Behavioural and Neurochemical Effects of Acute (±) 3,4 methylenedioxymethamphetamine (MDMA) in the Dopamine D1 Receptor Mutant Rat

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List of Abbreviations

2C-B	2,5-dimethoxy-4-bromophenethylamine
2C-I	2C-I 2,5-dimethoxy-4-iodophenethylamine
5-HT	5-Hydroxytryptamine; serotonin
6-OHDA	6-hydroxydopamine
AMPH	Amphetamine
ANOVA	Analysis of variance
ATS	Amphetamine-type stimulants
cDNA	Complementary DNA
CNS	Central nervous system
CPA	Conditioned place aversion
CPP	Conditioned place preference
CS	Conditioned stimulus
DA	Dopamine
$DAD1^{-/-}$	Homozygous dopamine D1 mutant
$DAD1^{+/-}$	Heterozygous dopamine D1 mutant
$DAD1^{+/+}$	Homozygous dopamine D1 wild-type
DAT	Dopamine re-uptake transporter
DL	Dorsolateral (part of the striatum)
DM	Dorsomedial (part of the striatum)
DMTS	Delayed matching to sample
DNMTP	Delayed nonmatching to position
ENU	N-ethyl-N-nitrosourea
EP	Epinephrine
IHC	Immunohistochemistry
i.p.	Intraperitoneal injection
HPS	Hippocampus
KO	Knock-out
L-DOPA	Levodopa
LID	Levodopa-induced dyskinesia
LSD	Lysergic acid diethylamide

LTD	Long-term depression
LTP	Long-term potentiation
M	Mean
MDA	3,4-methylenedioxyamphetamine
MDEA	3,4-methylenedioxy-N-ethyl-amphetamine
MDMA	(±) 3, 4-Methylenedioxymethamphetamine; ecstasy
METH	Methamphetamine
mPFC	Medial prefrontal cortex
Nacc	Nucleus accumbens
NE	Norepinephrine
NET	Norepinephrine re-uptake transporter
NZ	New Zealand
PCPA	*p*-chlorophenylalanine
PD	Parkinson's disease
PTSD	Post-traumatic stress disorder
PFC	Prefrontal cortex
RPM	Revolutions per minute
SD	Standard deviation
SEM	Standard error of the mean
SERT	Serotonin re-uptake transporter
SNPc	Substantia nigra pars compacta
SSRI	Selective serotonin re-uptake inhibitor
UCS	Unconditioned stimulus
UNODC	The United Nations Office on Drugs and Crime
VTA	Ventral tegmental area
w/v	Weight/volume percent

Abstract

Rationale: (±) 3,4-methylenedioxymethamphetamine (MDMA; 'ecstasy') is a recreationally abused psychostimulant that leads to detrimental effects on memory performance. MDMA's acute effects on memory are often attributed to a working memory impairment resulting from compromised serotonin systems. However, recent evidence from non-human animal experimental studies suggests that acute MDMA may impair memory performance through an MDMA-induced increase in dopamine (DA) release, leading to overstimulation of DA D1 receptors. The overstimulation of D1 receptors during acute MDMA exposure is thought to indirectly impair memory by increasing a subject's susceptibility to proactive interference, leading to a perseverative pattern of responding during memory tasks.

Objective: This project investigates the hypothesis that acute MDMA impairs memory performance via overstimulation of D1 receptors. The acute actions of MDMA will be assessed using DA D1 mutant ($DAD1^{-/-}$) rats which possess a selective down-regulation in functional DA D1 receptors. On the basis that acute MDMA impairs memory function via overstimulation of D1 receptors it is predicted that, compared to control rats, $DAD1^{-/-}$ rats will be protected from the acute memory deficits caused by MDMA. Due to the novelty of the $DAD1^{-/-}$ rat model, prior to the assessment of the acute effects of MDMA on memory performance in these rats, behavioural and neurochemical characterisations will be conducted.

Methods: Firstly, a behavioural characterisation was conducted to explore the tendencies of $DAD1^{-/-}$ rats, compared to controls, in a drug free state. Behaviours relevant for motivation and reward, movement, and memory were the focus of the behavioural investigation due to evidence suggesting a role for D1-like receptors in these functions. Secondly, a neurochemical assessment of $DAD1^{-/-}$ and controls rats in response to MDMA (3 mg/kg) was assayed using *c-fos* expression, a marker for neuronal activity, in several brain regions with known DA innervation. Thirdly, to assess the acute effects of MDMA on memory performance, $DAD1^{-/-}$ and control rats were trained on a spatial working memory T-maze task, delayed non-matching to position (DNMTP), over 25 sessions. Once trained, rats were administered either MDMA (1.5, 2.25 and 3 mg/kg) or saline fifteen minutes prior to testing on DNMTP, with all subjects experiencing all drug doses three time each. In addition, to further investigate the hypothesis that overstimulation of D1 receptors impairs memory performance, the effects of a D1

receptor agonist, SKF 81297 (0.5, 1, 1.5, 3, 4.5 mg/kg) on DNMTP performance were also assessed.

Results: The behavioural characterisation revealed that DAD1$^{-/-}$ rats are capable of performing many behaviours relevant for reward processing, movement and memory function. However, DAD1$^{-/-}$ rats were impaired with regard to some reward-related behaviours, such as the acquitision of lever pressing for sugar pellets. The assessment of *c-fos* expression demonstrated that DAD1$^{-/-}$ rats express less *c-fos* in the medial prefrontal cortex, striatum and nucleus accumbens compared to control rats following MDMA administration. Lastly, the effects of acute MDMA administration on memory performance were tested. During the third block of MDMA administration, control rats demonstrated decreased accuracy on the DMNTP task at both the 2.25 and 3 mg/kg doses. The decrease in accuracy during MDMA exposure in control rats was driven by an increase in perseverative errors. On the contrary, DAD1$^{-/-}$ rats were not impaired on the DNMTP task following acute MDMA at any of the doses tested. Administration of SKF 81297 did not lead to any systematic changes in performance, but at the 3 mg/kg dose DAD1$^{-/-}$ rats displayed increased accuracy compared to control rats.

Conclusions: DAD1$^{-/-}$ rats were protected from an MDMA-induced decrease in accuracy on the DNMTP task compared to control rats. This finding challenges the assumption that MDMA's acute effects on memory performance are wholly due to serononergic mechanisms. Specifically, the current study provides evidence for the hypothesis that acute MDMA exposure impairs memory performance in rats via overstimulation of D1 receptors, caused by an increase in proactive interference.

Chapter 1: General Introduction

The pharmacological and behavioural actions of the psychostimulant (±) 3,4 methylenedioxymethamphetamine (MDMA; 'ecstasy') are unique when compared to other drugs of abuse, including related amphetamine (AMPH) derivatives. Whereas compounds with high abuse liability such as AMHP, cocaine or heroin stimulate substantial release of mesocorticolimbic dopamine (DA) and to a lesser extent brain serotonin (5-HT; 5-Hydroxytryptamine), recreational doses of MDMA preferentially stimulate the release of 5-HT (Baumann, Clark, & Rothman, 2008; Johnson, Hoffman, & Nichols, 1986; Steele, McCann, & Ricaurte, 1994; Schmidt, 1987). This characteristic has led to a great deal of research into MDMA's effects with a focus on the contributions made by 5-HT (Colado, O'Shea, & Green, 2004). For example, acute MDMA exposure can lead to a pattern of symptoms known as 5-HT syndrome (Piper, Fraiman, & Meyer, 2005; Spanos & Yamamoto, 1989; Tao, Shokry, & Callanan, 2015), and high or repeated doses of MDMA can lead to long-term depletions in 5-HT and 5-HT axons in rats, which many argue reflects 5-HT neurotoxicity (Battaglia et al .,1987; Benningfield & Cowan, 2013; McCann, Szabo, Dannals, & Ricaurte, 1998; Molliver et al., 1990; Parrott, 2002; Schmidt & Kehne, 1990; Souza, Battaglia, & Insel, 1990; although see Baumann, Wang, & Rothman, 2007 for a review to the contrary). Furthermore, compared to addictive drugs that primarily exert their effects on DA systems, it has been hypothesised that MDMA's increased potency for brain 5-HT stimulation underlies the lower acquisition rates of MDMA self-administration observed using animal models (Bradbury et al., 2013; Wang & Woolverton, 2007).

Although it is well documented that MDMA exposure can lead to long-term depletions of markers of 5-HT neurotransmission, the risks stemming from consumption of MDMA are not necessarily known by MDMA users (Carlson, Falck, McCaughan, & Siegal, 2004; Strote, Lee, & Wechsler, 2002; Uosukainen, Tacke, & Winstock, 2015). Despite the possible neurotoxic effects of MDMA, hospital admissions and deaths associated with MDMA use are relatively rare (European Monitoring Centre for Drugs and Drug Addiction, 2015; Landry, 2002; although rates do appear to be increasing: Global Drug Survey, 2015), leading to the concern that MDMA may be perceived as a relatively safe drug (Kahn, Ferraro, & Benveniste, 2012; Parrott, 2014; Rivas-Vazques & Delgado, 2002). This is worrying in the face of widespread reports that MDMA exposure leads to adverse effects on cognitive performance which likely severely impact the lives

of MDMA users, for example on tests of memory and attention (e.g. Jacobsen, Mencl, Pugh, Skudlarski, & Krystal, 2004; Kalechstein, Garza, Mahoney, Fantegrossi, & Newton, 2007; Kuypers & Ramaekers, 2005; 2007; McCann, Mertl, Eligulashvili, & Ricaurte, 1999; Murphy, Wareing, Fisk, & Montgomery, 2009; Quednow et al., 2006; Rendell, Gray, Henry, & Tolan, 2007; Wareing, Murphy, & Fisk, 2004; Zakzanis & Young, 2001). Due to MDMA's pronounced effects on 5-HT mechanisms and likely 5-HT neurotoxicity, many researchers broadly attribute the cognitive deficits associated with MDMA administration to 5-HT dysfunction (Galizio, McKinney, Cerutti, & Pitts, 2009; Kalechstein et al., 2007).

With a focus here on MDMA's ability to impair learning and memory, while 5-HT is possibly involved there are several caveats to the position that alterations in 5-HT neurotransmission can account for all of MDMA's effects on memory performance. Firstly, chronically administered regimens of MDMA that reliably and extensively compromise long-term 5-HT function in animal models do not necessarily lead to lasting memory deficits (e.g. Byrne, Baker, & Poling, 2000; Frederick et al., 1998; Moyano, Frechilla, & Rio, 2004; Robinson, Castaneda, & Whishaw, 1993; Taffe et al., 2001). Secondly, in a study by Moyano et al. (2004) systemic application of the 5-HT synthesis inhibitor *p*-chlorophenylalanine (PCPA), which depleted hippocampal 5-HT transmission by > 90%, did not lead to memory retention deficits on the passive avoidance task. MDMA administration still resulted in memory deficits subsequent to the treatment of PCPA, suggesting that 5-HT mechanisms were not a necessary cause for these MDMA-induced impairments (Moyano et al., 2004). Thirdly, regimes of MDMA that are non-toxic to 5-HT neurons have still been demonstrated to impair learning (Arias-Cavieres et al, 2010). Fourthly, memory impairments following acute MDMA exposure tend to emerge at a dose (~3 mg/kg via intraperitoneal (i.p.) injection) (Galizio, Byrd, Robinson, Hawkey, Rayburn-Reeves, & April, 2014; Galizio et al., 2009; Harper, 2013; Harper, Hunt, & Schenk, 2006; Harper, Wisnewski, Hunt, & Schenk, 2005; Kay, Harper, & Hunt, 2010) at which there is a relatively greater increase in extracellular DA than 5-HT (Baumann et al., 2007). This dose effect is further demonstrated using the 3-lever drug discrimination paradigm in rats, where the subjective effects of MDMA after a dose of 3 mg/kg appear similar to the potent DA agonist, AMPH, yet at lower doses MDMA and AMPH are successfully discriminated (Harper, Langen, & Schenk, 2014). Often, doses of MDMA lower than 3 mg/kg preserve memory function (Harper et al., 2005; Kay et al.,

2010) despite still stimulating substantial 5-HT release (Baumann et al., 2008), with higher doses altering more general aspects of behaviour that interfere with performance on many behavioural memory tasks (e.g. Galizio et al., 2014; Kay et al., 2010). Lastly, recent studies have directly implicated DAergic substrates in MDMA's effects on memory processes (Harper, 2013; Harper, Kay, & Hunt, 2011; Rozas et al., 2012). For example, in our laboratory it was demonstrated that acute MDMA (3 mg/kg) impaired performance on an operant delayed matching-to-sample (DMTS) working memory task, with this MDMA-induced deficit being ameliorated via concurrent administration of the DA D1-like receptor antagonist, SCH 23390 (Harper, 2013). Harper (2013) concluded that MDMA's acute effects on memory performance may involve its agonist actions at DA D1 receptors. As discussed in more detail later in this chapter, the finding that D1 receptors may be involved in MDMA's effects on memory is not all that surprising given that this receptor is considered an important neural substrate for learning and memory (Castner, Williams, & Goldman-Rakic, 2000; Hotte et al., 2006; McNab et al., 2009; Nai et al., 2010).

The caveats presented above suggest that some of MDMA's effects on memory may be mediated by non-5-HTergic mechanisms. Possible sources of ambiguity in the interpretation of MDMA's effects on memory are that the effects are typically attributed to 5-HT mechanisms even when markers of 5-HT integrity were not determined or deliberately manipulated (e.g. following MDMA administration, compromised 5-HT transmission is assumed and then associated with the observed cognitive impairments); and regardless of whether the drug regime was 'acute' (while under the influence of the drug), 'sub-chronic' (unclearly defined but approximately 1-4 days following drug treatment), 'binge' (high dose exposure) or 'chronic' (long-term effects). Due to previous findings in our laboratory centring on the *acute* actions of MDMA on memory performance (Harper, 2013; Harper et al., 2005; 2006), this PhD project further explores the hypothesis that *acute* MDMA acts to impair memory via its agonist actions at DA D1 receptors from a behavioural neuroscience perspective.

Although it is inaccurate to claim that all of MDMA's effects on 5-HT are understood, by comparison MDMA's effects on DA mechanisms have received considerably less attention. *In vitro* assay and *in vivo* microdialysis demonstrate that acute MDMA administration leads to pronounced increases in extracellular DA in the prefrontal cortex (PFC), striatum, nucleus accumbens (Nacc) and hippocampus (HPS)

(Baumann et al., 2008; Crespi, Mennini, & Gobbi, 1997; Gough, Ali, Slikker, & Holson, 1991; Johnson et al., 1986; Shankaran & Gudelsky, 1998) and it has been suggested that the repeated use of MDMA may lead to a sensitised DA response that contributes to the transition from use to abuse (Schenk, 2011). The idea that DA may play a role in MDMA-induced memory deficits (Harper, 2013; Rozas et al., 2012) is in line with research suggesting that potent DA exerting drugs, such as methamphetamine (METH) and cocaine, also appear to impair memory via DA mechanisms (Macaskill, Harrow, & Harper, 2015; Shoblock, Maisonneuve, & Glick, 2003; Tomasi et al., 2007), and corroborates the large body of literature demonstrating that intact DA mechanisms, involving activation of D1-like receptors in mesocorticolimbic pathways, are required for normal memory function (Goldman-Rakic & Williams, 1995; Nai et al., 2010; Valentim Jr, Gontijo, Peres, Rodriques, & Nakamura-Palacios, 2009; Vijayraghavan, Wang, Birnbaum, Williams, & Arnsten, 2007).

There is substantial evidence indicating that acute MDMA exposure can impair performance on learning and memory tests (Braida, Pozzi, Cavallini, & Sala, 2002; Bryne et al., 2000; Galizio et al., 2009; 2014; Harper, 2013; Harper et al., 2005; 2006; Hawkey et al., 2014; Kay et al., 2010; Kay, Harper, & Hunt, 2011; Kuypers & Ramaekers, 2005; 2007; Marston, Reid, Lawrence, Olverman, & Butcher, 1999; Moyano et al., 2004; Moyano, Rio, & Frechilla, 2005). A central question of this project and a subject currently under consideration in the literature is whether these MDMA-induced performance deficits reflect a direct disruption of working memory processes or rather an indirect interference of working memory performance via alternative mechanisms (Galizio et al., 2014; Harper, 2013; Harper et al., 2005; Hawkey et al., 2014; Kay et al., 2011). Initially, the finding that MDMA administration can cause impairments on tests used to assess working memory function led to the idea that MDMA directly impairs working memory processes (e.g. Braida et al., 2002; LeSage, Clark, & Poling, 1993; Marston et al., 1999). More recently, researchers probing the basis of MDMA's effect on memory performance have implicated non-working memory processes as the source of the acute deficits.

On many behavioural tests of memory designed for rodents, accurate responding is thought to involve two dissociable cognitive components: working memory and reference/procedural memory. Working memory can be thought of as task accuracy in situations where the appropriate response changes frequently (i.e. multiple times

throughout a session), which requires remembering episodic events occurring on a trial-by-trial basis. In line with Baddeley's (1986) conceptualisations, working memory involves the generation of internal representations of information, which are held and manipulated 'online' by executive systems, in order to guide action. By contrast, reference memory function is reflected by accurate responding when the appropriate response remains constant indefinitely across sessions. In this case, a pattern of behaviour is learned which does not need to be adapted trial-by-trial. The fact that many behavioural tests of memory confound assessment of reference versus working memory likely underlies the attribution of MDMA's acute effects on performance to deficits in working memory. Using procedures that allow for the separation of working and reference memory components, recent studies have indicated that acute MDMA impairs reference memory processes (Braida et al., 2002; Galizio et al., 2014; Harper et al., 2011; Kay et al., 2010). The finding that acute MDMA impairs reference memory to a comparatively much greater extent than working memory is also consistent with several previous studies using non-acute regimes of MDMA administration (Able, Gudelsky, Vorhees, & Williams, 2006; Skelton et al., 2008; Sprague, Preston, Leifheit, & Woodside, 2003; Vorhees, Reed, Skelton, & Williams, 2004) as well as one study that did not explicitly tease apart working memory versus reference memory errors (Braida et al., 2002).

However, the emerging theme that acute MDMA impairs reference rather than working memory does not provide a complete picture of MDMA's acute effects on memory performance. Identifying the type of memory errors caused by acute MDMA is certainly useful, yet we also require a description of how the subjects' pattern of responding is disrupted during MDMA exposure. To address this, a possible mechanism by which MDMA impairs memory in rodents has been presented on the basis of data collected in our laboratory (Harper and colleagues, 2005; 2006; 2011; 2013). Using the DMTS task, acute MDMA appears to decrease accuracy by increasing proactive interference. Specifically, when exposed to MDMA rats displayed response perseveration whereby they tended to repeat their response given on the directly previous trial, rather than performing the currently required response (Harper and colleagues, 2005; 2006; 2011; 2013). The finding that MDMA increases perseveration has been reported elsewhere (Galizio et al., 2009; Montgomery, Fisk, & Newcombe, 2005; Verrico et al., 2008), yet this phenomenon has not been fully characterised. Related to the previously mentioned possible role of DA D1 receptors in MDMA's effect on memory performance,

Harper (2013) found that D1-like receptor antagonism attenuated MDMA's effect on accuracy by directly decreasing perseverative responding. Therefore, this project will explore the idea that MDMA impairs performance on memory tests by increasing perseverative responding caused by MDMA's agonist actions at DA D1 receptors.

The current investigation expands on the finding that DA D1-like receptors are involved in MDMA's impact on memory processes by examining D1 receptors in isolation from other binding sites, which are targeted by non-selective DA D1 receptor ligands. To isolate D1 receptors, a novel animal model with down-regulated DA D1 receptor function – the DA D1 mutant ($DAD1^{-/-}$) rat – will be used. As there are no published behavioural studies using the $DAD1^{-/-}$ rats to date, the first data chapter (*chapter 3)* presents a behavioural assessment of the $DAD1^{-/-}$ rats in a drug-free state, with regard to behaviours that are thought to engage D1 receptor processes. This behavioural profile is necessary in order to account for any baseline performance differences that may be present between the $DAD1^{-/-}$ rats and controls ($DAD1^{+/-}$ or $DAD1^{+/+}$ rats). The next data chapter (*chapter 4*) investigates the $DAD1^{-/-}$ rats' neurochemical response to acute MDMA using immunohistochemical analysis of *c-fos* expression in several brain regions with known DAergic innervation. This explores whether the $DAD1^{-/-}$ rats display an altered neurochemical response to MDMA, and allows for correlation between *c-fos* expression and MDMA-induced locomotor activity. Subsequent to the behavioural and neurochemical profiling of $DAD1^{-/-}$ rats, the last data chapter (*chapter 5*) presents an exploration of MDMA's acute effects on memory performance and the role of D1 receptors.

The following sections of the current chapter commence with a background to the use and effects of MDMA, with particular attention paid to MDMA's acute actions observed in rat subjects. The involvement of DA and D1-like receptors in MDMA's cognitive and behavioural effects are then outlined. The latter half introduces the $DAD1^{-/-}$ rat model and discusses the use of genetic animal models in behavioural neuroscience. Relevant behavioural and neurological functions involving DA and D1-like receptors are then summarised, which serve to guide the experimental direction of this project. Lastly, the three ensuing data chapters are outlined and the central questions of this thesis are stated.

