensitised locomotor activation, and may have modulated a common dopaminergic mechanism. This raises a question as to what, if any, circumstances could decrease latency to acquisition following MDMA administration? As mentioned above, different administration schedules of amphetamine lead to different behavioural consequences. Previous investigations that have looked at changes in potency to amphetamine (Piazza et al, 1990) and cocaine (Schenk & Partridge 1997) self administration did not use an escalating dosing regimen of stimulant. This may suggest that an escalating pre-treatment would not be hypothesised to

change the potency of latency to MDMA self-administration acquisition. It may have been that the self administration doses selected in the current investigation were too high and future studies that commence acquisition on a lower dose may show a change in potency resulting in a decrease in latency to acquisition.

Throughout the current set of experiments neurochemical changes have been induced from exposure to MDMA. This was demonstrated by the reduction in 5-HIAA levels. The deficits observed in the current investigation are mirrored in a number of MDMA studies that identify neurochemical changes following MDMA administration (Battaglia, Yeh, et al., 1988; Commins, et al., 1987; Goñi-Allo, et al., 2007; Nair & Gudelsky, 2006). It has become increasingly clear that long lasting and perhaps permanent changes in the brain underlie maladaptive alterations associated with compulsive drug craving (Nestler, 2001). The current testing was conducted under steady state conditions whereas most neurochemical adaptations are examined in response to a further drug administration (Kalivas, et al. 1998).

Neurochemical analysis under drug conditions may reveal differing neurochemical levels to those revealed in the current investigation. During amphetamine sensitisation there was a significantly elevated DA release in dorsal and ventral striatum (Paulson, Robinson, 1995; Robinson, Jurson, Bennett, & Bentgen, 1988) however there was no reported change in DA levels in the caudate or accumbens (Segal & Kuczenski, 1992). In future investigations, recording of DA release in dorsal and ventral striatum as well

as caudate and accumbens brain regions, during the MDMA sensitisation regimen, may reveal if there is any overlap in alterations of brain amine levels.

Taken together, the current research shows that repeated administration of MDMA produces sensitisation to its locomotor activating effects that induced lasting dopaminergic and serotonergic neural adaptations.

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