A Background to MDMA Use and Abuse

MDMA is a recreationally administered ring-substituted AMPH with both stimulant- and hallucinogenic- eliciting properties (Gold, Koob, & Meyer, 1988; Kalant, 2001). MDMA is the active compound in street-sold ecstasy and, despite being a controlled substance in much of the world since the 1980s, is widely used in the club and rave scenes (Schwartz & Miller, 1997). High use of MDMA among the party scene likely reflects its unique profile of subjective effects which are viewed as complimentary to this setting. In general, a standard dose of MDMA (~ 75-120 mg) is reported to produce AMPH-like stimulation, perceptual alterations, and alertness, coupled with feelings of emotional closeness to others, euphoria and reduced anxiety (Baylen & Rosenberg, 2006; Peroutka, Newman, & Harris, 1988; Solowij, Hall, & Lee, 1992). Negative effects are also reported, including depersonalisation, anxiety, tachycardia and bruxism (Peroutka et al., 1988; Vollenweider, Gamma, Liecht, & Huber, 1998). This experience typically peaks 30-90 minutes after administration, before wearing off after 3-4 hours, sometimes followed by an extended comedown period of anhedonia (Cami et al, 2000; Cohen, 1995).

The interchangeable terms ‘entactogen’ and ‘empathogen’, denoting a class of psychoactive drugs, were coined in reference to the distinct spectrum of psychological effects produced by MDMA and related compounds such as 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxy-*N*-ethyl-amphetamine (MDEA), mephedrone, 2,5-dimethoxy-4-bromophenethylamine (2C-B) and 2C-I 2,5-dimethoxy-4-iodophenethylamine (2C-I). Stemming from MDMA’s acute ability to engender emotional openness in this regard, there is a subset of psychotherapists and clinicians who have explored the use of low doses of MDMA as an in-session therapeutic tool for the treatment of post-traumatic stress disorder (PTSD) and related disorders (Bouso, Doblin, Farre, Alcazar, & Gomez-Jarabo, 2008; Hysek et al., 2013; Sessa, 2007). Evidence that MDMA may inflict 5-HT neurotoxicity, and that MDMA use is associated with cognitive impairments and other negative effects, warrants caution going forward with further pre-clinical research necessary to elucidate the short- and long-term effects of MDMA prior to human trials.

A brief history of MDMA/”the truth of MDMA” (Wolfson, 1986). MDMA has been among the most popular psychotropic drugs since the mid-1980s (Freudenmann,

Öxler, & Bernschneider-Reif, 2006), yet it was synthesised considerably earlier than this.

In the medical literature, it is often reported that MDMA was originally patented in Germany in 1912 by a pharmaceutical company, Merck, as an appetite suppressor or anorectic drug (Climko, Roehrich, Sweeny, & Al-Razi, 1986; Kalant, 2001). Contrary to this assertion, however, and after examination of primary documents from Merck's archives, Freudenmann et al. (2006) concluded that although MDMA was synthesised by Merck in 1912, it was developed as a precursor in a novel chemical pathway for a blood clotting agent. During their investigation into the origin of MDMA, Freudenmann et al. (2006) also uncovered the first two documented instances, in 1927 and 1959, of basic pharmacological testing of this compound, which at the time was called 'Methylsafrylamin'. The first instance of a regular scientific publication on MDMA was not until 1960 (Biniecki & Krajewski, 1960), yet merely a decade later MDMA tablets were first seized and identified from the streets of Chicago (Gaston & Rasmussen, 1972).

The first known animal experiments using MDMA were conducted over 1953-54 but were not published until 1973 due to being a classified project supported by the US army (Hardman et al., 1973). These experiments used MDMA as one of seven chemical analogues of lysergic acid diethylamide (LSD) in order to test the toxicity and behavioural correlates of LSD in five species of lab animals: the mouse, rat, guinea pig, dog and monkey. Based on these data, it was later concluded that MDMA was less toxic than MDA but more toxic than LSD (Shulgin, 1986).

It took another five years after the publication of the initial toxicology study, reported by Hardman et al. (1973), before the first non-clinical reports of the effects of MDMA were described in humans (Shulgin & Nichols, 1978). However for at least two years prior, MDMA's entactogenic properties were being employed by clinical psychologists who freely administered this psychostimulant to patients during therapy sessions to aid in the establishment of rapport between patient and therapist and to elicit an openness of emotional expression (Greer, 1985; Shulgin, 1990; Wolfson, 1986). Wolfson (1986) eloquently conveyed the opinion of MDMA-proponent therapists in his account of "The Truth of MDMA" (pp. 331):

"The fundamental truth is that MDMA provides in its totality an unprecedented access to an experience that human beings value and may wish to have the opportunity to repeat at a future date. The second part of this truth is the almost uniform observation

that those who have had the MDMA experience wish to share it with others and believe it has the ability to alter lives, even societies, positively. Nor has anyone been able to say otherwise after hundreds of thousands of experiences with MDMA. This is the completion of the fundamental truth: there are almost no critics of the experience itself."

Wolfson's assertion that people wish to repeat the MDMA experience appears to inadvertently prophesise our current conceptualisations of MDMA dependence (described below). Considering MDMA's acute subjective effects, it is not surprising that during the late 1970s in the USA MDMA use acquired fervent support among rave goers, despite public controversy and outcry from those opposed to this new psychostimulant (Shulgin, 1990). Early reports of MDMA use were limited to case studies and prevalence data, with popular opinion spreading that MDMA was not addictive and that it was infrequently used for recreational purposes (Peroutka, 1990; Solowij et al, 1992). The term 'ecstasy' was coined in California in 1984, only one year before the American Drug Enforcement Agency, listed MDMA as a Schedule I controlled substance, officially coming into effect on 1 July 1985 (The New York Times, 1985) despite little evidence of reported MDMA harm at that stage. Soon after, many other countries followed America's lead and also prohibited MDMA, including New Zealand in 1987 (www.drugfoundation.org.nz).

The recreational use of MDMA and ecstasy began escalating worldwide from the late 1990s (Schifano, Corkery, Deluca, Oyefeso, & Ghodse, 2006). The United Nations Office on Drugs and Crime (UNODC) estimated that, globally, around 19 million (between 10 and 28 million people or 0.2 - 0.6 per cent of the population) aged 15-64 used ecstasy at least once in 2012 (UNOCD, 2015). Although worldwide ecstasy use appears to be in gradual decline, prevalence rates for Europe, North America and Oceania (including New Zealand at 2.9 per cent) remain above the global average (UNOCD, 2015).

MDMA use in New Zealand. MDMA is the second most frequently used illegal substance in New Zealand, after cannabis. The detrimental repercussions stemming from use of ecstasy have been identified as a significant area of concern for New Zealand (NZ) (Wilkins et al., 2004). The prevalence of MDMA use and manufacture in NZ has rapidly increased over recent years (Ministry of Health, 2010; Wilkins, 2002; Wilkins, Bhatta, Pledger, & Casswell, 2003; Wilkins & Sweetsur, 2008), with a corresponding surge in the amount of MDMA seized by customs and a rise in the number of clandestine drug

laboratories that manufacture MDMA (and other AMPH-type substances; ATS) being detected by the NZ police (Wilkens, 2002). The most recent data available on ecstasy use in New Zealand was sampled during the 2007/08 period (Ministry of Health, 2010) which estimates that, across 16 – 64 year olds, 2 – 3.1 per cent of people used ecstasy at least once in the previous year. By comparison, the global prevalence of people aged 15 - 64 years, also surveyed in 2007, was estimated as 0.2 per cent of people aged between 15 – 64 year old (UNODC, 2008). This clearly illustrates the high rate of ecstasy use in New Zealand compared to the global scene.

The rise in consumption of MDMA throughout NZ has been linked to parallel increases in a myriad of social and health consequences, including hospital admissions for drug-induced psychosis, violent crimes and thefts (Wilkins, 2002), cases of mental illness, injury while under the influence of MDMA, incidences of domestic violence, relationship breakdown, and child neglect (Wilkins & Sweetsur, 2008). Despite our awareness of these detrimental outcomes stemming from the manufacture, sale and use of MDMA, efforts to regulate MDMA have been met with limited success. An improved understanding of the effects of MDMA would allow for informed and evidence-based decision making about, and attitudes towards, the use of MDMA.

Studying MDMA's actions: Problems with using human participants.

Studying human users to examine the effects of MDMA is problematic. Firstly, ecstasy users are often consumers of a variety of other drugs, such as cannabis, alcohol, opiates and other ATS (Schifano, Di Furia, Forza, Minicuci, & Bricolo, 1998). This makes it difficult to separate the effects that derive from MDMA use from those of other drugs. Secondly, whilst the main stimulant ingredient of street-sold ecstasy tablets is purported to be MDMA, the purity of ecstasy can change dramatically (UNODC, 2015; Parrott, 2004; Schifano et al., 2006) meaning that the perception of an ecstasy users' MDMA consumption may not reflect their actual MDMA consumption. Thirdly, the use of human subjects in MDMA trials may be confounded by the characteristics of the people that volunteer their participation in such research, with regard to their past drug histories, personality attributes and attitudes. Fourth, it is not feasible to detect subtle neurological pathologies caused by MDMA, or to map the drug's mechanism of action, in living subjects. Fifth, necessary ethical considerations regarding the administration of MDMA to human subjects can constrain the ability of researchers to carry out controlled studies using large subject numbers or high doses of MDMA, leading to self-report measures of

drug use being a main source of data in this area (Schifano et al., 1998). Lastly, studies that report measures of brain activity in human drug users (in which no drugs are administered) necessarily rely on correlational analysis to determine whether drug use is associated with a particular pattern of activity which in turn is associated with assessments of cognitive ability. This means that the possibility that there are pre-existing differences between drug-using versus non drug-using participants which influenced drug taking behaviour cannot be ruled out.

In light of the above limitations, it can be difficult to interpret findings from human studies that investigate the impacts of drugs on brain function. As such, the use of animal models to investigate the neurological actions of drugs of abuse has been invaluable. Due to shared ancestry between mammalian genomes and the subsequent structural and functional similarities at the levels of anatomy, cell biology and physiology, the appropriate use of animal testing to study human pathology is highly relevant. This approach also affords stringent control over the test subjects' genetic and environmental backgrounds, which minimises confounds and reduces statistical variance. Within the context of MDMA research the use of rats is favoured over the use of mice subjects due to mice demonstrating a response to MDMA characterised by long-term DA depletions rather than 5-HT depletions, which is typical of rats, humans and non-human primates (for a review see Colado et al., 2004). The following section deals with evidence pertaining to the pharmacology and physiological effects of MDMA that has, unless specified, been determined using rats.

The pharmacology and physiological effects of MDMA. MDMA is a synthesised 'designer drug', with early clandestine laboratories seeking to create a blend of stimulant 'AMPH-like' and hallucinogenic 'mescaline-like' effects (Climko et al., 1986). MDMA is a derivative of METH – its parent compound is AMPH – however its chemical structure differs from these related stimulants in one vital respect (see figure 1). MDMA is "ring substituted" meaning that it has a methylenedioxy (-O-CH2-O) group attached to positions 3 and 4 of the aromatic ring of the AMPH molecule (Kalant, 2001). MDMA is a substrate for monoamine transporters (Baumann et al., 2007), and broadly speaking its biological effects appear similar to 5-HT, DA and epinephrine (EP) stimulation (Kalant, 2001).

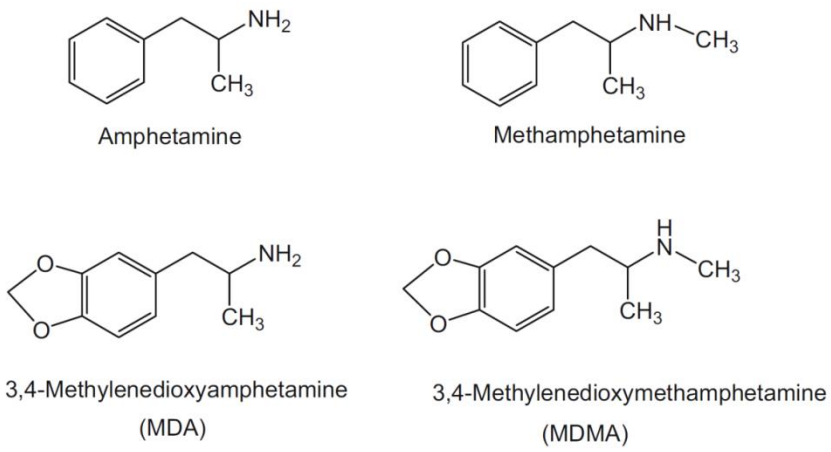

Figure 1. Comparison of amphetamine (AMPH), methamphetamine (METH), 3,4-methylenedioxyamphetamine (MDA) and 3,4-methylenedioxymethamphetamine (MDMA) molecules. Sourced from Baumann et al., 2007.

Although MDMA has in common with its AMPH analogs (and other drugs of abuse) the ability to stimulate action of brain DA, MDMA has a unique pharmacological profile compared to other drugs. *In vivo* rat brain slice and *in vitro* microdialysis experiments demonstrate MDMA's enhanced potency for 5-HT stimulation, and decreased potency for DA release (Baumann et al., 2005; 2007; Gudelsky & Nash, 1996). Dialysate samples from rat Nacc collected 20 minutes after i.p. injection of 1 mg/kg MDMA showed a tenfold rise in 5-HT, but only a twofold rise in DA (Baumann et al., 2007). The mechanism by which MDMA stimulates the release of monoamines is largely through binding to transporter proteins (5-HT: SERT; DA: DAT; NE: NET) and stimulating reverse-transport, non-exocytotic release of 5-HT, DA and NE (Berger, Gu, & Azmitia, 1992; Crespi et al., 1997; Green, Mechan, Elliot, O'Shea, & Colado, 2003). Furthermore, MDMA prevents vesicular uptake of cytosolic 5-HT (Battaglia, Brooks, Kulsakdinun, & Souza, 1988; Rudnick & Wall, 1992).

The acute physiological effects of MDMA administration vary, depending on dose and route of administration and the users' immediate environment. Typically, human users ingest MDMA orally (ecstasy or 'E' tablets) or by snorting the powder. A standard dose administered is 1-2 ecstasy tablets or approximately 80-100 mg of MDMA (although the dose can vary considerably), equating to around 1-3 mg/kg (Green et al., 2003; Schifano, 2004). Given that the acute neurochemical, endocrine and behavioural effects

of MDMA are similar across rats and humans when using comparable doses (e.g. 1-3 mg/kg) (Baumann et al., 2007), the physiological effects of MDMA as measured in rats will be described below.

Preferential stimulation of 5-HT is thought to be at the root of MDMA's primary acute physiological effects (Colado & Green, 1994; Green, O'Shea, & Colado, 2004) with the two most widely reported being hyperthermia (Dafters, 1995; Gordan, Watkinson, O'Callaghan, & Miller, 1991; Nash, Meltzer, & Gudelsky, 1988) and symptoms of '5-HT syndrome' (Parrott, 2002; Slikker et al., 1989; Spanos & Yamamoto, 1989). Although limited research has looked at the hyperthermic response to MDMA, it can be reversed through pre-treatment with 5-HT antagonist ketanserin (Nash et al., 1988). 5-HT syndrome, however, has been the subject of intense investigation and its mechanisms are still unclear. It is characterised by hyperlocomotion, reciprocal forepaw treading, head weaving, piloerection, flattened body posture, hind limb abduction, proptosis, ataxia, unawareness, and when particularly severe, leading finally to convulsions and death (Green & Heal, 1985). Other physiological effects include elevated heart rate and mean arterial pressure (O'Cain, Hletko, Ogden, & Varner, 2000).

Administration of MDMA can lead to chronic selective dysfunction in markers of 5-HT transmission after only a single high dose, and certainly after repeated doses (Battaglia et al.,1987; Benningfield & Cowan, 2013; McCann et al., 1998; Molliver et al., 1990; Parrott, 2002; Schmidt & Kehne, 1990; Souza et al., 1990). There is on-going debate over whether the observed 5-HT dysfunction reflects 5-HT neurotoxicity or another mechanism that disrupts 5-HT transmission more transiently (see Baumann et al., 2007). Common findings used to support the hypothesis that MDMA is neurotoxic to 5-HT neurons are that MDMA administration reduces 5-HT nerve terminal integrity (Sprague et al., 2003), causes persistent inactivation of tryptophan hydroxylase activity (the rate limiting step in 5-HT synthesis), depletes brain tissue 5-HT and reduces SERT binding and functions (Battaglia et al., 1987; Schmidt et al., 1987). Using immunohistochemistry O'Hearn et al. (1988) found cortical and subcortical areas displayed 5-HT neuron axon and terminal loss after MDMA treatment, and axon and terminals that remained demonstrated structural damage. Despite these markers of compromised 5-HT nerve terminal integrity, Baumann et al. (2007) argue that there are several caveats to the hypothesis that MDMA causes 5-HT neurotoxicity. Briefly, MDMA-induced reductions in 5-HT function and SERT binding have been found to

recover (Scanzello, Hatzidimitriou, Martello, Katz, & Ricaurte, 1993). Also, other drugs that are not considered to be neurotoxins, such as selective 5-HT re-uptake inhibitors (SSRIs), demonstrate similar effects such as sustained depletions of brain tissue 5-HT and decreased SERT binding. SSRIs have even been found to cause structural damage to 5-HT terminals, resembling MDMA damage (Kalia, 2000). While collectively these data do suggest that 5-HT terminals are not destroyed subsequent to MDMA exposure, the debate remains open.

Evidence for MDMA dependence. The focus of this thesis is on the acute effects of MDMA and as such it is only partially relevant to provide an analysis of MDMA dependence. However, possibly the most important reason we study drugs of abuse, at all levels of analysis, is because some people transition from casual drug use to compulsive drug taking, leading to a myriad of health risks and psychological and social harms (Everitt & Robbins, 2005). Evidence for dependency, then, further necessitates an understanding of MDMA's acute effects.

Although MDMA was originally conceived of as a non-addictive substance (Peroutka, 1990), there is now evidence that some human users meet criteria for MDMA dependency, albeit to a lesser extent than for other drugs of abuse that elicit greater DA stimulation (Degenhardt, Bruno, & Topp, 2010; Jansen, 1999; von Sydow, Lieb, Pfister, Höfler, & Wittchen, 2002). An important paradigm used to model addiction in animal subjects is the self-administration paradigm (described in more detail by Schenk, Gittings, Johnstone, & Daniela, 2003). When used with rats, a typical procedure involves placing the subject in an operant chamber where they are presented with two levers: an active lever that, upon depression, leads to infusion of drug directly into the subject's jugular vein, and an inactive lever, the pressing of which leads to an infusion of saline. Animals will self-administer drugs that are abused by humans (Pickens & Harris, 1968; Schenk & Partridge, 1997; Schenk et al., 2003) and invariably these drugs stimulate substantial release of mesolimbic DA, particularly in the Nacc (Chiara & Imperato, 1988). Under normal conditions, 80 – 100 % of rats will acquire self-administration of classic drugs of abuse such as cocaine and AMPH (e.g. Carroll & Lac, 1997), whereas only 50 – 60 % of rats will reliably self-administer MDMA (Bradbury et al., 2014; Schenk, Colussi-Mas, Do, & Bird, 2012; Schenk et al., 2007).

One hypothesis for why MDMA self-administration is lower than for other drugs of abuse predicts that the massive release of 5-HT following MDMA administration inhibits or flattens the reinforcing effects of MDMA (Bradbury et al., 2013; Oakly et al., 2014; Schenk, 2011). Neurotoxic lesions to 5-HT systems have been found to greatly enhance the reinforcing properties of MDMA (Bradbury et al., 2013), and using *in vivo* microdialysis it was demonstrated that subjects which self-administer MDMA have a correspondingly smaller 5-HT response to MDMA than subjects which failed to meet the criteria for MDMA self-administration (Bradbury et al., 2013). With repeated exposure to MDMA, subjects can develop tolerance (when a substance has a progressively diminished effect) to the 5-HTergic properties of MDMA (Do & Schenk, 2013), with behavioural markers of acute 5-HT syndrome following MDMA in rats appearing less pronounced over time (Marston et al., 1999). Simultaneously, subjects develop sensitisation (when a substance has a progressively increased effect) to the DAergic component of MDMA (Bradbury, Gittings, & Schenk, 2012). Repeated exposure to acute MDMA administration in rats leads to incremental increases in locomotor activity across sessions, with this increase in activity being associated with DA stimulation (Bradbury et al., 2012; Schenk & Bradbury, 2015). A sensitised response to the DA-stimulating effects produced by drugs of abuse is thought to increase *wanting* for the drug, rather than *liking*, resulting in escalated drug taking (see below for discussion of the incentive salience hypothesis which provides a description of DA's role in reward – Berridge & Robinson, 1998). Thus, the neuroadaptions underlying both tolerance and sensitisation to MDMA appear to interact to increase the likelihood that a subject will develop MDMA self-administration, or dependence, over time.

Consistent with animal studies, investigations of patterns of MDMA use in human subjects have found that with repeated exposure, more experienced users gradually increase their MDMA dose often by several times over. Initially, users typically administer 1-2 tablets, whereas more experienced users may administer 2-3 and some people have admitted to using 10-25 tablets in a single session (Parrott, 2005). Worryingly, compared to cocaine or heroin users, MDMA-dependent individuals were found to report less desire to decrease their drug consumption, less desire to receive help for their drug taking, and fewer risks associated with their drug taking (Uosukainen et al., 2015). Given that MDMA is considered an addictive substance, and in combination with the detrimental effects of MDMA use, especially, as discussed below, on cognitive

functions, comprehensive analysis of the behavioural and neurochemical effects of MDMA is warranted.

MDMA-induced cognitive changes.

Cognitive changes associated with chronic or binge MDMA exposure. Even after long periods of abstinence, human users of MDMA often exhibit persistent cognitive changes, most notably on tasks of memory such as deficits in verbal recall (Bolla, McCann, & Ricaurte, 1998; McCardle, Luebbers, Carter, & Croft, 2004; Morgan, Impallomeni, Pirona, & Rogers, 2006; Parrot & Lasky, 1998; Parrott, Lees, Garnham, Jones, & Wesnes, 1998; Quednow et al., 2006), increased impulsivity (Clark, Robbins, Ersche & Sahakian, 2006; Morgan, McFie, Fleetwood, & Robinson, 2002; Morgan et al., 2006; Quednow et al., 2007) and impaired executive functions including decision making and attention (Morgan et al., 2006; Quednow et al., 2007). As previously noted, the most consistent neurochemical outcome stemming from repeated or high doses of MDMA exposure in rat subjects is a reduction in markers of 5-HT neurotransmission. It has been commonplace for researchers to draw on these neurochemical studies using rats and attribute MDMA's disruptive effects on cognition in human subjects to compromised 5-HT function. For example, Quednow et al. (2007) investigated impulsivity and decision-making in a group of heavy ecstasy users. This study employed remarkably strict inclusion criteria, recruiting individuals that used MDMA 50 or more times over the past year, had less history with other psychotropic drugs other than MDMA, had no family history of a severe mental illness, no current mental illness and no history with legitimate psychotropic medication as well as produce a clean drug test by way of urine sample. Using the go/no go task to measure impulsivity, and an adaptation of the Iowa gambling task to measure decision making, they found that heavy ecstasy users were significantly impaired on both tasks compared to drug naïve participants. Quednow et al. (2007) interpreted these findings in line with neurochemical studies using animal subjects that present evidence of 5-HTergic dysfunction following chronic MDMA exposure and argued that the deficits observed were consistent with a 5-HT deficit. However, without the ability to directly measure 5-HT integrity when employing human participants, it is difficult to be confident when considering the source of the various cognitive deficits associated with MDMA use. Furthermore, neurochemical studies suggesting that MDMA is neurotoxic to 5-HT neurons in rats use extremely high doses of MDMA which are generally well higher than those taken by human MDMA users (e.g. Battaglia et

al.,1987). Behavioural studies in rats using doses of MDMA that are comparable to those taken by human users are therefore important for investigating the neurological changes responsible for the chronic cognitive deficits observed following MDMA exposure.

Although some of the evidence is mixed, cognitive changes stemming from prior administration of MDMA in rats is fairly consistent with the human literature. Previous MDMA exposure in rats has been associated with decreased sustained attention (Dalley et al., 2007; Piper et al., 2005), impaired effort-based decision making (Schulz, Becker, Nagel, Ameln-Mayerhofer, & Koch, 2013) and impaired memory performance, which is characterised by an increase in reference as opposed to working memory errors (Able et al., 2005; Harper et al., 2013; Kay et al., 2011; Piper & Meyer, 2004; Skelton et al., 2008; Sprague et al., 2003; Vorhees et al, 2004). Again, although researchers typically attribute these cognitive impairments to 5-HT dysfunction, there is evidence that MDMA's effects on memory performance involve non-5-HT mechanisms. For example, rats repeatedly administered MDMA for four consecutive days demonstrated a pronounced 5-HTergic deficit seven days after the drug treatment, yet these 5-HT depleted animals do not display a memory deficit on passive avoidance learning when tested at this time (Artaiz, Del Rio, & Lasheras, 1996; Barrionuevo, Aguirre, Del Rio, & Lasheras, 2000; Moyano et al., 2005), although impairments were observed on the days on which rats were treated with MDMA. What is more, other experimental manipulations causing compromised 5-HT integrity, such as treatment with the 5-HT synthesis inhibitor PCPA, have been found to have no disruptive effects on memory performance using passive avoidance learning (Misane, Johansson, & Ove Ogren 1998; Moyano et al., 2004).

While prior MDMA exposure may not always cause lasting memory deficits, such as with passive avoidance learning, indicating that memory function may not be impaired by MDMA-induced disruptions of 5-HT, other studies have found lasting memory impairments following repeated administration of MDMA which may relate to compromised 5-HT function (e.g. Able et al., 2005; Harper et al., 2013; Piper et al., 2004; Skelton et al., 2008; Vorhees et al, 2004). Furthermore this could indicate that the passive avoidance learning task is not sensitive enough to detect long-term cognitive changes caused by MDMA. Thus it is valuable to further investigate the relationships between chronic MDMA exposure, 5-HT function and memory performance using a wider range of behavioural paradigms. However, there may be other factors contributing to MDMA's

disruptive effects on memory given that neurotoxic regimens of MDMA and 5-HT disruptive compounds do not necessarily produce learning and memory deficits in rats.

One marked discrepancy between investigations of human users of MDMA and rats treated with MDMA relates to measures of long-term changes in impulsivity. Despite long-term increases in impulsivity being a major finding in studies of human MDMA users, rats treated with MDMA do not exhibit long-term changes in impulse control (Bird & Schenk, 2013; Dalley et al., 2007; Saadat, Elliott, Green, & Moran, 2006), even after MDMA administration led to long-term decreases in markers of 5-HT transmission (Saadat et al., 2006). However, what animal studies can contribute to the debate regarding chronic MDMA exposure and impulsivity is that individuals with high basal levels of impulsivity (i.e. trait impulsivity rather than manipulating impulsivity by way of lesions or pharmacological application) may be more vulnerable to developing addiction-like behaviours (Bird & Schenk, 2013). Indeed, that impulsivity is a predisposing factor to addiction-like behaviours has been demonstrated using a wide range of drugs of abuse (Jupp, Caprioli, & Dalley, 2013; Molander et al., 2011).

The inconsistent conclusions deriving from human versus rat subjects in terms of MDMA's long-term effects on impulsivity illustrates the limitations to studying long-term drug effects in human users. The inability to control for poly-drug use and pre-existing individual traits when employing human subjects means that the direction of causal relationships, i.e. whether a drug causes a particular effect or conversely whether a trait increases the likelihood of drug taking, may be misinterpreted. With this in mind, the acute effects of MDMA on cognition, discussed below, were gleaned from studies using non-human animal subjects.

Cognitive changes associated with acute MDMA exposure: Proactive interference (perseveration) and DA D1-like receptors. The most frequent cognitive deficit reported subsequent to acute MDMA exposure in rats is impaired memory performance. Memory performance deficits following acute MDMA administration have been observed using a variety of procedures including DMTS (Harper, 2013; Harper et al., 2005; 2006), the radial arm maze (Braida et al., 2002; Harper et al., 2011; Kay et al., 2010) passive avoidance learning (Moyano et al., 2004; 2005), the double Y-Maze (Young, McGregor, & Mallet, 2005), repeated acquisition procedures (Galizio et al., 2009; 2014) and the olfactory Span Task (Hawkey et al., 2014). As previously noted, the

memory deficits seen on these tasks tend to emerge, or are at the greatest magnitude, when MDMA is administered systemically at a dose of ~3 mg/kg (Galizio et al., 2009; 2014; Harper, 2013; Harper et al, 2005; 2006; Hawkey et al., 2014; Kay et al., 2010), with lower doses often leaving performance intact (Frederick, Gillam, Allen, & Paule, 1995) and higher doses often causing a more general behavioural impairment, meaning subjects can no longer perform the task (Galizio et al., 2014; Kay et al., 2010). Of particular relevance is that when MDMA is administered at a dose of 3 mg/kg, as opposed to at a lower dose, there is a relatively greater increase in MDMA-induced synaptic DA relative to 5-HT measured using microdialysis (Baumann et al., 2007). Correspondingly, the discriminative stimulus properties of MDMA appear to be more DAergically mediated when administered at 3 mg/kg (Harper et al., 2014).

Consistent with the effects of chronic administration of MDMA, acute MDMA has been found to increase reference memory-type errors (Braida et al., 2002; Galizio et al., 2014; Kay et al., 2010). The idea that MDMA impairs reference rather than working memory is relatively recent, possibly because many behavioural tests of memory confound working and reference memory processes. By using carefully designed behavioural studies, researchers can tease apart working and reference memory components in order to study MDMA's effects on memory performance. Harper et al. (2005) used an operant DMTS task in which rats were reinforced when they responded on the lever that they were presented with at the beginning of the trial. Harper et al. (2005) found that MDMA's detrimental effects on accuracy were delay-independent meaning that when there was no delay between sample presentation and choice selection, rats still demonstrated a deficit in performance during acute MDMA exposure. Presumably under conditions of no delay working memory processes are not required for accurate responding. Given that the nature of the memory impairments caused by MDMA could not always be attributed to working memory deficits, alternative avenues have been explored.

Using a version of the radial arm maze that allows for separation of reference versus working memory errors, Kay et al. (2010) found that acute MDMA significantly increased reference memory errors to a greater extent than working memory errors. Akin to reference memory errors on the radial arm maze, Galizio et al. (2014) found that acute MDMA disrupted responding on the stable-response component during a repeated acquisition procedure. By employing alternating multiple schedules, rats were tested on

'place acquisition learning' (where the correct response changes across sessions) as well as 'performance' (where the correct response remains constant across sessions). Galizio et al (2014) found that doses of MDMA which impaired accuracy of place acquisition learning also impaired performance of the previously well-learned response, with the authors interpreting this finding as reflecting a reference memory deficit. That MDMA produces an increase in reference memory errors implies that MDMA interferes with the general rules or strategies required for accurate responding on memory tasks (Kay et al., 2010) while leaving episodic memory for events and stimuli encountered during a session or trial (i.e. working memory) relatively intact.

A possible mechanism underlying the reference memory impairment observed during MDMA exposure has been proposed by Harper and colleagues (2005; 2006; 2013) on the basis of findings using an operant-based DMTS task with rats. At the start of each DMTS trial, one lever was inserted into the chamber (either on the left or right side of the chamber) and rats were trained to press on this lever. After an intervening second lever press on the back chamber lever, rats were presented with both the left and the right front levers (choice phase) and were required to respond on the lever that they were presented with during the sample phase. Acute MDMA administration produced a pattern of errors indicative of perseverative responding, or proactive interference, in that rats made response errors characterised by the repetition of a choice made on the immediately preceding trial, as opposed to the current trial's required response (Harper, 2013; Harper et al., 2005; 2006). When the delay between the sample and choice phases was increased, acute MDMA-induced proactive interference was alleviated, suggesting that the rats could still respond accurately under MDMA exposure, again demonstrating intact working memory processes. Furthermore, Harper (2013) found that the MDMA-induced perseverative pattern of responding was ameliorated via concurrent administration of the DA D1-like receptor antagonist SCH 23390. This finding is interesting because it provides the starting point for a novel exploration of a mechanism by which MDMA may impair behavioural responses at the acute level. That D1-like receptors may be molecular targets through which MDMA induces response perseveration also coincides with previous literature suggesting that D1-like receptors may mediate perseverative responding (Diekamp, Kalt, Ruhm, Koch, & Güntürkün, 2000; Ralph, Paulus, Fumagalli, Caron, & Geyer, 2001; Zahrt, Taylor, Mathew & Arnsten, 1997).

In contrast to the finding that chronic MDMA administration *does not* lead to long-term increases in impulsivity in rats, acute and sub-chronic MDMA exposure *does* tend to increase behavioural impulsivity in rats (Bird & Schenk, 2013; Dalley et al., 2007; van Wel et al., 2012). Impulse control critically depends on intact monoaminergic corticostriatal projections, and in particular on DA systems in the frontal cortex (for a review see Jupp et al., 2013), suggesting that MDMA's acute effects on DA transmission may contribute to the increase in impulsive behaviour observed during drug treatment. Related to the previous discussion of MDMA-induced perseveration, impulsive responding and perseverative responding may involve overlapping neural mechanisms in that they are both intimately related to frontally-mediated executive functions, including attention and decision-making processes (Chadasama et al., 2003; Robbins, Weinberger, Taylor, & Morris, 1996).

The current project aims to expand on the finding that DA D1 receptors may be a target through which MDMA induces response perseveration. However, there are limitations to using pharmacological agents that block or potentiate action at the DA D1 receptor site (discussed below). Recently, a novel genetic animal model was generated that provides an exciting new avenue regarding isolation of DA D1 receptor function. The following sections of this thesis will 1. provide background to genetic animal models used frequently in behavioural neuroscience; 2. present the novel animal model used here – the DAD1$^{-/-}$ rat; 3. discuss relevant functions of D1-like receptors and finally, 4. describe the present investigation.

Using the DAD1$^{-/-}$ Rat Model to Investigate the Acute Effects of MDMA on Memory Performance

A brief history of transgenic mice models and the introduction of rat genetic models. During the 1980s behavioural neuroscientists and geneticists witnessed the development and proliferation of targeted gene mutation technologies in mice. There are now a number of procedural variations used to create transgenic mice, one main procedure being the insertion of mutated complementary DNA (cDNA) into embryonic stem cells (Crawley, 2007). Genetic engineering in mice has enabled researchers to create behavioural models of human psychiatric, neurodegenerative and neurodevelopmental diseases and to further our understanding of how genes shape behaviour (Crawley, 2007). Despite these methodological advances using mice, the ability to target genes in rats has

been hindered, in part, due to their lack of suitable embryonic stem cells. Rats are an extremely valuable model organism and have been used extensively in behavioural and biological research. Rats are larger than mice and have more accessible brains, and many organ specific disease models have been tailored to the rat. Many behavioural paradigms are specially designed and standardised for use with rats and considering the wealth of data generated using such paradigms, genetically modified rat models would be an important tool for behavioural neuroscientists and geneticists alike. What is more, rats and mice have been found to differ in their pharmacological responses to some drugs, such as MDMA, with rats' responses often corresponding to those of human and non-human primate subjects. For example, a well-documented finding is that in rats, humans and non-human primates, exposure to high doses of MDMA compromises 5-HT, but not DA, systems (Battaglia et al .,1987; Benningfield & Cowan, 2013; McCann et al., 1998; Molliver et al., 1990; Parrott, 2002; Schmidt & Kehne, 1990; Souza et al., 1990), yet the converse is true for mice (Colado et al., 2001; Logan, Laverty, Sanderson, & Yee, 1988). Considering these differences, it would be a major advantage to have both rat and mice gene mutant and knockout (KO) models available in order for researchers to select the most appropriate model to suit their investigation.

Using selective inbreeding programmes, many phenotypic rat models have been developed that exhibit biomedical traits relevant for human pathological conditions (Jacob & Kwitek, 2002). It would greatly aid our understanding and treatment of such conditions if we were able to identify the underlying gene mutations. In the early to mid-2000s, the genome of the laboratory rat *Rattus norvegicus* was sequenced by the Rat Genome Sequencing Consortium, highlighting a recent effort to establish the rat as a genetic model. At a similar time, the first strains of KO rat models using N-ethyl- N-nitrosourea (ENU)-driven target selected mutagenesis were created (Smits et al., 2006; Smits, Mudde, Plasterk, & Cuppen, 2004; Zan et al., 2003). The current research serves to report a battery of behavioural experiments conducted using one of these novel rat models: the DA D1 receptor mutant ($DAD1^{-/-}$) rat (Smits et al., 2006). The phenotypic characterisation of this novel rat genotype is of great importance, as it is likely to become a useful tool with which to investigate the behavioural functions of DA D1 receptors.

Dopamine, D1-like receptors and the $DAD1^{-/-}$ rat. DA is the predominant catecholamine neurotransmitter in the mammalian brain where it is involved in the regulation of memory, impulsivity and decision making, reinforcement and motivation, endocrine

regulation, and locomotion. There are four major DA pathways in the central nervous system (CNS; figure 2). DA cell bodies located in the ventral tegmental area (VTA) project to limbic regions, including the Nacc and septal nuclei, and to the frontal cortex, comprising the mesolimbic and mesocortical pathways, respectively. Primarily, mesocorticolimbic systems mediate DA's role in reward and the attribution of incentive salience to reward-related stimuli (Berridge, 2007; Pierce & Kumaresan, 2006), as well as learning and memory processes (Cools & D'Esposito, 2007). Projections originating from DA cell bodies located in the substantia nigra pars compacta (SNPc) innervate the dorsal striatum and form the nigrostriatal pathway. The nigrostriatal pathway is involved in motor control and has been extensively studied due to its role in movement disorders such as Parkinson's disease (Deumens, Blokland, & Prickaerts, 2002). A fourth, less infamous, DA tract is the tuberoinfundibular pathway which projects DA from the hypothalamus to the pituitary gland (Carson et al., 1977), playing a role in hormone regulation and maternal behaviour.

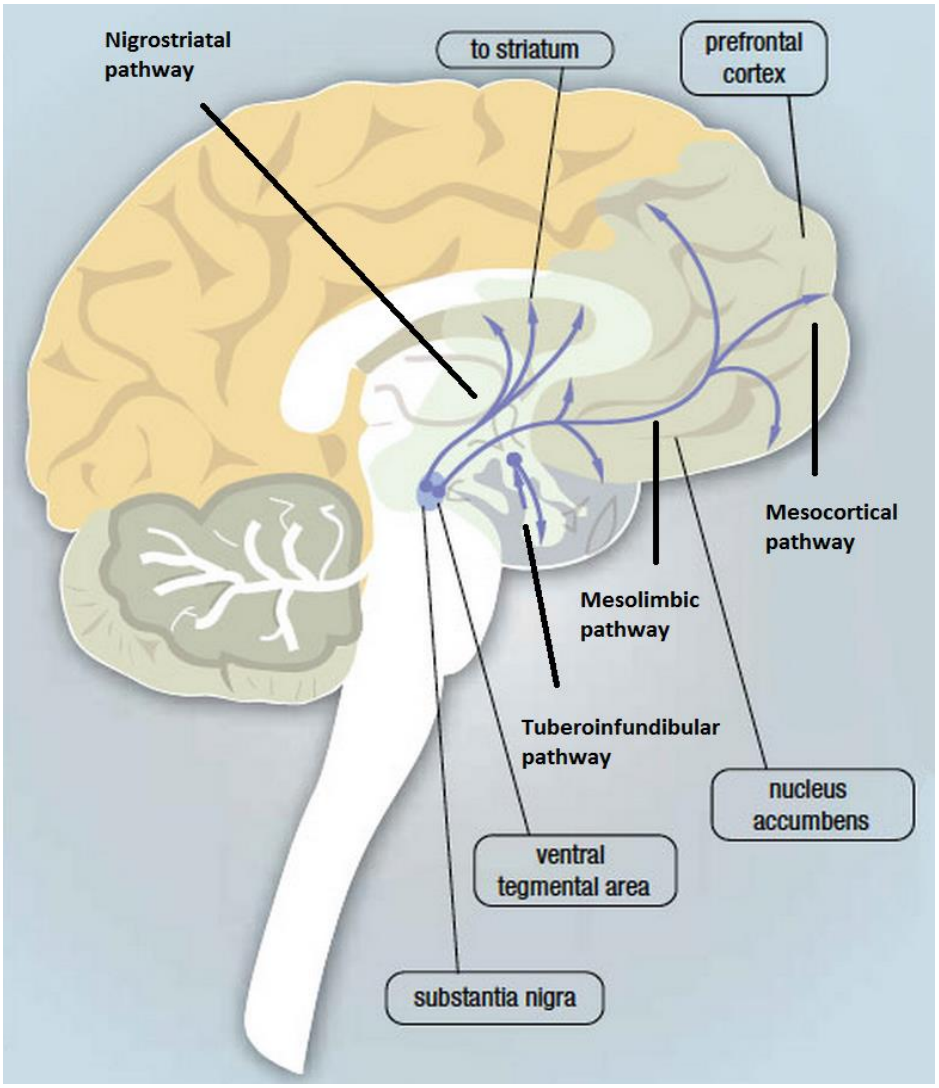

Figure 2. Schematic of the four main DA projection pathways as viewed from the mid-sagittal section in a human brain. Image was adapted from National Institutes of Health (2014).

DA is regulated by five isolated G-protein coupled receptors, which have been subdivided into two receptor sub-families based on these receptors' biochemical and pharmacological properties (reviewed in Missale, Nash, Robinson, Jaber, & Caron, 1998; Jaber, Robinson, Missale, & Caron, 1996; Vallone , Picetti, & Borrelli, 2000). The D1-like receptor sub-family comprises the D1 and D5 receptors; whereas the D2-like receptor sub-family includes the D2, D3 and D4 receptors.

D1- and D2-like receptors often appear to regulate DA through antagonistic, or opponent, processes. Firstly, D1- and D2-like receptor activation has contrasting effects on second messenger systems. While D1-like receptors activate adenylate cyclase and are

therefore positive regulators of intracellular cyclic AMP (cAMP), ultimately facilitating neurotransmitter release (Cameron & Williams, 1993), D2-like receptors inhibit adenylate cyclase and the production of cAMP, thereby decreasing cellular firing (Missale et al., 1998). Secondly, DA neurons display two modes of transmitter release, characterised by either tonic (sustained or background) cellular firing, versus phasic (transient or bursting) cellular firing (Goto, Otani & Grace, 2007; Grace, 1991; 1995). Tonic DA levels are found to be consistently present in low concentrations in the synaptic cleft, whereas phasic spike-dependent DA release results in high concentrations of extracellular DA which is rapidly inactivated via re-uptake (Cohen, Braver, & Brown, 2002; Goto et al., 2007). There is evidence to suggest that these dissociable patterns of cell firing differentially involve D1-and D2-like receptors. Pharmacological manipulation of D1-like receptors results in changes in tonic DA release, whereas phasic bursts of DA release may more readily activate D2-like receptors (Cohen et al, 2002). While the precise contributions to behaviour made by tonic versus phasic firing remain elusive, it has been proposed that phasic firing may occur in response to behaviourally relevant external stimuli, with tonic DA predominantly playing a regulatory role, and being active under basal conditions (Cohen et al., 2002; Grace, 1995).

A final example of the antagonistic relationship between D1- and D2-like receptors relates to the two separate striatal populations of GABA-containing medium-sized spiny projection neurons (MSNs). Striatonigral MSNs comprise what is known as the 'direct pathway' which contains neurons that selectively express D1-like receptors and substance P. By contrast, striatopallidal MSNs form the 'indirect pathway' and express D2-like receptors and enkephalin (Smith, Beyan, Shink, & Bolam, 1998; Yung, Smith, Levey, & Bolam, 1996). These pathways have been found to play opposing roles in various functions that are orchestrated by basal ganglia structures, including motor control (Groenewegen, 2003), reward and aversive learning (Hikida, Kimura, Wada, Funabiki, & Nakanishi, 2010; Kravitz, Tye, & Kreitzer, 2012) and drug-related behaviours (Lobo & Nestler, 2011). As one example, behavioural sensitisation produced by repeated administration of AMPH was attenuated following transient inhibition of D1-like expressing MSNs, yet facilitated following inhibition of D2-like expressing MSNs (Ferguson et al., 2011).

Given the close functional ties between D1- and D2-like receptors, as well as the complex nature of DA signalling more generally, determining the behavioural functions of individual DA receptors can be difficult. Recognising this caveat, our current understanding of the roles played by DA and D1-like receptors in behavioural processes is presented below.

D1 receptors are the most highly expressed of the DA receptors (Dearry et al., 1990; Weiner et al., 1991) and have widespread distribution throughout the CNS, in particular within DA's projection pathways. D1 receptor mRNA has been detected in many subcortical brain structures, including the caudate-putamen, Nacc and olfactory tubercle, and to a lesser extent in the limbic system, cerebral cortex, amygdala, thalamus and hypothalamus (Fremeau et al., 1991). These findings were further substantiated using D1 subtype-specific antibodies, with the exception of the thalamus and hypothalamus (Levey et al 1993). It is not surprising, then, that D1 receptors have been implicated in a broad range of cognitive and behavioural processes such as working memory (Rieckmann, Karlsson, Fischer, & Backman, 2011, Muller, Cramon, & Pollmann, 1998), motivation (Spina et al., 2010) and movement (Plaznik, Stefanski, & Kostowski, 1989).

The most widely used method to investigate the behavioural functions of brain receptors in rats is the assessment of behaviour following administration of pharmacological agents, or ligands, that potentiate (i.e. an agonist) or block (i.e. an antagonist) the function of a given receptor. A limitation of this approach when applied to D1 receptors is that D1 receptor ligands will bind to both D1 and D5 receptors, as well as other non-DA receptors (e.g. Ramos, Goni-Allo, & Aguirre, 2005), and currently there are no pharmacological agents that can selectively bind to D1 receptors. The lack of pharmacological specificity of D1-like receptor ligands constrains our ability to determine the separate roles of the D1 and D5 receptors despite the fact that these receptors appear to perform distinct functions in the brain (e.g. Centonze et al., 2003).

The DAD1$^{-/-}$ rat provides researchers with an unprecedented method by which to examine the behavioural functions of D1 receptors in rats. Using [3H]SCH23390 autoradiography, Homberg et al. (in press) observed down-regulated D1 receptor function in the DAD1$^{-/-}$ rats, with [3H]SCH23390 binding to DAD1$^{-/-}$ brain slices decreased by 50% in the PFC, subcortical regions, substantia nigra (SN) and by 20% in the olfactory tubercle. D5 receptors in this rat are intact meaning that DAD1$^{-/-}$ rats can be used to examine the behavioural roles that D1 receptors normally performs devoid of D5 receptor involvement. Down-regulated D1 receptor function in DAD1$^{-/-}$ rats is further supported by the finding that cocaine-induced locomotor activity was strongly reduced in these animals (unpublished data, reported in Muller, Olivier, & Homberg, 2010). Not only does the DAD1$^{-/-}$ rats model circumvent the issue posed by a lack of pharmacological agents specific for D1 vs. D5

receptors, but by being a mutant model leading to reduced D1 function rather than a complete KO, this tool might also be of more relevance to humans.

Although the DAD1$^{-/-}$ rat is a novel model of down-regulated D1 function and there are no published articles using this strain as of yet, there are some preliminary findings on the nature of the DAD1$^{-/-}$ rats reported from our laboratory and a collaborating laboratory. Physically, DAD1$^{-/-}$ rats weigh 15-25% less than littermate wild type (DAD1$^{+/+)}$ and heterozygous DA D1 (DAD1$^{+/-)}$ rats but no other gross physically abnormalities are apparent. Behaviourally, compared to wild types the DAD1$^{-/-}$ rats performed normally on the elevated plus maze, implying no differences between groups in levels of anxiety. Performances in the Morris water maze did differ, however, with DAD1$^{-/-}$ demonstrating slower swimming speeds and a possible memory deficit. However, overall there is a paucity of behavioural data using the DAD1$^{-/-}$ rats. Therefore, the current project will conduct a behavioural battery to investigate the behavioural tendencies of this strain and to assess their usefulness as a behavioural animal model. We selected tasks based on evidence, described below, suggesting that D1-like receptors play a regulatory role in motivation and reward, movement, and memory processes.

Dopamine & D1-like receptors in motivation and reward: Attribution of incentive salience. Despite mesolimbic DA projections often being branded the 'reward pathway' of the brain, this label has been heavily criticised as it fails to recognise the specific nuances of DA's role in reward processing (Salamone & Correa, 2012). Many stimuli perceived as pleasurable or rewarding activate mesolimbic DA (Di Chiara & Imperato, 1988; Phillips, Robbins, & Everitt, 1994; Tanda & Di Chiara, 1998) yet the precise role played by DA in reward has been highly contested for several decades. In a review by Berridge (2007) it was argued that there are three main explanatory categories of the causal role played by DA in reward, namely which argue that mesolimbic DA either mediates 1. the hedonic impact of reward ('*liking*'); 2. learned predictions of future rewards and prediction-error signals ('*learning*') or; 3. motivation to pursue rewards through the attribution of incentive salience to reward-related stimuli ('*wanting*').

The anhedonia hypothesis (Wise, 1982; 1985), which held that mesolimbic DA stimulation produced subjective feelings of pleasure, was formed on the basis of several observations. Firstly, in 1954, Olds and Milner presented the earliest demonstration of brain stimulation reward. Olds and Milner (1954) showed that electrical stimulation of the

septal area, a limbic region that receives DA projections from the VTA, produced rewarding effects, or positive reinforcement, which gave rise to acquisition and extinction curves for operant lever pressing in much the same way as a primary reward. This landmark finding appeared to suggest that there were 'reward centres' in the brain and provided a basis for studying the neurological mechanisms of reward. Secondly, Wise (1982) reported that acute administration of neuroleptic drugs, which decrease mesolimbic DA transmission, in rats led to anhedonia-like behaviours, and disrupted positively reinforced responding. The evidence in favour of DA as a substrate for reward led to the notion that DA signals correspond to the hedonic properties of rewards (Wise, 1982; 1985). In the 1980s the hedonia hypothesis of DA function was very influential in neuroscience and indeed remnants of the idea that DA acts as a 'liking' signal (or reward neurotransmitter) is still present in popular culture and even some scientific literature today.

One major caveat of the hedonia hypothesis is that it cannot account for the observation that the onset of a DA signal in response to the presentation of a rewarding stimulus changes with repeated exposure to that reward. When a rewarding stimulus is encountered for the first time, limbic neurons are activated with close temporal proximity to the presentation of the rewarding stimulus (thought to signal 'liking') (Wise, 1982; 1985). Upon successive presentations of the same rewarding stimulus, the corresponding DA signal can come to occur in anticipation of the reward, in response to the presentation of predictive conditioned stimuli (CS) (de la Fuente-Fernandez et al., 2002; Schultz, 1998; 2004; Schultz, Dayan, & Montague, 1997). Given that the reward is still 'liked' despite the presentation or consumption of the reward not corresponding to DA signalling implies that the hedonic component of reward is mediated by neural mechanisms other than mesolimbic DA.

The observation that mesolimbic DA neurons respond to stimuli that are predictive of reward formed the basis of prediction error hypotheses of DA function (Schultz, 1998; 2004; Schultz et al., 2007). Largely based on electrophysiological, neurotransmitter release and imaging data, prediction error hypotheses suggest that DA codes for reward learning. In other words, learning accounts of DA's function in reward hold that DA modulates synaptic plasticity that supports the neural representations of S-S (stimulus-stimulus) or S-R (stimulus-response) associations (Everitt, Dickinson, & Robbins, 2001; Schultz, 1997; 2004; Schultz et al., 1997). Such theories imply that

normal learning could not occur in the absence of DA. However, using a genetic mouse model lacking the enzyme tyrosine hydroxylase (meaning that these mice cannot synthesise DA), Hnasko, Sotak, and Palmiter (2005) and Robinson, Sandstrom, Denenberg, and Palmiter (2005) demonstrate that S-S and S-R links can be formed in the absence of intact DA systems in T-maze and conditioned place preference (CPP) tasks. Furthermore, pharmacologically-induced mesolimbic lesions in rats did not inhibit the production of conditioned taste aversion (Berridge & Robinson, 1998), further suggesting that DA is not necessary for new reward learning. Additionally, there is evidence indicating that manipulations affecting mesolimbic DA transmission lead to behaviour changes consistent with the idea that DA mediates 'wanting' for rewards, rather than learning about rewards. For example, hyper-DAergic mice display a greater willingness to work for rewards on a progressive ratio schedule, yet did not appear to like the food rewards more and did not display enhanced learning, compared to normal mice (Cagniard, Balsam, Brunner, & Zhuang, 2005; Yin, Zhuang, & Balleine, 2006).

The incentive salience hypothesis of DA's role in reward can account for the fact that many manipulations of brain DA seem to alter 'wanting' for rewards, whilst leaving 'liking' and 'learning' intact. Incentive salience posits that DA mediates the attribution of motivational value to rewards and reward-related stimuli, or 'wanting' for rewards (Berridge 2007; Berridge & Robinson, 1998).Within the incentive salience framework, mesolimbic DA is a neural signal which assigns conditioned motivation to conditioned stimuli (CS), or stimuli that are associated with rewards. In the first instance, 'wanting' is attributed to a neutral CS due to an associated unconditioned stimulus (UCS; i.e. the reward) producing a 'liked', hedonic response (via non-DA mechanisms). Once a CS has acquired motivational value, it can act to powerfully modulate cue-triggered responding (e.g. priming; Berridge, 2004), as well as acting to enhance a subject's motivation to acquire the associated reward (Di Ciano, Underwood, Hagan, & Everitt, 2003). A consequence of the attribution of incentive salience to a CS is that this CS can also become 'wanted' by the subject, sometimes resulting in approach or consummatory behaviour directed toward the CS (e.g. Uslaner, Acerbo, Jones, & Robinson, 2006).

As well as accounting nicely for behaviours relating to the receipt of 'natural' primary rewards, the incentive salience hypothesis has been extended to apply to drug rewards in a theory called the incentive sensitisation hypothesis (Robinson & Berridge, 2008; Robinson et al., 1993). Incentive sensitisation explains the finding that mesolimbic

DA systems develop neural sensitisation in response to repeated drug exposure, corresponding to parallel increases in 'wanting' or craving (behavioural sensitisation) for the drug and drug-associated stimuli (Anderson & Pierce, 2005; Lett, 1989; Vezina, 2004).

While certain aspects of mesolimbic DA functioning are still contested, the incentive salience account certainly presents a good case for DA acting to mediate the attribution of motivational value to relevant reward stimuli. What then is the current picture of the contributions made by D1-like receptors in the context of reward? When applied systemically, D1-like receptor antagonists decrease operant responding for rewards including food, deep brain stimulation, self-administered drugs and conditioned rewards, as well as the expression of CPP (Acquas, Carboni, Leone, & Di Chiara, 1989, Anderson, Bari, & Pierce, 2003; Beninger et al., 1987; Beninger & Miller, 1998; Nakajima, 1986). Intracerebroventricular D1-like receptor blockade has also been found to prevent CPP which was previously elicited using both ethanol and acetaldehyde in rats (Spina et al., 2010). Similarly in D1 receptor deficient mice, El-Ghundi, O'Dowd, Erclik, & George (2003) found an attenuation of responding for sucrose in an operant conditioning task. These findings broadly suggest that D1-like receptor activation is necessary for the performance of reward-directed behaviours. It is interesting that the converse effect on behaviour is not found using D1-like receptor agonists. In general, systemic administration of D1-like receptor agonists disrupts operant responding for rewards (Beninger & Rolfe, 1995; Hoffman & Beninger, 1989a; Katz & Witkin, 1992; Ranaldi, Pantalony, & Beninger, 1995), often does not produce CPP and can even produce conditioned place aversion (CPA) (Hoffman & Beninger, 1989b; White, Packard, & Hiroi, 1991). However, there have been exceptions regarding findings using CPP, with Abrahams, Rutherford, Mallet, and Beninger (1998) testing an array of D1-like receptor agonists at various doses, and finding that SKF 82958 at a dose of 0.05 mg/kg produced CPP. Furthermore, it has been argued that CPA by systemic D1-like agonists may be caused by the non-specific effects of these agents (White et al., 1991).

On the face of it, the finding that systemic administration of D1-like receptor antagonists *and* agonists can attenuate the performance of reward-related behaviour appears contradictory. However, Beninger and Miller (1998) present a compelling account for why activation of D1-like receptors using systemic D1-like agonists might impair responding on some reward paradigms. Briefly, Beninger and Miller (1998) argue

that under drug-free conditions the receipt of a reward or the presentation of CS that predict reward generate an intense and short-lived peak of mesolimbic DA release in the synapse which subsequently interacts with D1-like receptors – this is termed a 'DA signal'. When a direct acting D1-like receptor agonist is administered, it continuously binds to D1-like receptors rather than mimicking the time-course of a genuine DA signal produced by, or associated with, reward stimuli. In this way, Beninger and Miller (1998) suggest that medium to high doses of D1-like receptor agonists obscure the DA signal associated with natural reward stimuli. They argue that some reward paradigms are therefore more likely to be disrupted by D1-like agonists, such as in operant reinforcement where the presentation of a given stimulus comes to control behaviour and the timing of the DA signal is crucial. On the contrary a paradigm such as CPP, which does not rely on the timing of the DA signal to elicit a reward response, would be less disrupted by D1-like agonists. This explanation can account nicely for findings from studies using systemic application of D1-like receptor agonists, which find that D1-like agonists disrupt operant responding for reward (Beninger & Rolfe, 1995; Hoffman & Beninger, 1989a; Katz & Witkin, 1992; Ranaldi et al., 1995), yet that a D1-like agonist has been reported to produce CPP (Abrahams et al., 1998).

As well as reward, D1-like receptors are also involved in movement and memory processes (discussed below). Because D1-like receptor ligands are not solely selective for D1 receptors, potential global effects of systemic administration of D1-like receptor agents cannot always be ruled out. By using local administration of D1-like receptor agonists or antagonists, the role of D1-like receptors in reward have been more finely dissected (Andrzejewski, Spencer, & Kelley, 2006; Baldo, Sadeghian, Basso, & Kelley, 2002; Ikemoto, Glazier, Murphy, & McBride, 1997; Koch, Schmid, & Schnitzler, 2000; Nowend, Arizzi, Carlson, & Salamone 2001; White et al., 1991). In particular, activation of D1-like receptors in the Nacc, and more so in the shell than in the core, appears to be important for reward processing. The Nacc is in a prime anatomical position to act as an interface between motivation and action (Robbins & Everitt, 1996), given that it receives DAergic innervation from the VTA and projects via the ventral pallidum to premotor and cortical motor areas (Mogenson, Brudzynski, Wu, Yang, & Yim, 1993). Intra-accumbal administration of the D1-like receptor antagonist SCH 23390 disrupts conditioned operant responding for palatable rewards (i.e. sugar pellets), but also coincides with a compensatory increase in consumption of freely available, but less preferable, chow (Koch et al., 2000;

Nowend et al., 2001), indicating that D1-like receptors in the Nacc are not required for consummatory behaviour. Nowend et al. (2001) also found that after intra-accumbal infusion of SCH 23390 rats still displayed a preference for palatable food in a free choice test suggesting that the hedonic evaluation of the reward is intact. Furthermore, although systemic application of SKF 38393 produced CPA, intra-accumbal infusion of this drug produced CPP (White et al., 1991). Taken together, these studies suggest that mesolimbic D1-like receptors are required for behaviours relevant for the receipt of natural rewards.

Extending these findings to addiction research, low doses of the D1-like receptor agonist SKF 81297 were self-administered by rhesus monkeys (Weed, Vanover, & Woolverton, 1993). However, Ikimoto et al. (1997) found that SKF 38393 (a less selective D1-like receptor agonist compared to SKF 81297) was not self-administered by rats and only when SKF 38393 was mixed with equimolar proportions of quinpirole, a D2-like agonist, would the rats acquire self-administration. Although D1-like receptors are often thought to play a more critical role in reward than D2-like receptors (reviewed in Beninger & Miller, 1998) some studies demonstrate synergistic effects of D1- and D2-like receptors in the Nacc, for example in terms of primed reinstatement of drug-seeking using self-administration (Schmidt, Anderson, & Pierce, 2006). However, SCH 23390 administered into the Nacc shell attenuated cocaine-primed drug seeking in rats without the co-administration of a D2-like receptor antagonist (Anderson et al., 2003). These findings indicate that D1-like receptors are involved in drug-associated reward behaviours.

Taken together, mesolimbic D1-like receptors appear critical for reward processing in a manner consistent with the incentive salience hypothesis (Berridge 2007; Berridge & Robinson, 1998). To determine whether the down-regulation of functional D1 receptors in DAD1$^{-/-}$ rats leads to an impairment in reward processing, specifically in terms of reward 'wanting', DAD1$^{-/-}$ rats will be compared with controls in terms of sucrose preference, latency to consume freely available sugar pellets, and lever pressing for sugar pellet reinforcement in an operant chamber.

Dopamine & D1-like receptors in movement. Research investigating the motor deficits caused by Parkinson's disease (PD) has firmly established a role for DA and D1-like receptors in movement processes. PD is characterised by the neurodegeneration of DA neurons, mainly in the SNPc, leading to drastically reduced DA neurotransmission in basal ganglia motor circuits leading to severe disturbances of voluntary motor control

(Anglade et al., 1997; Jankovic, 2008). As well as highlighting an important role for DA in the execution of movement, PD research has also shown that D1 signalling is an essential component of motor output.

Firstly, rats with unilateral 6-hydroxydopamine (6-OHDA) lesions of the nigrostriatal pathway are widely used as a model for PD-like symptoms (Deumens et al., 2002; Lundblad et al., 2002; Truong, Allbutt, Kassiou, & Henderson, 2006). Using 6-OHDA lesioned rats, Gulwadi et al. (2001) found that the selective D1-like receptor agonist, dinapsoline, effectively reversed impaired motor control. Although human PD patients do not respond as positively to D1-like receptor treatment, possibly due to a lack of ligand specificity as well as the development of rapid tolerance to some D1-like compounds (Asin & Wirtshafter, 1993), some clinical trials using selective D1-like agonists to treat PD patients have been met with success (e.g. Rascol et al., 1999).

Secondly, levodopa (L-DOPA), a precursor for DA which can cross the blood-brain barrier, is a common drug treatment for PD. While initial L-DOPA administration is effective at alleviating PD symptoms, chronic use can result in the development of L-DOPA-induced dyskinesia (LID), which is a debilitating side-effect of L-DOPA treatment. LID is characterised by involuntary motor fluctuations where movement is flowing and dance-like, with patients appearing to be writhing or twisting. The expression of LID is linked to over-active D1 receptor signalling in the direct striatonigral pathway (Aubert et al., 2005; Darmopil, Martin, de Diego, Ares, & Moratalla, 2009), demonstrating that both under- and over-activation of D1-like receptors can lead to movement disturbances.

Evidence from PD research strongly suggests that nigrostriatal D1-like receptor activation is required for controlled movement. Consistent with this evidence is that pharmacological manipulation of D1-like receptors via application of D1-like receptor agonists and antagonists produce dose-dependent increases (Ralph & Caine, 2005; Schindler & Carmona, 2002) or decreases (Hoffman & Beninger, 1985; Meyer, Cottrell, Van Hartesveldt, & Potter, 1993), respectively, in spontaneous locomotor activity in rats. That D1-like receptors are an important neural substrate for movement is relevant for the current investigation of the acute behavioural effects of MDMA for several reasons. Firstly, many (if not all) behavioural tasks, including those used for the current investigation of memory performance, involve a movement component. In the context of a maze- or operant-

based memory task, if the DAD1$^{-/-}$ rats were to have altered baseline levels of motor output, for instance if they moved less than control rats, then this could decrease their accuracy on the memory task by increasing the delay between acquisition and retrieval of memories for task-relevant stimuli. Secondly, acute MDMA administration reliably produces hyperlocomotion, with D1-like receptors playing a role in this effect (Ball, Budreau, & Rebec, 2003; Bubar, Pack, Frankel, & Cunningham, 2004). If MDMA-induced hyperlocomotion were to interfere with the subjects' performance on the behavioural tasks, and if DAD1$^{-/-}$ rats have a reduced locomotor response to acute MDMA compared to control rats, task performance could be more greatly affected in the control rats than DAD1$^{-/-}$ rats through mechanisms other than memory function or proactive interference. Given these potentially confounding factors, *chapter 4* will investigate the locomotor tendencies of DAD1$^{-/-}$ rats compared to controls in a drug-free state, and *chapter 5* will examine whether the DAD1$^{-/-}$ rats display an altered locomotor response to acute MDMA compared to controls in the context of an immunohistochemical analysis of MDMA-induced *c-fos* expression.

Dopamine & D1-like receptors in memory. Memory involves the set of processes by which organisms encode, store and retrieve information (Melton, 1963). Both the HPS and PFC have been extensively studied for their roles in memory and cognition, with mesocorticolimbic DA systems having important neuromodulatory functions in these areas (PFC: Goldman-Rakic, 1995; HPS: Lisman & Grace, 2005). D1-like receptors are expressed in both the HPS (Fremeau et al., 1991) and PFC (Muly, Szigeti, & Goldman-Rakic, 1998) where they mediate diffuse inputs from DA midbrain neurons and contribute to memory function.

Investigators of memory function seek to understand how experience comes to modify the brain in order to form a neural representation of this in-coming information. One form of neuroadaption relevant for the encoding of memories is synaptic plasticity. The strengthening or weakening of synaptic connections in the HPS by way of long-term potentiation (LTP) or long-term depression (LTD), respectively, is thought to underlie the encoding of long-term memories (Bliss & Collingridge, 1993; Lemon & Manahan-Vaughan, 2006). There is clear evidence that DA affects the induction of LTP and LTD (Lisman & Grace, 2005; Thomas & Malenka, 2003; Sajikumar & Frey, 2004) and that activation of D1-like receptors is involved in these processes. For example, application of D1-like agonists can enhance LTP in the HPS (Li, Cullen, Anwyl, & Rowan, 2003), whereas the administration of D1-like antagonists block the expression of LTP (Frey,

Matthies, Reymann, & Matthies, 1991). Similarly, in vitro analysis of CA1 neurons in rat HPS slices demonstrates that activation of D1 receptors enhances LTD and D1-like antagonism blocks the induction of LTD (Chen et al., 1996). In sum, these studies suggest that D1-like receptors in the HPS are important for synaptic plasticity that may underlie the formation of long-term memories.

In the PFC, which orchestrates working memory processes (Braver et al., 1997; Goldman-Rakic, 1995), the level or amount of DA transmission appears to be critical for optimal working memory function. Specifically, too much or too little DA stimulation in the PFC leads to impairments in working memory in humans (Cools & D'Esposito, 2011), non-human primates (Vijayraghavan, Wang, Birnbaum, Williams, & Arnsten, 2007; Williams & Goldman-Rakic, 1995) and rats (Seamans, Floresco, & Phillips, 1995; 1998; Zahrt et al., 2007). That under- or over-stimulation of DA in the PFC impairs working memory forms the basis of the inverted-U theory of PFC DA transmission and it has since been demonstrated that, in particular, D1-like receptor stimulation mediates this effect. In a seminal study, Williams and Goldman-Rakic (1995) used local iontophoretic application of the D1-like receptor antagonist SCH 39166 in the dorsolateral PFC of monkeys to investigate the cellular basis of working memory. When SCH 39166 was applied at a dose just above threshold for effect, this resulted in the enhancement of neuronal memory fields by increasing firing selectively for the preferred direction of pyramidal cells during the delay period of the oculomotor delayed response task. Memory fields have been defined as the maximal firing of a neuron during a working memory task delay period, in response to the presentation of a stimulus in a given location of the subjects' visual field. Using the same procedure, they then found that SCH 39166 actually abolished these memory fields when further iontophoretic application was applied. These findings led Williams and Goldman-Rakic (1995) to suggest that dopaminergic signalling within the dorsolateral PFC must fall between an optimum range in order to be beneficial to resulting memory performance.

Since the discovery that D1-like receptors may mediate the relationship between DA transmission in the PFC and working memory performance in primates, this finding has also been supported using rats. Zahrt et al., (1997) trained rats on a delayed-alternation task in a T-maze, where subjects were reinforced for alternating their arm visits with a delay period between each arm visit. The length of the delay period was adjusted for each subject to produce ~ 80 % accuracy (delays ranged from 5-30 seconds)

in order to be able to detect both improvements and impairments following drug. The rats were then administered microinfusions of saline, 0.05 or 0.5 μg/0.5 μl of the D1-like agonist SKF 81297 into the PFC prior to the start of testing sessions. Zahrt et al. (1997) found that after the highest dose of SKF 81297 performance was significantly impaired, yet after the lower dose of SKF 81297 performance was not significantly affected. The impairment of delay-related spatial working memory was subsequently reversed when the 0.5 μg/0.5 μl dose of SKF 81297 was concurrently applied with the D1-like antagonist SCH 23390 (0.03 mg/kg i.p.), suggesting that the effects on memory were due to excess stimulation of D1-like receptors rather than non-specific effects. Importantly, when SCH 23390 0.03 mk/kg was administered alone performance was unimpaired yet using a higher dose, 0.035 mg/kg, SCH 23390 significantly impaired accuracy (Zahrt et al., 1997). Other studies using rats have further demonstrated that when SCH 23390 is administered directly into the PFC, spatial working memory performance is impaired (Seamans et al., 1995; 1998). Taken together, these findings suggest that over- and under-stimulation of D1 receptors in the PFC can impair working memory processes in primates and rats. In light of evidence suggesting that D1-like receptors are integral for memory processes, the memory performance of DAD1$^{-/-}$ rats while in a drug-free state will be compared to control rats in *chapter 3.*

The inverted-U relationship between D1-like receptor activation in the PFC and working memory function has shed light on the potential role of DA and D1-like receptors in working memory. In order for accurate responding on working memory tasks, stimuli are required to be held 'online' during the delay period because there are no stimuli presently available to guide responding. During the brief delay periods on working memory tasks PFC DA neurons are highly active, and appear to contribute to the active retention of task-relevant information (Kubota & Niki, 1971; for a review see Goldman-Rakic, 1995). As well as possibly contributing to the generation of an internal representation of to-be-remembered stimuli, DA neurons in the PFC inhibit spontaneous or background activity within the PFC (Ferron, Thierry, Le Douarin, & Glowinski, 1984; Mantz, Milla, Glowinski, & Thierry, 1988). These findings suggest that PFC DA, mediated by D1-like receptors, may act to selectively enhance task-relevant or salient information relative to background activity or spontaneous inputs in the PFC (Seamans et al., 1998). Consistent with this idea, application of D1-like receptor antagonists decrease the effectiveness of delay-period activity relative to background noise activity, leading to

more random modes of behaviour (Seamans et al., 1998). On the contrary, over-stimulation of D1-like receptors would be expected to strongly inhibit PFC inputs in a manner that might restrict behavioural output. Accordingly, in Zahrt et al.'s, (1997) study, outlined above, it was reported that D1-like agonist administration into the PFC impaired delayed alternation by increasing response perseveration. That D1-like receptor activation in the PFC induced response perseveration is notably consistent with the main hypothesis of the current study – that acute MDMA impairs memory by increasing response perseveration via over-activation of D1 receptors. In order to investigate this hypothesis, memory performance following acute MDMA administration will be examined in *chapter 5.*

The Current Study

The present investigation employs the novel DAD1$^{-/-}$ rat model of down-regulated DA D1 receptor function to investigate the roles played by DA D1 receptors in some of the behavioural and neurochemical effects of acute MDMA administration. In the three ensuing experimental chapters (summarised below) the central questions explored are:

1. When in a drug free state do DAD1$^{-/-}$ rats display an altered behavioural phenotype compared to control rats? (*Chapter 3*)
2. Does a genetic reduction of functional D1 receptors lead to an altered neurochemical response to systemically applied acute MDMA compared to control rats? (*Chapter 4*)
3. Do DAD1$^{-/-}$ rats show expression of MDMA-induced hyperlocomotion? (*Chapter 4*)
4. Does acute MDMA administration impair memory performance via its agonist actions at the DA D1 receptor? (*Chapter 5*)
5. Does acute MDMA administration impair memory performance by increasing susceptibility to proactive interference? If so, does this increase in susceptibility to proactive interference appear to be mediated by MDMA's agonist actions at DA D1 receptors? (*Chapter 5*)

Phase 1 – behavioural characterisation of the DAD1$^{-/-}$ rats. Currently, little is known about this novel strain of rat and there are no published studies investigating MDMA exposure using DAD1$^{-/-}$ rats. As such, phase 1 (*chapter 3*) of experimentation involves conducting a battery of behavioural tasks to gain insight into whether the DAD1$^{-/-}$ rats differ

from controls in a drug-free state with regard to: 1. motivation to consume freely available sugar pellets; 2. the ability to autoshape to press a lever for food reinforcement; 3. locomotor tendencies in an open field; 4. balance performance on a narrow, elevated beam 5. memory performance in a T-maze and 6. novel odour recognition in their home cage. This 'behavioural profiling' of the DAD1$^{-/-}$ rats is important in identifying the nature of subsequent behavioural changes predicted in Phase 2.

Phase 2 – neurochemical assessment. Phase 2 (*chapter 4*) investigates a neurochemical response to MDMA that might be altered in the DAD1$^{-/-}$ rats. Immunohistochemistry (IHC) will be undertaken to investigate the role of the D1 receptor in the expression pattern of the immediate-early gene *c-fos* during acute MDMA exposure. *C-fos* expression is a widely used marker for neuronal activity and will be assayed in response to an acute dose of MDMA 3 mg/kg administered via i.p injection in several brain regions, namely the medial prefrontal cortices (mPFC; infralimbic cortex, cingulate cortex and prelimbic cortex), the striatum (dorsolateral and dorsomedial), Nacc (core and shell), VTA and SNPc.

Phase 3 – MDMA, memory/response perseveration and the D1 receptor. In phase 3 (*chapter 5*) we originally planned to employ the DMTS procedure in order to investigate the central question of this research: *is the D1 receptor implicated in the memory performance errors that are observed during acute MDMA exposure? Specifically, it is proposed that if MDMA interferes with memory via its agonist actions at the D1 receptor site then DAD1$^{-/-}$ rats should display relatively less response perseveration than controls during acute MDMA exposure.* The initial choice of the DMTS task stemmed from its prior use with 'normal' rats in the original research conducted by Harper and colleagues (2005, 2006 and 2013). However, data from the behavioural assessment (*chapter 3*) suggests the need to consider alternate behavioural tasks for assessing memory function in the DAD1$^{-/-}$ rats. Instead of the DMTS task conducted in an operant chamber, this chapter will present data from a T-maze based memory task: delayed non-matching-to-position (DNMTP).

Chapter 2: General Method

Subjects

Across the three data chapters presented, 78 male rats from the outbred Wistar strain were used. This comprises 41 DAD1$^{-/-}$ rats, four DAD1$^{+/-}$ rats and 33 DAD1$^{+/+}$ rats. On the basis of unpublished observations (personal communication with B. Ellenbroek, 2012) indicating that the heterozygous (DAD1$^{+/-}$) animals are both physically and behaviourally analogous to that of wild type (DAD1$^{+/+}$) rats, heterozygous and wild type rats were used as control groups. Breeding and housing space constraints limited the ability to compare all three groups in any given experiment. Rats were between 3 - 12 months old at the start of each experiment with the experimental and control groups being matched for age. Experimental and control groups were always matched in terms of their experimental histories. See tables 1, 2 and 3 for breakdowns of the information pertaining to the subjects used for each experiment.

Consistent with Muller et al. (2010), the DAD1$^{-/-}$ rats' free feeding weights (M = 341 grams) were 15-20% less than the DAD1$^{+/-}$ (M = 408 grams) and DAD1$^{+/+}$ (M = 398 grams) control rats. Rats were bred and housed at Victoria University of Wellington's animal facility. All experimental procedures were conducted in accordance with Victoria University of Wellington's animal care principles and were approved by the Victoria University of Wellington's Animal Ethics Committee.

Housing conditions. The rats were housed in pairs or triples (matched for genotype). Rats had access to laboratory grade animal chow and water *ad libitum* except during the autoshaping, consumption of sugar pellets and reinforced T-maze experiments, which relied on motivation to consume food. During these experiments, rats were put on a restricted food regime designed to maintain their weights at 85% of their free feeding weights (adjusted for growth). Their wire-top home cages (21.5cm x 19cm x 44cm) were lined with saw dust and contained a white tube enrichment item. The housing room was on a reverse 12-hour light/dark cycle (lights off between 7am and 7pm) and was kept at a constant temperature of 22.C. All experimental procedures took place during the rats' dark cycle, between 9am and 6pm.

Generation of the DAD1 mutant strain. DAD1$^{-/-}$ and DAD1$^{+/-}$ rats were generated using *N*-ethyl-*N*-nitrosourea (ENU)-driven target-selected mutagenesis, as described in detail by Smits et al. (2006). Administration of ENU, and generation of the

initial litter of mutant rats, was conducted at Radboud University in Nijmegen, the Netherlands. In 2011, a sample of the mutant progeny was transported to Victoria University of Wellington, NZ, where further offspring were reared.

To briefly outline the ENU process and the generation of the DAD1 gene mutation, male outbred Wistar rats, 11 weeks of age, were administered three intraperitonial injections of ENU, one per week, in the following dose order: 30, 35 and 40 mg of ENU/kg bodyweight. Three weeks after the last injection, the injected males were paired with wild type ($DAD1^{+/+)}$ outbred Wistar females, producing F1 progeny. Next, and because this mutagenesis technique can produce distinct and co-existing genetic mutations in the F1 offspring, each F1 juvenile was analysed for the presence of a genetic mutation using a tail sample. Of these offspring, one rat was identified that possessed the dopamine D1 mutant gene (i.e. $DAD1^{-/-).}$ This subject was subsequently bred with a $DAD1^{+/+}$ rat of the same outbred Wistar strain, creating heterozygous dopamine D1 mutant ($DAD1^{+/-)}$ F2 progeny. Next, these $DAD1^{+/-}$ offspring were mated, procuring litters that included $DAD1^{-/-}$ rats (F3 progeny).

Note regarding graphical figures. For all graphs, error bars represent standard error of the mean.

Table 1

Genotype, weight and age information pertaining to all rat subjects in cohort one. Rats in cohort one were subjects in the autoshaping, free access to sugar pellets and balance beam experiments.

Subject numbers	Genotype	Free feeding weight in grams	Age at start of experiment/s
1	$DAD1^{-/-}$	336	5-6 months
2	$DAD1^{-/-}$	250	5-6 months
3	$DAD1^{-/-}$	285	5-6 months
4	$DAD1^{-/-}$	328	5-6 months
5	$DAD1^{+/-}$	407	5-6 months
6	$DAD1^{+/-}$	422	5-6 months
7	$DAD1^{+/-}$	434	5-6 months
8	$DAD1^{+/-}$	368	5-6 months

Table 2

Genotype, weight and age information pertaining to all rat subjects in cohort two. Rats in cohort two were subjects in the immunohistochemical assay of c-fos expression experiment.

Subject numbers	Genotype	Free feeding weight in grams	Age at start of experiment/s
9	DAD1$^{-/-}$	342	11-12 months
10	DAD1$^{-/-}$	322	11-12 months
11	DAD1$^{-/-}$	312	11-12 months
12	DAD1$^{-/-}$	297	11-12 months
13	DAD1$^{-/-}$	308	9-10 months
14	DAD1$^{-/-}$	332	9-10 months
15	DAD1$^{-/-}$	318	9-10 months
16	DAD1$^{-/-}$	296	9-10 months
17	DAD1$^{-/-}$	309	9-10 months
18	DAD1$^{-/-}$	343	9-10 months
19	DAD1$^{-/-}$	348	9-10 months
20	DAD1$^{-/-}$	317	9-10 months
21	DAD1$^{+/+}$	384	7-8 months
22	DAD1$^{+/+}$	402	7-8 months
23	DAD1$^{+/+}$	398	7-8 months
24	DAD1$^{+/+}$	411	7-8 months
25	DAD1$^{+/+}$	420	11-12 months
26	DAD1$^{+/+}$	417	11-12 months
27	DAD1$^{+/+}$	443	11-12 months
28	DAD1$^{+/+}$	475	11-12 months
29	DAD1$^{+/+}$	378	11-12 months
30	DAD1$^{+/+}$	434	11-12 months

Table 3

Genotype, weight and age information pertaining to all rat subjects in cohort three. Rats in cohort three were subjects in the sucrose preference test, delayed non-matching-to-position, open-field and rotarod experiments.*

Subject numbers	Genotype	Free feeding weight in grams	Age at start of experiment/s
31(2)	$DAD1^{-/-}$	374	3-4 months
32(1)	$DAD1^{-/-}$	333	3-4 months
33(2)	$DAD1^{-/-}$	372	3-4 months
34(2)	$DAD1^{-/-}$	344	3-4 months
35(2)	$DAD1^{-/-}$	416	5-6 months
36(3)	$DAD1^{-/-}$	334	4-5 months
37(2)	$DAD1^{-/-}$	375	4-5 months
38(2)	$DAD1^{-/-}$	408	4-5 months
39(2)	$DAD1^{-/-}$	388	4-5 months
40(3)	$DAD1^{-/-}$	373	4-5 months
41(2)	$DAD1^{-/-}$	445	4-5 months
42(2)	$DAD1^{-/-}$	361	4-5 months
43(1)	$DAD1^{-/-}$	307	5-6 months
44(1)	$DAD1^{-/-}$	375	5-6 months
45(1)	$DAD1^{-/-}$	281	5-6 months
46(1)	$DAD1^{-/-}$	392	5-6 months
47(1)	$DAD1^{-/-}$	330	5-6 months
48(2)	$DAD1^{-/-}$	346	5-6 months
49(2)	$DAD1^{-/-}$	350	5-6 months
50(3)	$DAD1^{-/-}$	345	5-6 months
51(3)	$DAD1^{-/-}$	362	5-6 months
52(1)	$DAD1^{-/-}$	374	5-6 months
53(1)	$DAD1^{-/-}$	372	5-6 months
54(1)	$DAD1^{-/-}$	291	5-6 months
55(1)	$DAD1^{-/-}$	308	5-6 months
56(1)	$DAD1^{+/+}$	288	3-4 months

57(3)	DAD1$^{+/+}$	338	3-4 months
58(2)	DAD1$^{+/+}$	396	3-4 months
59(1)	DAD1$^{+/+}$	461	3-4 months
60(2)	DAD1$^{+/+}$	404	4-5 months
61(2)	DAD1$^{+/+}$	487	4-5 months
62(2)	DAD1$^{+/+}$	330	4-5 months
63(2)	DAD1$^{+/+}$	307	4-5 months
64(3)	DAD1$^{+/+}$	364	4-5 months
65(2)	DAD1$^{+/+}$	413	4-5 months
66(1)	DAD1$^{+/+}$	482	4-5 months
67(2)	DAD1$^{+/+}$	373	4-5 months
68(1)	DAD1$^{+/+}$	480	5-6 months
69(1)	DAD1$^{+/+}$	460	5-6 months
70(2)	DAD1$^{+/+}$	510	5-6 months
71(2)	DAD1$^{+/+}$	422	5-6 months
72(2)	DAD1$^{+/+}$	409	5-6 months
73(2)	DAD1$^{+/+}$	444	5-6 months
74(1)	DAD1$^{+/+}$	325	5-6 months
75(2)	DAD1$^{+/+}$	354	5-6 months
76(1)	DAD1$^{+/+}$	301	5-6 months
77(1)	DAD1$^{+/+}$	300	5-6 months
78(2)	DAD1$^{+/+}$	319	5-6 months

*All subjects in cohort three were used in the sucrose preference test. This cohort was then split into three groups: The 1st group (rats marked with (1)) comprises subjects used for the 1st rotarod test (minimal handling group), the 2nd group (rats marked with (2), n = 24) comprises rats who completed the delayed non-matching-to-position, open-field and 2nd rotarod (extensive handling group) experiments, and the 3rd group (rats marked with (3), n = 6) comprises subjects used in the delayed non-matching-to-position task who did not acquire the task.

Chapter 3: A Behavioural Characterisation of DAD1$^{-/-}$ Rats

DA's actions in the brain are mediated by five receptors (D1-D5), but a lack of selective ligands for these receptors has hindered our ability to determine their specific functions. As a result, DA receptors have been categorised into two families of receptors based on similar molecular structures and pharmacology - the D1-like (D1 and D5) and D2-like (D2, D3 and D4) families. D1-like receptor ligands stimulate both D1 and D5 receptors (Tiberi et al., 1991) and for the most part the contributions to behaviour made by D5 receptors are overlooked (Khan et al., 2000; Rivera et al., 2002). The relatively few studies that have assessed D1 and D5 receptors separately suggest that they play different roles in DA neurotransmission. For instance, D1 and D5 receptors are distributed differently throughout the rat brain (Tiberi et al., 1991), and additionally, D5 receptors display a 5 to 10-fold higher affinity for DA than D1 receptors (Grandy et al., 1991; Sunahara et al., 1991) suggesting that D5 receptors may participate highly effectively in DA mediated functions (Khan et al., 2000). Using D1 receptor knockout mice, Centonze et al. (2003) found that D1 and D5 receptors exert distinct actions with regard to striatal synaptic plasticity and spontaneous motor activity. Centonze et al. (2003) suggest that D1 receptors may be relatively more important than D5 receptors for the induction of striatal long-term potentiation (LTP), yet D5 receptors may play a larger role than D1 receptors in striatal long-term depression (LTD). What is more, SCH 23390 (a D1/D5 antagonist) decreased spontaneous motor activity in rats with ablated D1 receptors as well as control animals suggesting that D5 receptors may be critical neural substrates for movement (Centonze et al., 2003).

Not only do D1-like receptor ligands bind to D1 and D5 receptors, but in general they will also bind to non-DAergic receptors as well. For example, approximately a quarter of cortical binding by SCH 23390 (considered a *selective* D1 antagonist) was displaced by prior 5-HT_{2A} receptor antagonism as measured by PET in non-human primates (Ekelund et al., 2007). A further limitation of using pharmacological manipulations to examine the functions of D1 receptors is that medium-high doses of D1-like receptor agonists can flood synapses with DA, thereby obscuring the natural DA signal required for the performance of DA mediated behaviours (Beninger & Miller, 1998).

A novel aspect of the current project is the use of DAD1$^{-/-}$ rats to investigate the role of D1 receptors in some of MDMA's acute effects. DAD1$^{-/-}$ rats have a selective genetic down-regulation (~ 50%) in functional D1 receptors, but intact D5 receptors. This model stands to be an incredibly useful tool with which to further our understanding of the behavioural and neurochemical functions of D1 receptors. Because of the novelty of this rat model, and due to the fact that D1 receptors are involved in a range of conceptually distinct functions, a necessary starting point is to conduct a behavioural characterisation of the DAD1$^{-/-}$ rat phenotype in a drug-free state. Rather than conducting an exhaustive characterisation, this behavioural profile will focus on behaviours that involve D1-like receptors, and that are relevant to our later investigation into the nature of D1 receptor function in the effects of acute MDMA on memory (i.e. the focus of the *chapter 5*). There are three broad areas of behaviour that have been selected for analysis, specifically consummatory and reward-related behaviours, movement and spontaneous locomotor activity, and memory performance.

Firstly, based on extensive evidence suggesting a role for D1-like receptors in reward processes (Beninger & Miller, 1998), it is hypothesised that the DAD1$^{-/-}$ rats will display altered reward-related behaviours. To test this, food deprived DAD1$^{-/-}$ rats will be compared to controls (either DAD1$^{+/-}$ or DAD1$^{+/+}$ rats) with regard to 1. whether they display a preference for sucrose solution over water when both are freely available in the home cage; 2. latency to consume ten freely available odourless sugar pellets used in operant tasks in our lab and; 3. autoshaping to press a lever for reinforcement (sugar pellets). Because DA and D1 receptors have been implicated in behaviours consistent with *wanting* rather than *liking* rewards (Berridge 2007; Berridge & Robinson, 1998), it is predicted that the DAD1$^{-/-}$ rats will perform similarly to control rats on the first two tasks (i.e. sucrose preference and consuming sugar pellets) which aim to detect hedonic liking of palatable rewards. However, with regard to autoshaping to press a lever for palatable rewards, it is predicted that the DAD1$^{-/-}$ rats may be impaired in acquiring this behaviour, manifesting as fewer lever presses than controls. In support of this prediction, El-Ghundi et al. (2003) reported that D1 receptor deficient mice demonstrated attenuated lever pressing for sucrose. In addition, D1-like receptor antagonism disrupts the acquisition and maintenance of operant responding for palatable food rewards (Baldwin, Sadeghian & Kelley, 2002; Beninger et al., 1987; Beninger & Miller, 1998; Koch et al., 2000;

Nakajima, 1986; Nowend et al., 2001). In sum, it is expected that DAD1$^{-/-}$ rats will demonstrate intact *liking*, but impaired *wanting* for rewards.

Secondly, this chapter provides an assessment of movement in the DAD1$^{-/-}$ rats. A movement assessment is important because there is strong evidence that D1-like receptors are involved in movement (Aubert et al., 2005; Darmopil, Martin, de Diego, Ares, & Moratalla, 2009; Bergquist, Shahabi, & Nissbrandt, 2003; Drago et al., 1994; Hoffman & Beninger, 1985; Meyer et al., 1993; Ralph & Caine, 2005). Motor proficiency is required for the investigation of MDMA's acute effects on memory performance presented in *chapter 5*. Therefore it is relevant to examine the motor capabilities of the DAD1$^{-/-}$ rats compared to controls in order to take into account any baseline movement differences that may exist between the groups. The movement assessment will compare DAD1$^{-/-}$ rats to controls with regard to: 1. locomotor tendencies and spontaneous activity in an open field; 2. crossing a narrow, elevated balance beam and; 3. motor coordination on a rotarod. Firstly, due to D1-like receptor antagonists reducing spontaneous movement in rats (e.g. Hoffman & Beninger, 1985; Meyer et al., 1993), it is predicted that DAD1$^{-/-}$ rats will display reduced movement compared to controls on the two tasks in which voluntary movement is measured, namely in the open field and balance beam tasks. In addition, a benefit of measuring locomotor tendencies in an open field is the ability to assess for potential differences in anxiety-like behaviour by separately analysing activity that is performed in the periphery versus centre zones of the arena. Although in general rats display pronounced thigmotaxis (a tendency to prefer the periphery relative to the centre of an open field), anxiolytic agents have been found to preferentially increase activity in the central zone, relative to increases observed in the periphery (Treit & Fundytus, 1988). As an explorative assessment, it will be useful to compare the activity patterns of DAD1$^{-/-}$ rats and controls to assess whether DAD1$^{-/-}$ rats display altered levels of anxiety-like behaviour in a novel environment. Secondly, regarding the rotarod test in which rats are placed on a rotating rod to measure co-ordination, D1 knockout mice demonstrated impaired performance on the rotarod (Drago et al., 1994), and microinjection of SCH 23390 into the SN of rats led to impaired rotarod performance (Bergquist et al., 2003) suggesting that D1-like receptors are required for skilled rotarod performance. Thus it is predicted that the DAD1$^{-/-}$ rats will demonstrate impaired performance on the rotarod test compared to control rats.

Lastly, D1-like receptors are critically involved in memory function. While the roles of D1-like receptors in memory are still being clarified, they appear to be involved in the formation of memories via mechanisms of synaptic plasticity such as LTP and LTD, as well as guiding behaviour during working memory tasks (Frey et al., 1991; Li et al., 2003; Williams and Goldman-Rakic, 1995; Zahrt et al., 1997). Given that D1-like receptors are important for memory, it is possible that a reduction in functional D1 receptors will result in the DAD1$^{-/-}$ rats demonstrating impaired baseline memory function compared to control rats. Alternatively, and with the inverted-U theory of DA transmission in the PFC in mind (which posits that too much or too little PFC DA impairs memory function) (Williams and Goldman-Rakic, 1995; Zahrt et al., 1997), it is possible that the DAD1$^{-/-}$ rats possess sufficient D1 receptor function to support DA neurotransmission within the optimum levels required for memory function.

Planned experiments for *chapter 5* included conducting the delayed nonmatching to sample (DMTS) test of working memory in an operant chamber in order to investigate the acute effects of MDMA on memory performance in the DAD1$^{-/-}$ rats. Prior to testing the effects of MDMA on memory function in the DAD1$^{-/-}$ rats, it is necessary to assess the ability of DAD1$^{-/-}$ rats on tasks that are required for performance on the DMTS task. As previously mentioned, the ability for DAD1$^{-/-}$ rats to autoshape to press a lever will be investigated. Learning to lever press for sugar pellet reinforcers is required if memory performance following acute MDMA administration is to be assessed using the DMTS task. If the DAD1$^{-/-}$ rats do not autoshape to lever press we will need to consider alternative memory tasks to access memory function in the DAD1$^{-/-}$ rats. Therefore, DAD1$^{-/-}$ and controls rats will also be compared with regard to performance on a reinforced spatial memory task using the delayed nonmatching to position (DNMTP) paradigm in a T-maze. Although the DNMTP task is conducted in a T-maze and does not require an operant lever press in order for the subject to receive a reward, the trial structure of the DNMTP task is similar to that of the DMTS task. The DNMTP paradigm involves a sample phase, a delay period, and a choice phase, with the subject being required to remember which arm to enter during the choice phase in order to attain reinforcement. During the sample phase one of the T-maze arms is blocked off, and the opposite arm is baited with a food reinforcer for the subject to consume. Once the subject has consumed the reinforcer, there is a brief delay period after which the subject is returned to the maze for the choice phase. During choice, both arms are open but only the

opposite arm to the previously baited arm contains a food reinforcer. Thus to attain maximum reinforcement the rat is required to learn to always alternate their arm entry during the choice phase.

In T-maze experiments similar to DNMTP rats display a tendency to spontaneously alternate their arm visits on successive trials (Dember & Fowler, 1958; Lalonde, 2002; Rodriguez, Gomez, Alonso & Afonso, 1992). The tendency to alternate is hypothesised to reflect an attempt to maximise exploration of the environment and it is thought to involve memory in that by alternating their arm choice the rat is remembering which arm it visited previously and visiting a different arm on this occasion (Dember & Fowler, 1958; Lalonde, 2002; Rodriguez et al., 1992). Thus, an assessment of memory performance using the DNMTP paradigm will be two-fold. Firstly, whether the $DAD1^{-/-}$ and $DAD1^{+/+}$ rats demonstrate spontaneous alternation in training trial one will be examined (i.e. before the subject has learnt that during the choice phase the arm opposite to the sample arm will be baited). Based on previous studies, it is predicted that the control animals will tend to alternate their arm visits ~70% of the time (e.g. Rodriguez et al., 1992). Due to a correct response during the choice phase requiring subjects to alternate their arm visit relative to the sample arm, an alternation rate of ~70% translates to an accuracy score of ~70%. Secondly, whether the $DAD1^{-/-}$ rats improve their accuracy over the 25 training sessions in a manner similar to control rats will be examined.

Method

See the general method section (*chapter 2*) for information pertaining to generation of the $DAD1^{-/-}$ and $DAD1^{+/-}$ rat genotypes, housing conditions, and for a summary table of the subject groups employed for each experiment.

Behavioural Paradigms

Sucrose preference test.

Subjects. Forty-eight adult male Wistar rats (25 $DAD1^{-/-}$ and 23 $DAD1^{+/+}$ - cohort three) were subjects in this experiment. The subjects were housed individually during this experiment which took place over five consecutive days, 24 hours per day. Following the completion of this experiment subjects were re-housed into groups of three, matched by genotype. Laboratory grade animal chow was available *ad libitum*.

Apparatus. The experiment was conducted in the rat's assigned cage, which was identical to their home cage, and they were housed in their usual housing room which had a reversed light-dark cycle (lights on at 7 am). Due to space restrictions, the rats were split into five groups – four groups of 10 (five DAD1$^{-/-}$ rats and five DAD1$^{+/+}$ rats per group) and one group of eight (five DAD1$^{-/-}$ rats and three DAD1$^{+/+}$ rats) which were run through the experiment one group at a time (25 days total). The sucrose solution (10 % w/v) was made fresh each day by dissolving 100 grams of white cane sugar in 1 litre of water. Water and sucrose solutions were dispensed in white plastic drinking bottles which contained leak-stoppers in the drinking tube in order to ensure the consumption measurements were as accurate as possible.

Procedure. This experiment investigated whether DAD1$^{-/-}$ rats demonstrate a preference for sucrose solution over water when they are simultaneously available in the home cage. The first four days of the experiment involved the subjects having free access to sucrose solution and water, on separate days, which alternated over the four days (habituation period). For example, a subject may have had access to water on day one, sucrose on day two, water again on day three and sucrose on day four - half of the rats received this order, with the other half receiving the reverse order. The side of the cage (left or right) on which sucrose and water were presented was counterbalanced for each rat to avoid position preferences being formed. On day five (choice day), subjects had simultaneous access to both sucrose solution and water. The position of the bottle, left or right, was randomly assigned for each rat and counterbalanced across genotype. Drink bottles, containing either water or sucrose solution, were weighed before and after each day (24 hour intervals) in order to record consumption.

Performance measures. Although consumption during the first four days (habituation period) was examined to ensure subjects were drinking the water and sucrose solutions, data for analysis were collected on the choice day. Four measures were calculated for each subject:

1. amount of sucrose solution consumed in grams;
2. amount of water consumed in grams;
3. total amount consumed (i.e. consumption of sucrose solution + water) and;
4. percentage of sucrose solution consumed relative to total consumption.

Planned data analysis. Group means were obtained for each of these four measures as a function of genotype. The amount of liquid consumed in grams (of each sucrose and water) was then analysed using a 2 (genotype) x 2 (solution) mixed measures analysis of variance (ANOVA) with genotype as a between-subjects variable and solution as a within-subjects variable. Next, the total amount of liquid consumed and the percentage of sucrose consumed relative to water were separately analysed using independent samples *t*-tests with genotype as a between-subjects variable. All statistical analyses were conducted using IBM SPSS Statistics version 19.0 for windows. The alpha level for statistical significance was set at $p < 0.05$. Greenhouse-Geisser corrections were used in the event of Mauchly's test of sphericity being violated.

Consumption of freely available sugar pellets.

Subjects. Eight adult male Wistar rats (four DAD1$^{-/-}$ and four DAD1$^{+/-}$ – cohort one) were subjects in this experiment. The subjects were on a restricted food regime to maintain their weights at 85% of their free feeding weights. This involved feeding each cage of two rats once per day, seven days a week, approximately 24 grams of rat chow.

Apparatus. A clean cage which was identical to the home cages was used as the testing arena in all trials. In between each trial, the cage was wiped clean with Virkon 'S' disinfectant (Southern Veterinary Supplies, Palmerston North, NZ to remove any olfactory cues, and fresh saw dust was laid down to line the cage. At one end of the cage, ten 45 mg Bio-serv® sugar pellets were placed in a round, white ramekin dish (diameter = 9cm).

Procedure. This experiment assessed whether white odourless sugar pellets, used in lever-operated operant chambers in our lab, are consumed within a similar time-frame by both DAD1$^{-/-}$ and DAD1$^{+/-}$ rats when the pellets were freely available. Each session began by placing the rat in the test cage at the opposite end from the sugar pellets, and facing in the direction of the pellets. Once the rat was placed in the cage, the lid was immediately placed on top of the cage and a timer was started. The trial terminated once the rat had consumed all of the sugar pellets, or after 25 minutes had passed. If the sugar pellets were not consumed within 25 minutes, the data from this trial were not included in the final analysis. Once the trial had ended, the rat was removed from the testing cage and returned to their home cage. Each rat completed three trials, with approximately seven days separating each testing day.

Performance measure. The time taken in seconds to consume all ten sugar pellets was recorded for each of the three sessions, per rat.

Planned data analysis. Group averages of the time taken to consume all ten pellets were calculated as a function of genotype and session. These data were analysed using a 2 (genotype) x 3 (session) mixed measures ANOVA, with genotype as a between-subjects variable and session as a within-subjects variable. In the event that an interaction between genotype and session is detected, independent samples *t*-tests will be conducted *post hoc* for each session separately with genotype as a between-subjects variable and time taken to consume the pellets as the dependent measure. All statistical analyses presented in this chapter were conducted using IBM SPSS Statistics version 19.0 for windows. The alpha level for statistical significance was set at $p < 0.05$ across all analyses. Lastly, for repeated measures analyses, Greenhouse-Geisser corrections were used in the event of Mauchly's test of sphericity being violated.

Autoshaping to lever press, FR-1 reinforcement.

Subjects. Eight adult male Wistar rats (four DAD1$^{-/-}$ and four DAD1$^{+/-}$ – cohort one) were subjects in this experiment. During this experiment the rats were on a restricted food regime to maintain their weights at 85% of their free feeding weights. This involved feeding each cage of two rats once per day, seven days a week, approximately 24 grams of rat chow. Prior to the start of this experiment these rats were subjects in the consumption of sugar pellets experiment reported above.

Apparatus. Rats were trained in Med Associates® standard rat operant chambers (ENV 008) measuring approximately 31 cm x 31 cm x 25 cm. The experiment was conducted in a room adjacent to the home cage room. Each rat was assigned to an experimental chamber in which it performed all training. There were two response levers situated on the front interface panel of each chamber, 5 cm above the floor, and 1 response lever located in the middle of the opposite wall. A sugar pellet dispenser (which delivered 45 mg Bio-serv® sugar pellets) was located at floor level between the two levers on the front panel. Lever presses were recorded on a PC that was located in the adjacent room.

Procedure. This experiment followed the standard autoshaping procedure used in our laboratory (Harper, 2013; Harper et al., 2005; 2006). The autoshaping procedure involved the following sequence of events: one of the three levers (randomly determined)

was inserted into the chamber and the corresponding light above it was illuminated. If after 12 seconds the rat had not responded on the lever, the lever was retracted, the light turned off and a sugar pellet was delivered. Thirty seconds later, one of the 3 levers was inserted and another trial began. If during the presence of the lever the rat made a single response then that lever was immediately retracted, the light turned off and a sugar pellet delivered.

Rats were exposed to nine autoshaping sessions each and the sessions lasted until 80 pellets were delivered or 30 minutes had elapsed, which ever occurred first. During training the room lights were switched off.

Performance measure. For each rat, the number of lever presses performed during each of the nine sessions was recorded.

Planned data analysis. Group averages of the number of lever presses per session were calculated as a function of genotype. The number of lever presses over each session were subsequently analysed using a 2 (genotype) x 9 (session) mixed measures ANOVA with genotype as a between-subjects variable and session as a within-subjects variable. In the event that an interaction between genotype and session is detected, independent samples *t*-tests will be conducted *post hoc* for each session separately with genotype as a between-subjects variable and the number of lever presses as the dependent measure.

Open field.

Subjects. Twenty-four adult male Wistar rats (11 $DAD1^{-/-}$ and 13 $DAD1^{+/+}$ – group two from cohort three) were subjects in this experiment. These rats were previously subjects in a maze task used for the current thesis (DNMTP – acquisition presented in the current chapter, drug doses presented in *chapter 5*) during which they experienced several acute drug administrations including non-neurotoxic doses of MDMA, SKF 81297 (D1-like receptor agonist) and saline. There was a two week washout period after the completion of the maze task and prior to starting the open field experiment to minimise carry-over effects from the drug treatments. Groups were matched with regard to their previous experiment and drug treatment histories. The maze experiment was conducted over a three and half month period, thus at the beginning of the open field experiment subjects were six - nine months old.

Apparatus. Horizontal movement and rearing were observed in eight Plexiglas open field chambers (Med Associates Inc, USA; model ENV-515) measuring 42 cm x 42 cm x 30 cm. The chambers were set in sound attenuating boxes. Horizontal movement (distance travelled in cm) was recorded using four sets of 16 infra-red sensors spaced evenly on the walls of the chamber which created a photobeam lattice, squares of the dimension 25 mm x 25 mm, which was located 4 cm above the floor of the chamber. A second photobeam lattice was located 15 cm above the floor of the chamber which detected vertical movement (rearing counts). A PC located in the experimental room recorded distance travelled and rearing counts.

Procedure. Each rat's activity was recorded on three separate sessions over the space of nine days (one session every three days) and on each session their activity was recorded for 30 minutes. At the start of a session, subjects were transported into the experimental room and habituated to the room for 30 minutes. Rats were then carefully placed into an assigned activity box which triggered the start of activity recording. During recording the main room light was switched off and a red light was used for illumination. To mask any extraneous noises, a white noise generator was used during the sessions. After 30 minutes rats were immediately removed from the chambers and placed back in their home cages in their home cage room. The chambers were thoroughly cleaned with Virkon 'S' disinfectant after each session.

Performance measures. In each of the three 30 minute sessions, activity was recorded in six 5 minute time intervals. Horizontal locomotor activity was measured as distance travelled in cm. Rearing counts were defined as the interruption of the upper photo beams, one count per interruption. Activity in the centre and periphery of the chamber was analysed separately, with the central zone defined as the inner 19 cm x 19 cm area, with the rest of the chamber being the periphery.

Planned data analysis. To generate time-course data, each subject's distance travelled in cm and rearing counts in the centre or periphery were separately averaged across the three sessions for each of the six 5 minute time intervals. Subject averages for each of the six 5 minute time intervals were then used to calculate group time interval averages as a function of genotype and location (periphery and centre). The group time-course data for both distance travelled in cm and rearing counts were analysed separately for each location using four 2 (genotype) x 6 (time) mixed measures ANOVAs with

genotype as a between-subjects variable and time as a within-subjects variable. In the event that an interaction between genotype and time is detected, independent samples *t*-tests will be conducted *post hoc* for each time interval separately with genotype as a between-subjects variable and distance travelled or rearing counts in either the centre or periphery as the dependent measures.

Balance beam.

Subjects. Eight adult male Wistar rats (four DAD1$^{-/-}$ and four DAD1$^{+/-}$ – cohort one) were subjects in this experiment. Laboratory grade animal chow and water was available in their home cages *ad libitum*. Prior to the start of this experiment these rats were subjects in the consumption of sugar pellets and autoshaping experiments reported above.

Apparatus. See figure 3 for a diagram of the beam apparatus. The beam was 80 cm in length and raised 80 cm off the ground using plastic supports to suspend both ends. The beam was suspended as such that at one end, the starting end, there was a sheer drop and at the opposite end, the finishing end, there was a ledge, on top of which sat the subject's home cage. At the starting end of the beam a line was marked across the beam at 20 cm from the sheer edge (on the opposite end to the home cage). Foam padding 10 cm in thickness was on the ground beneath the beam in order to prevent injury in the event that an animal fell off the beam.

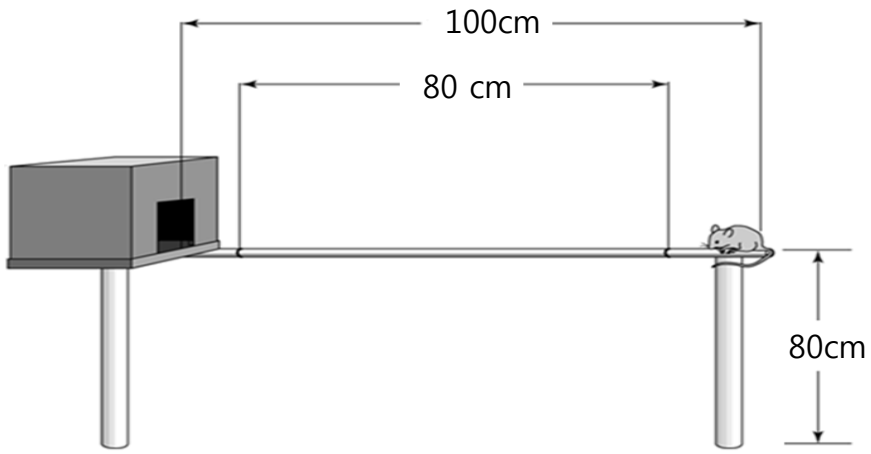

Figure 3. Diagram of the balance beam apparatus.

Procedure. The balance beam task has been employed to study the movement deficits in Parkinson's disease using rat and mice models. Performance on the balance beam requires balance and co-ordinated movement (Truong, Allbutt, Kassiou, & Henderson, 2006).

A trial on the balance beam began when the experimenter carefully placed a subject on the beam, with all four of its feet behind the starting the line located at the sheer end of the beam and with the rat facing the home cage. Once the rat was secure on the beam, the experimenter released the rat and started a timer. To complete the trial the subject was required to walk the full length of the beam and enter their home cage within 2 minutes. There were two balance beam sessions over the space of one week. Both sessions involved all rats experiencing three trials (i.e. there was a total of six trials per rat).

Performance measures. Initially, there were two timing measures recorded during each trial:

1. latency to step over the 20 cm line, determined once a weight bearing footstep was placed entirely over the 20 cm line, representing the latency to begin the task and;
2. latency to reach the home cage (determined once all four feet were off the beam and in the home cage).

However, so few trials were completed by DAD1$^{-/-}$ rats that in the place of latency measures, the dependent variable used was whether or not the trial was completed within the 2 minute time frame.

Planned data analysis. Given that the dependent measure used generates a dichotomous categorical variable (was the trial completed, yes or no?) and given the small sample size, Fishers exact test was used to analyse the data which was grouped by genotype. Separate tests were calculated for each of the six trials with genotype as a between-subjects factor and whether or not the trial was completed as an outcome variable.

Rotarod.

Subjects. This experiment was conducted twice with two different groups of rats. The apparatus and procedure used were identical across both replications, but the two groups differed in the amount of handling they experienced prior to the rotarod

experiment. The minimally handled group comprised 18 adult male Wistar rats (ten DAD1$^{-/-}$ and eight DAD1$^{+/+}$ – group one from cohort three) which were handled for five consecutive days prior to the start of the experiment. The extensively handled group comprised 24 adult male Wistar rats (11 DAD1$^{-/-}$ and 13 DAD1$^{+/+}$ – group two from cohort three) which were subjects in a previously conducted maze task (DNMTP - acquisition presented in the current chapter, drug doses presented in *chapter 5*) and experienced three and a half months of handling, four – five days per week prior to the start of the rotarod experiment. Handling was manipulated due to the control rats in the first group that was run (minimally handled group) not performing the task well.

Apparatus. The rotarod apparatus was constructed in-house based on standard rotarod dimensions used frequently in the literature. The rotarod consisted of a white plastic cylindrical rod which had a 30 cm circumference and was 10 cm wide. During the experiment the subjects perched on the rod which mechanically rotated along its axis. To improve traction for the rats there was sand paper covering the exterior of the rod which was attached with adhesive. At each length of the rod there were white, round Plexiglas sheets attached which created walls on the rod measuring 22 cm from the axis of the rod. These walls ensured that the subject could not climb off of the rod during the experiment. The rod was suspended 21 cm above the floor from the highest point of the rod where the rats would sit. There was a small enclosed space underneath the rod in which the animals were contained in, in the event that they came off of the rod. The rotation speed of the rod could be increased or decreased manually using a dial which was located on the external motor used to power the rod.

Procedure. On each day of the experiment the subjects were transported in their home cages to the experiment room. The home cages sat on a large table and rats were habituated to the room for 30 minutes. The subjects were first habituated to the rotarod when it was stationary over the course of three sessions which were separated by a day off between each session. Habituation involved placing a rat on the rod several times and releasing the rat to ensure that it was able to sit on the rod unassisted. Next the subject was gently held next to the stationary rod and rats could voluntarily step onto the rod. After three sessions of habituation, the rats that reliably stepped onto the rod and sat unassisted advanced to the testing phase.

The experiment phase began 48 hours after the third habituation session. The experiment phase involved 6 sessions – three training sessions and three testing sessions using three different rod rotation speeds (10, 20 and 30 revolutions per minute (RPM)). Specifically, on day one of the experimental phase there was a training session using the 10 RPM speed. Forty-eight hours later there was a testing session using the 10 RPM speed. Forty-eight hours after the 10 RPM testing session, a second training session was conducted using the 20 RPM speed which was again followed up 48 hours later with a testing session using the 20 RPM speed. This structure was repeated for the 30 RPM speed. On the day of each session, rats were habituated to the experiment room for 30 minutes with the rotarod motor running so that the subjects habituated to the sound of the motor. Each training session began by gently holding the rat adjacent to the rod while it was stationary and allowing them to step onto the rod. They were then gently removed from the rotarod, the rotarod was turned on and the rat was held close to the rod once again to allow the rat to step onto the rod while it was rotating. A timer was started as soon as the rat was walking on the rod unassisted to record the time the rat spent on the rod. The trial was terminated in the event that the rat fell from the rod or until 2 minutes elapsed, which ever occurred first. If the rat did not voluntarily step onto the rod after several attempts, the rat was placed back into its cage and the next rat began its trial. On each training session the rats had five trials each, with approximately 15 minutes in between each of these five trials. On each training session the subjects were required to spend at least 10 seconds or more on two or more trials out of 5 to advance to the next session. Testing sessions were identical to training sessions except that only one trial was conducted, rather than five.

Performance measures. Data for analysis was collected on the three test days. The amount of time each rat spent on the rod at the three speeds tested was determined in seconds.

***Planned data analysis*.** Time spent on the rod in seconds was averaged by handling group (minimal versus extensive handling) and genotype. Time spent on the rod was to be separately compared in the two handling groups using two 2 (genotype) x 3 (RPM) mixed measures ANOVAs with genotype as a between-subjects factor and RPM (10, 20 or 30 RPM) as a within-subjects factor. In the event that an interaction between genotype and session is detected, independent samples *t*-tests will be conducted *post hoc*

for each RPM separately with genotype as a between-subjects variable and time spent on the rad as the dependent measure.

Delayed non-matching to position.

Subjects. Thirty adult male Wistar rats (15 DAD1$^{-/-}$ and 15 DAD1$^{+/+}$ rats – groups two and three from cohort three) were used in this experiment. Water and wood shavings were available *ad libitum* in the home cages. The subjects' weights were maintained at 85% of their free feeding weights with food pellets delivered to their home cages seven days a week straight after experimentation had been completed on experimental days. These rats were drug naïve, and were subjects in the sucrose preference test which is also presented in this chapter.

Apparatus. This paradigm was conducted using a wooden T-maze which had metal inners inserted into it to form the walls of the maze. This was done in order to create taller walls and shorter arm and stem lengths than provided by the wooden maze. The metal inserts were 30 cm high, the two arms were 30 cm long and the stem was 45 cm long. The width of the maze throughout was 9 cm. The stem was painted grey and the arms were painted black. Two pieces of black plexi-glas that were the same height as the maze walls were used to block the maze arms. These "doors" were attached at the top of the maze, and acted as sliding doors that were controlled manually when they were needed to block off either of the arms. Where the doors crossed the arms to block entry were defined as the entry lines to the arms.

White odourless sugar pellets (45 mg Bio-serv®) were used as reinforcement. The sugar pellets were delivered at the end of each T-maze arm, in round white plastic containers (3 cm across) which were secured to the floor of the maze using adhesive.

Procedure. This experiment was conducted on weekdays between the hours of 9 am and 6 pm. Rats were handled for 5 – 10 minutes each on five consecutive days prior to the start of this experiment. The day after the last handling day, rats began four days of habituation to the T-maze apparatus. Habituation sessions involved placing a rat in the stem of the maze and allowing them to explore the maze for 5 minutes. On days one and two of habituation there were five sugar pellets available to the rats in the maze – one in each of the containers at the end of the maze arms, one in the middle of each of the arms and one at the junction of the two arms and the stem. On days three and four of habituation there were four sugar pellets in the maze, two in each of the containers at the

end of the arms. On day four of habituation the subjects who reliably ate the sugar pellets from both the left and right maze arms advanced to the training stage of the experiment.

Training sessions began the day following the last habituation day. There were 25 training sessions conducted, with six trials per session. Each trial was separated by a 5 minute inter- trial interval. Each trial involved a sample presentation phase, a delay and a choice phase. During sample presentation either the left or right arm was baited with one sugar pellet and entry to this arm was unimpeded. Entry to the opposite arm was blocked using the Plexiglas door thus only the sample arm was able to be accessed by the rat. The rat was placed at the end of the stem of the maze, facing the junction between the stem and the arms, and was given 2 minutes to consume the pellet. Once the rat entered the baited arm (deemed the sample arm) defined as when all four feet had crossed the entry line, the door was slid closed to confine the subject to this arm. On the contrary, if the subject did not enter the sample arm within the allotted 2 minutes, the rat was removed from the maze and the trial was terminated. Once the rat had consumed the pellet, it was removed from the arm and placed in a holding cage for 10 seconds which was situated on a table behind the stem end of the maze. During this brief delay both doors were slid open and both arms appeared to be re-baited. In reality, only the previously blocked arm was baited and the sample arm was unbaited. Thus in order to retrieve the reward the rat was required to visit the opposite arm to the sample arm. The choice phase began after the delay period. The rat was placed in the stem of the maze and given 2 minutes to explore. If the rat entered one of the arms, the door to that arm was slid closed and the rat was confined to that arm. In the event that the arm entered was the previously baited arm (i.e. the sample arm) which was now unbaited, the rat was confined to this arm for 2 minutes. If the rat alternated their arm entry and entered the baited arm, the rat was allowed to consume the pellet and then removed from the maze and placed back into their home cage. If the rat did not enter either of the arms during the 2 minutes given then the rat was removed from the maze and the trial was terminated. Lastly, the experimenter monitored the consumption of the sugar pellets and in the event that a rat entered an arm but did not consume an available sugar pellet within 1 minute, the trial was terminated.

Whether the baited arm during the sample phase was on the left or right side was pseudo-randomly selected and counterbalanced across both groups of rats. Of the six trials conducted per session per rat, three of the sample arms were on the left, and three

were on the right. Between each trial the maze was wiped out thoroughly with Virkon 'S' disinfectant.

Performance measures. Three performance measures were collected:

1. whether the arm entered during the choice phase matched the sample arm (incorrect response) or did not match the sample arm (correct response);
2. latency in seconds to enter the forced choice arm during the sample presentation phase;
3. latency in seconds to enter an arm during the choice phase.

***Planned data analysis*.** Accuracy data was calculated by determining the percentage of correct trials performed by each rat during each of the 25 sessions. The individual accuracy scores were then averaged by genotype to allow for group comparison. Similarly, for both of the latency measures, each subject's performance on the six trials within a session were averaged, and then group means were calculated as a function of genotype.

Firstly, group accuracy scores on trial one were checked to ensure they were at or around 70 - 75% which would be indicative of a tendency to alternate their arm visits. Next, using group accuracy scores on trial one, an independent samples *t*-test was carried out with genotype as the between-subjects variable to assess for group differences in alternation behaviour prior to the DNMTP task being learned.

Secondly, each of the three dependent measures (accuracy, latency to enter the forced choice arm during the sample run and latency to enter an arm during the choice run) observed across the 25 training sessions were then subjected to 2 (genotype) x 25 (session) mixed measures ANOVAs, with genotype as a between-subjects variable and session as a within-subjects variable. The accuracy analysis was performed to assess for learning across the 25 sessions, and the latency comparisons were assessing for differences in movement. In the event that interactions between genotype and session are detected, independent samples *t*-tests will be conducted *post hoc* for each session separately with genotype as a between-subjects variable and accuracy as the dependent measure.

Results

Sucrose Preference Test

Figure 4 shows the total amount of water and sucrose consumed. As expected, a significant effect of solution was observed, $F_{1,44} = 248.59$, $p < .001$, with both genotypes consuming more sucrose solution ($M = 81.04$; $SD = 34.13$) than water ($M = 4.67$; $SD = 2.49$). In line with the finding that DAD1$^{-/-}$ rats are smaller than the control rats, there was a significant effect of genotype, $F_{1,44} = 5.34$, $p = .03$, with DAD1$^{-/-}$ rats ($M = 74.61$; $SD = 32.83$) drinking less overall compared to DAD1$^{+/+}$ rats ($M = 96.83$; $SD = 32.36$). There was also a significant interaction between solution and genotype, $F_{1,44} = 5.06$, $p = .03$, which, on the basis of follow-up independent samples *t*-tests indicates that the DAD1$^{-/-}$ rats consumed the same amount of water as controls, $t_{44} = -.29$, $p = .77$, yet significantly less sucrose than controls, $t_{44} = -2.29$, $p = .03$. However, due to the floor effect that can be observed with regard to the amount of water consumed, the interaction is likely driven by the finding that the DAD1$^{-/-}$ rats consumed less liquid overall than the DAD1$^{+/+}$ rats (i.e. the main effect of genotype reported above). Indeed, when the percentage of sucrose solution consumed, relative to the total amount consumed across water and sucrose solution, is compared across genotype, no significant difference is observed, $t_{44} = -.88$, $p = .38$, with both genotypes showing a strong preference for the sucrose solution over water. As depicted by figure 5, approximately 94 - 95% of the total liquid consumed by both genotypes was sucrose solution.

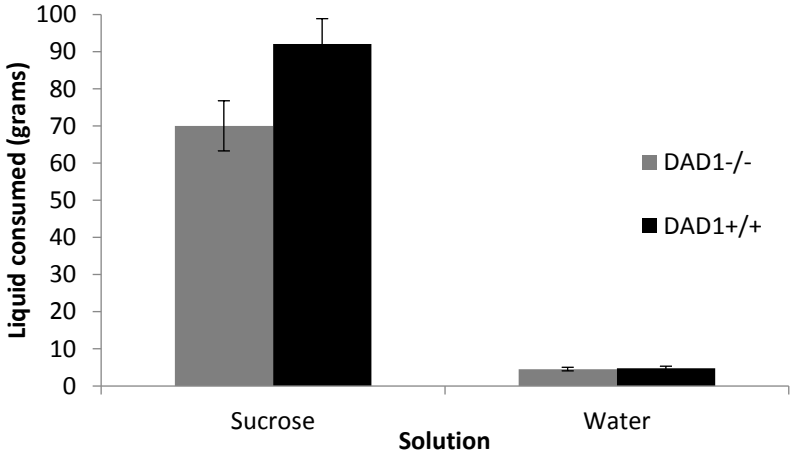

Figure 4. Amount of sucrose solution and water consumed in grams by DAD1$^{-/-}$ ($n = 25$) and DAD1$^{+/+}$ ($n = 23$) rats over a 24 hour period on the choice day of the sucrose preference test.

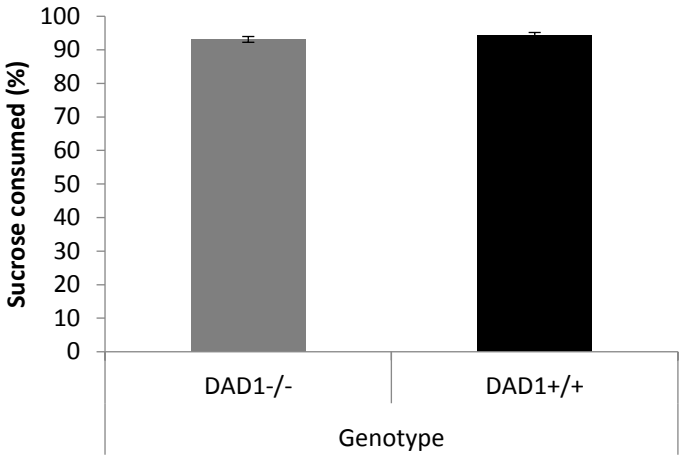

Figure 5. Mean percentage of sucrose solution consumed relative to the total liquid consumed (water + sucrose solution) by DAD1$^{-/-}$ (n = 25) and DAD1$^{+/+}$ (n = 23) rats over a 24 hour period on the choice day of the sucrose preference test.

Consumption of Freely Available Sugar Pellets

A group comparison of the time taken to consume ten freely available sugar pellets (figure 6) revealed no significant effect of session, $F_{1.01, 6.01} = 2.74$, $p = .10$, no significant effect of genotype, $F_{1,6} = 2.32$, $p = .18$, and no significant interaction between session and genotype, $F_{1.01,6.04} = 1.42$, $p = 0.28$. However, in the first session the environment was novel and the subjects were not aware of the presence of the sugar pellets (also, an independent samples t-test revealed no significant difference between genotype in session one latencies, $t_6 = 1.30$, $p = .24$). Thus, the first of the three sessions could be conceived of as qualitatively different from the following two sessions. As such, a 2 (session) x 2 (genotype) mixed measures ANOVA was conducted using latency to consume the ten pellets in sessions two and three. There was no significant effect of session observed, $F_{1,6} = 5.32$, $p = .06$, although the approaching significance p-value suggests there was a trend for rats to eat the pellets more slowly on session two (M = 27.13, SD = 9.45) compared to session three (M = 17.00, SD = 10.60). A significant difference between genotype was detected, with the DAD1$^{-/-}$ rats (M = 28.50, SD = 2.40) consuming the sugar pellets more slowly than the DAD1$^{+/-}$ rats (M = 15.63, SD = 2.40), $F_{1,6} = 14.3$, $p = .01$. There was no significant interaction between genotype and session, $F_{1,6} = 0.10$, $p = .77$.

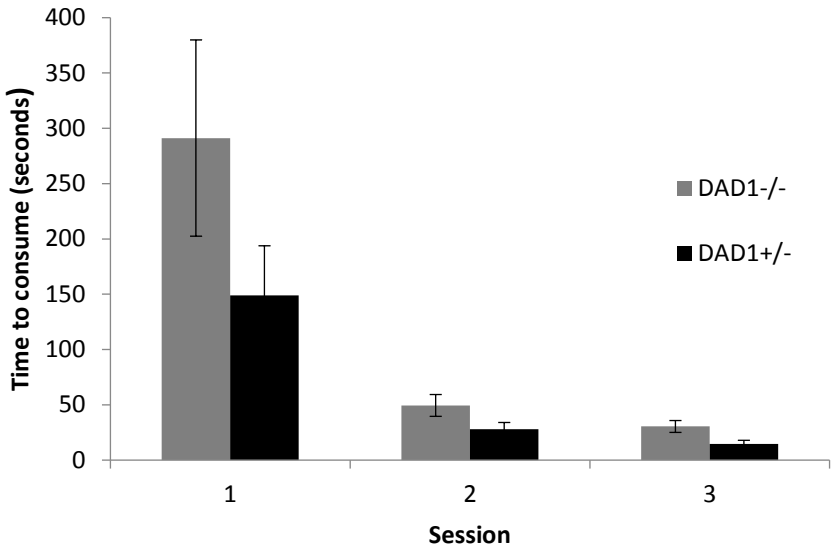

Figure 6. The time taken in seconds by DAD1$^{-/-}$ (n = 4) and DAD1$^{+/-}$ (n = 4) rats to consume ten freely available sugar pellets across three sessions.

Autoshaping to Lever Press, FR-1 Reinforcement

The number of lever presses performed across the nine autoshaping sessions was compared by genotype. A significant effect of genotype was found, $F_{1,6} = 30.09$, $p < .01$, with the DAD1$^{-/-}$ rats ($M = .83$, $SD = .49$) making fewer lever presses than the DAD1$^{+/-}$ rats ($M = 60.97$, $SD = 21.92$). There was no significant effect of session, $F_{8,48} = 1.31$, $p = .26$, and no significant interaction between session and genotype, $F_{8,48} = 1.26$, $p = .29$. Depicted by figure 7, these data indicate that the DAD1$^{-/-}$ rat's lever pressed markedly less than DAD1$^{+/-}$ rats which did not change as a function of session.

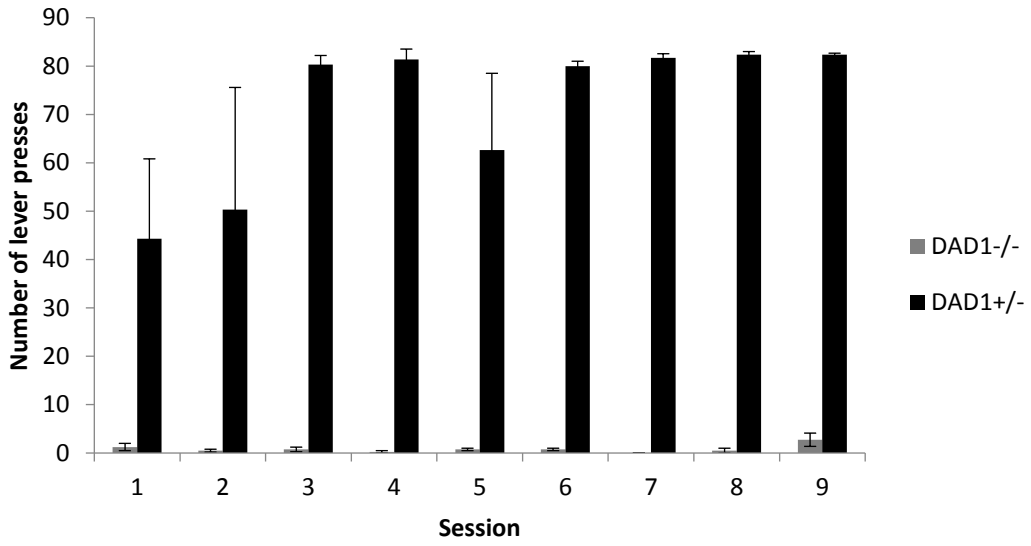

Figure 7. Operant responding for sugar pellets maintained under an FR-1 schedule of reinforcement. The number of lever presses made by DAD1$^{-/-}$ (*n* = 4) and DAD1$^{+/-}$ (*n* = 3) rats is plotted over nine training sessions.

Open Field

Horizontal activity – distance travelled (cm). See figure 8 for comparison of the mean distance travelled by DAD1$^{-/-}$ and DAD1$^{+/+}$ rats in both the centre and periphery of the chamber.

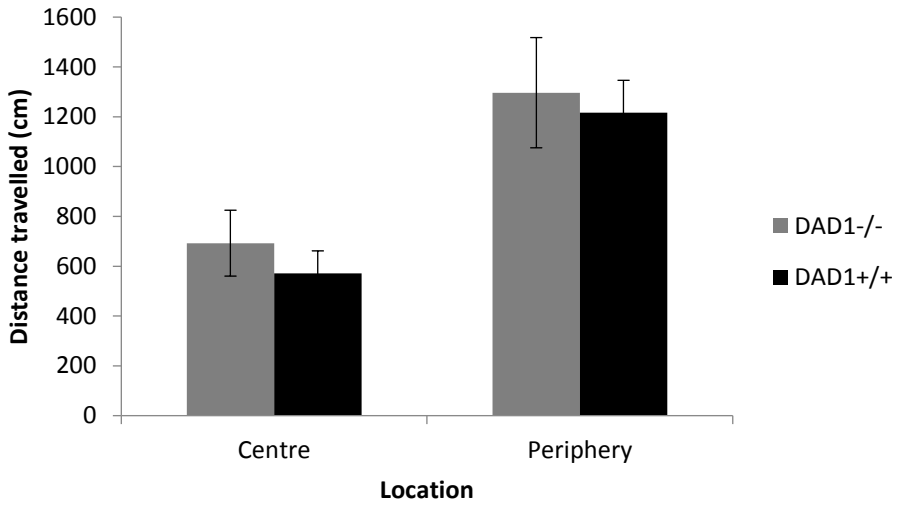

Figure 8. Mean distance travelled by DAD1$^{-/-}$ (n = 11) compared to DAD1$^{+/+}$ (n = 13) rats in the centre and periphery of the open field chamber. Data is averaged across three 30 minute sessions.

Centre. As shown in figure 9 the time-course of horizontal activity in the centre of the chamber was fairly similar in both DAD1$^{-/-}$ and DAD1$^{+/+}$ rats. While there was no significant effect of genotype, $F_{1, 22} = .80$, $p = .38$, and no significant interaction between genotype and time, $F_{2.2, 49.4} = .89$, $p = .43$, there was a significant effect of time, $F_{2.2, 49.4} = 70.19$, $p < .01$. As shown in figure 9 the rats moved more in the first 5 minute time interval compared to the other time intervals, with rats travelling around 250 cm during the first 5 minutes. Furthermore, activity was consistent from around 10 minutes through until the end of the 30 minutes, with rats travelling approximately 50 - 100 cm during the remaining time intervals.

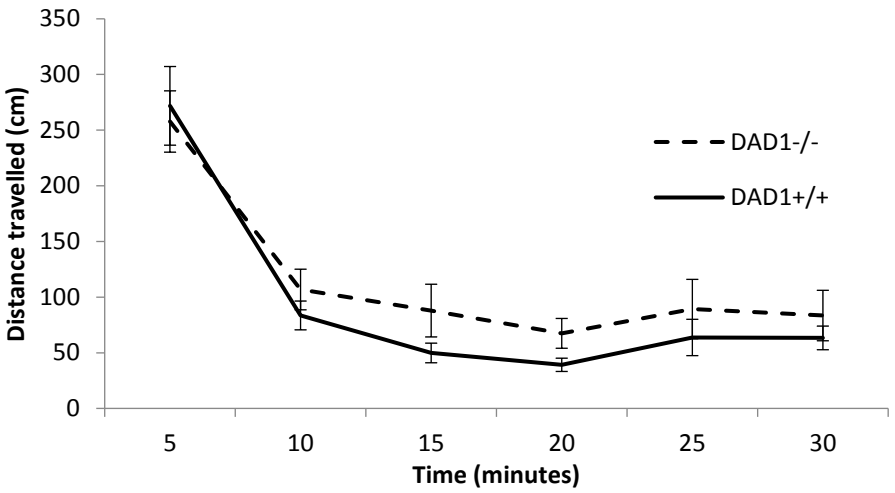

Figure 9. Time-course of distance travelled (cm) in the centre of the chamber by DAD1$^{-/-}$ (n = 11) and DAD1$^{+/+}$ (n = 13) rats, averaged across three 30 minute open field sessions.

Periphery. In a manner similar to distance travelled in the centre of the chamber, the time-course pattern of horizontal activity in the periphery was comparable across DAD1$^{-/-}$ and DAD1$^{+/+}$ rats (figure 10). There was no significant effect genotype, $F_{1, 22}$ = .15, p = .70, and no significant interaction between genotype and time, $F_{1.8, 39.7}$ = .85, p = .43, but there was a significant effect of time, $F_{1.8, 39.7}$ = 115.58, p < .01. As shown by figure 10, the rats were more active in the first 5 minute time interval, travelling approximately 550 cm, with the distance travelled diminishing to plateaux at around 100 - 200 cm during each of the remaining 5 minute intervals.

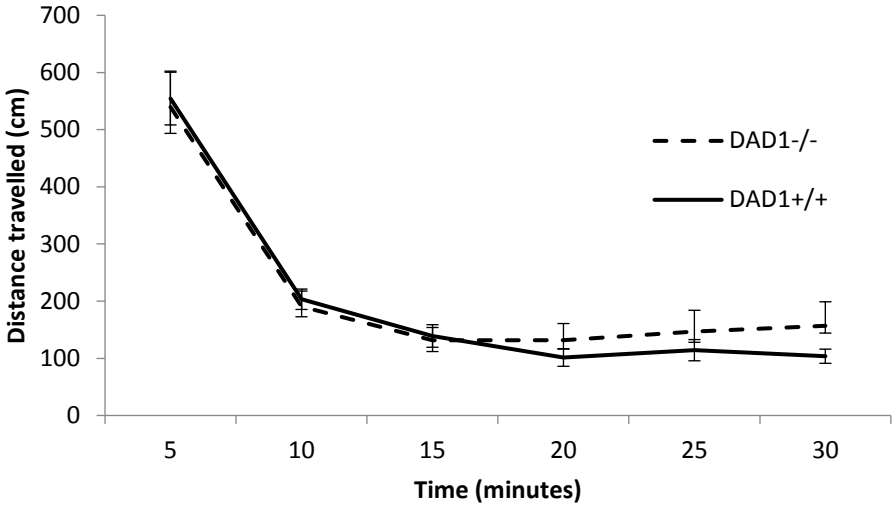

Figure 10. Time-course of the distance travelled in cm in the periphery of the chamber by DAD1$^{-/-}$ (n = 11) and DAD1$^{+/+}$ (n = 13) rats, averaged across three 30 minute open field sessions.

Rearing counts. See figure 11 for a comparison of mean rearing counts made by DAD1$^{-/-}$ and DAD1$^{+/+}$ rats in the centre or periphery of the chamber.

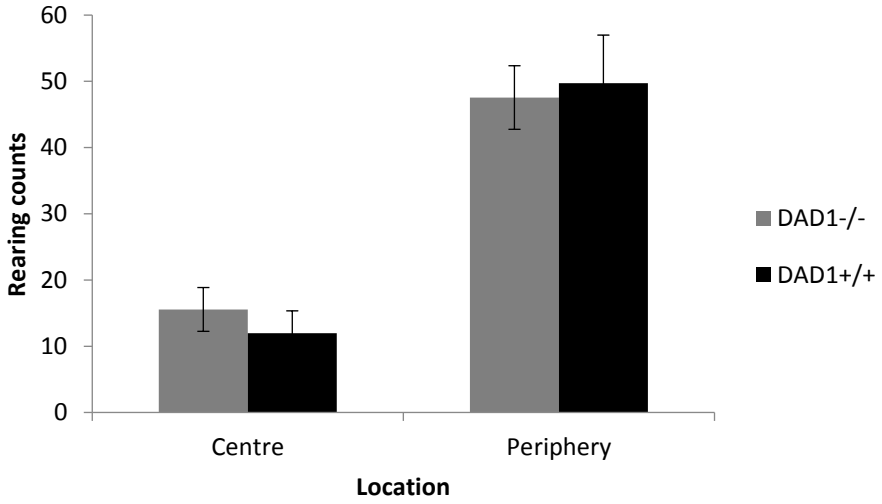

Figure 11. Mean rearing counts made by DAD1$^{-/-}$ (n = 11) compared to DAD1$^{+/+}$ (n = 13) rats in the centre and periphery of the open field chamber. Data is averaged across three 30 minute sessions.

Centre. As shown by figure 12, the time-course of rearing counts performed in the centre of the chamber was similar DAD1$^{-/-}$ and DAD1$^{+/+}$ rats. There was no significant effect of genotype, $F_{1, 22} = .05$, $p = .83$, and no significant interaction between genotype and time, $F_{2.7, 59.2} = .63$, $p = .59$, but there was a significant effect of time, $F_{2.7, 59.2} = 20.22$, $p < .01$. As with horizontal locomotor activity, figure 12 shows that rearing counts in the centre of the chamber peaked in the first 5 minute interval, at around 6 counts, and then plateaued fairly rapidly to around 1 - 2 counts for the remaining time intervals.

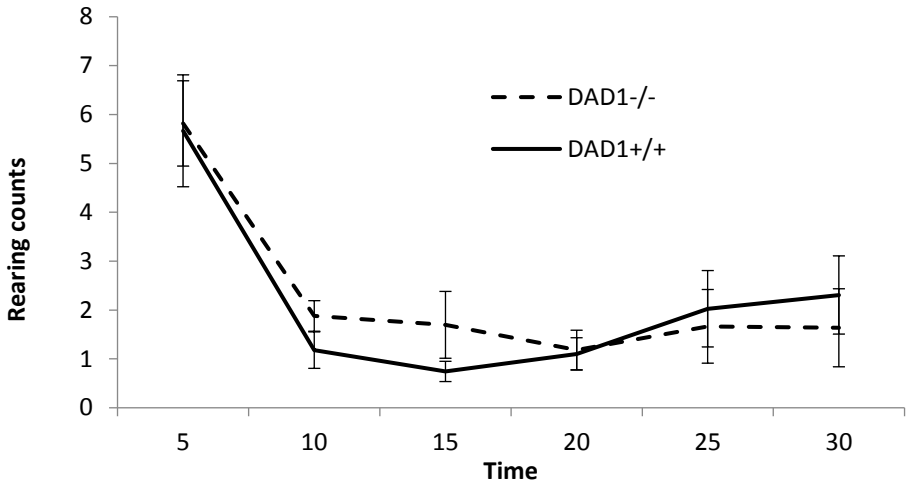

Figure 12. Time-course of rearing counts in the centre of the chamber made by DAD1$^{-/-}$ ($n = 11$) and DAD1$^{+/+}$ ($n = 13$) rats, averaged across three 30 minute open field sessions.

Periphery. See figure 13 for a depiction of the time-course of rearing counts performed in the periphery of the chamber. Once again, the DAD1$^{-/-}$ and DAD1$^{+/+}$ rats performed comparably. There was no significant effect of genotype, $F_{1, 22} = .03$, $p = .86$, no significant interaction between genotype and time, $F_{2.4, 51.7} = .29$, $p = .79$, but there was a significant effect of time, $F_{2.4, 51.7} = 67.92$, $p < .01$. Figure 13 displays the same pattern as above, with there being approximately 20 rearing counts in the first 5 minute time interval, with counts dropping to around 5 counts across all other intervals across genotype.

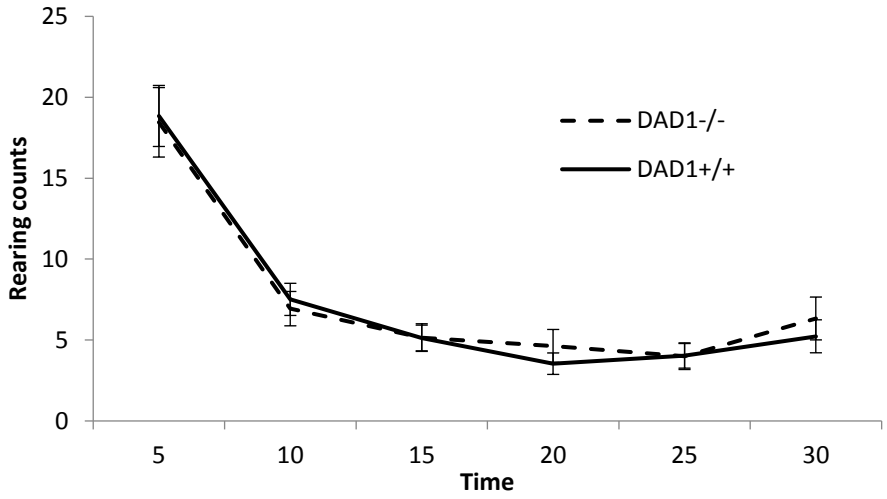

Figure 13. Time-course of rearing counts in the periphery of the chamber made by DAD1$^{-/-}$ (n = 11) and DAD1$^{+/+}$ (n = 13) rats, averaged across three 30 minute open field sessions.

Summary of open field data. DAD1$^{-/-}$ and DAD1$^{+/+}$ rats displayed very similar patterns of locomotor activity (in terms of distance travelled and rearing counts) in both the peripheral and central zones of the chamber. Taken together, these data suggest that the DAD1$^{-/-}$ rats did not display altered spontaneous locomotor tendencies or anxiety-like behaviours compared to control rats in an open field.

Balance Beam

The number of subjects that completed each of the six balance beam trials was compared by genotype (figure 14). As determined using Fishers exact test for dichotomous categorical variables, for trials one, two and three there were no significant differences between the genotypes, p = 1.00, p = .43 and p = .43, respectively. In contrast for trials four and five there were significant differences between the genotypes, p = .03 and p = .03 respectively, with no DAD1$^{-/-}$ rats completing either trial 4 or 5, yet all of the DAD1$^{+/-}$ rats completing both of these trials. However, the genotype comparison for trial six was non-significant, p = .14, despite the fact that only one DAD1$^{-/-}$ rat completed this trial compared to all four of the DAD1$^{+/-}$ rats completing it. Therefore, it is likely that trial 6 did not reach significance due to a lack of statistical power.

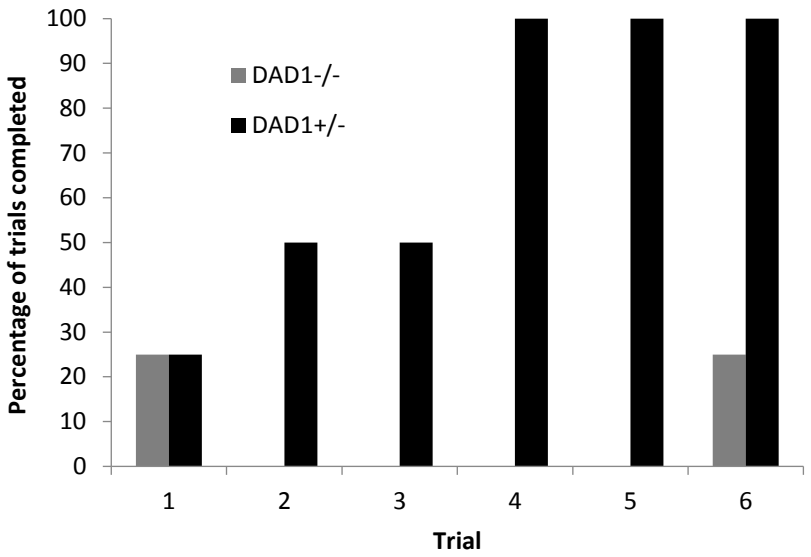

Figure 14. Mean percentage of balance beam trials completed by $DAD1^{-/-}$ (n = 4) and $DAD1^{+/-}$ (n = 4) rats across 6 trials. To complete a trial subjects were required to cross a narrow, elevated beam and enter their home cage within 2 minutes.

Rotarod

Minimally handled group. The rotarod task was used to compare motor coordination across genotypes at three different speeds (10, 20 and 30 RPM). Surprisingly, the control rats in the minimally handled group did not acquire this task well and there were high levels of attrition (attrition rates for $DAD1^{+/+}$ rats: 10 RPM - 3/8; 20 RPM - 6/8; 30 RPM - 7/8; and $DAD1^{-/-}$ rats: 30 RPM - 6/10). As such, for the highest RPM, only one control rat remained meaning that the data from this speed cannot be subject to means testing. Therefore, a 2 (genotype) x 2 (RPM; 10 or 20) mixed measures ANOVA was conducted with RPM as the within-subjects variable and with time spent on the rotarod as the dependent measure (figure 15). There was an approaching significant effect of genotype, $F_{1,10} = 4.42$, $p = .06$, suggesting that the $DAD1^{-/-}$ rats ($M = 71.60$; $SD = 30.49$) spent longer on the rotarod than the $DAD1^{+/+}$ rats ($M = 24.00$; $SD = 13.44$), although this needs to be interpreted with caution due to being just shy of statistical significance. No significant effect of RPM, $F_{1,10} = 1.84$, $p = .21$, was observed and no significant interaction between RPM and genotype, $F_{1,10} = .02$, $p = .90$, was found.

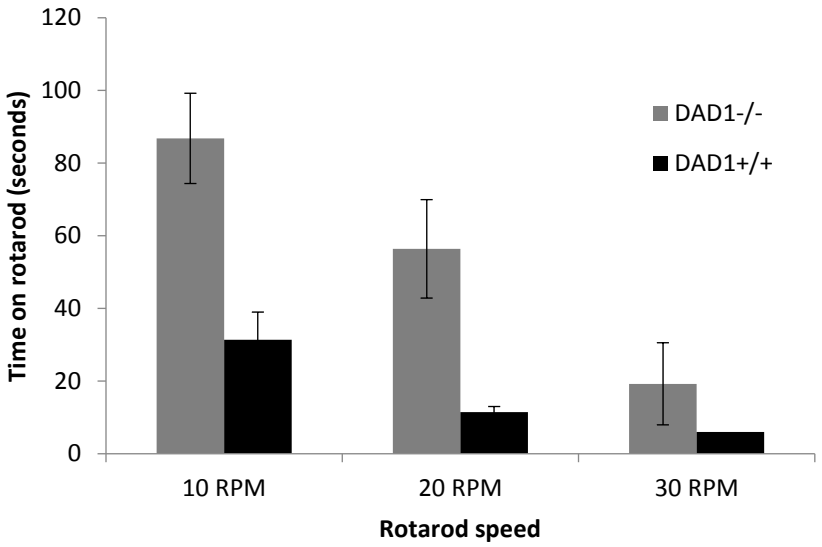

Figure 15. The time in seconds that the minimally handled rats spent on the rotarod at three speeds (10 RPM: n = 10 DAD1$^{-/-}$ & 5 DAD1$^{+/+}$; 20 RPM: n = 10 DAD1$^{-/-}$ & 2 DAD1$^{+/+}$; 30 RPM: n = 4 DAD1$^{-/-}$ & 1 DAD1$^{+/+}$).

Extensively handled group. Due to the high levels of attrition observed during the first rotarod experiment using minimally handled rats, a second cohort of rats who were extensively handled (four – five days per week for three and a half to four months) prior to this experiment were run through the same rotarod procedure. Again, however, high levels of attrition were observed with this being especially pronounced in the control animals, and the experiment was necessarily disbanded. Specifically, despite extensive habituation, too few control rats completed any of the speeds tested to conduct means testing on the data.

Delayed non-matching to position

Accuracy. Group accuracy as a function of genotype was compared across the 25 training sessions (figure 16). Firstly, on training trial one the DAD1$^{-/-}$ and DAD1$^{+/+}$ rats displayed comparable levels of accuracy (~72 - 73%) with no significant difference between the groups, t_{28} = -.19, p = .85. This finding suggests that both DAD1$^{-/-}$ and DAD1$^{+/+}$ tend to alternate their arm choices prior to learning the DNMTP task. Secondly, group analysis of accuracy across all 25 training trials revealed no significant effect of genotype, $F_{1, 28}$ = 2.85, p = .10, and no significant interaction between genotype and session, $F_{25,700}$ = .66, p = .89, indicating that the DAD1$^{-/-}$ and control rats performed the DNMTP task with a similar degree of accuracy over successive trials. There was a

significant effect of session, $F_{25, 700} = 1.84$, $p = .02$, which figure 16 indicates is due to the accuracy of both DAD1$^{-/-}$ and DAD1$^{+/+}$ rats increasing as the sessions progress. Accuracy across both groups increased from ~72 - 73% on the first trial to around ~85 - 90% on trial 25.

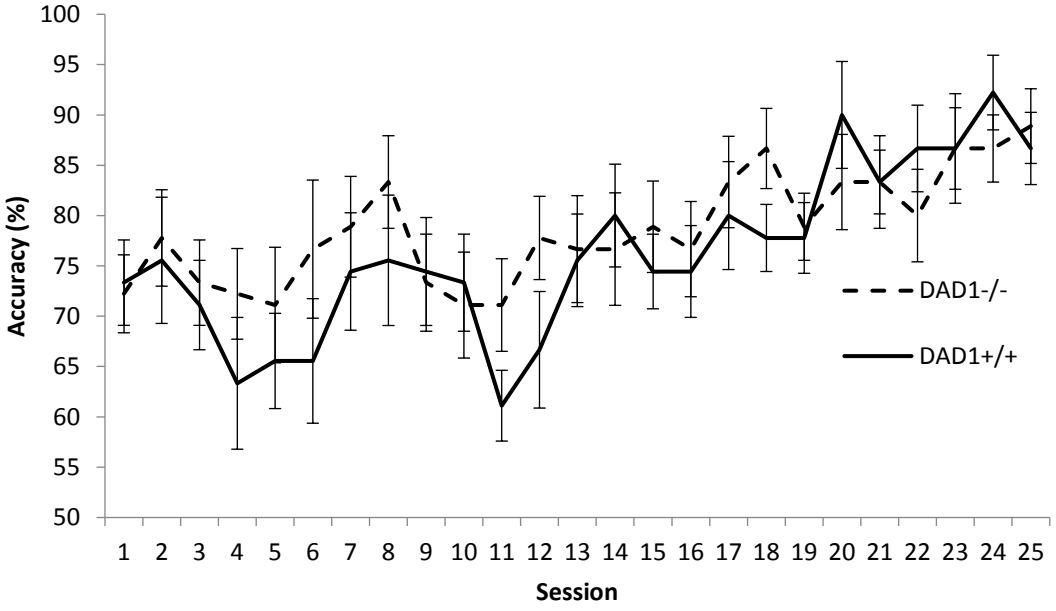

Figure 16. Accuracy of DAD1$^{-/-}$ (n = 15) and DAD1$^{+/+}$ (n = 15) rats on the DNMTP task over 25 sessions. Each session contained six trials, with an accurate response defined as when subjects entered the opposite arm during the choice phase to the arm entered during the sample phase.

Latency. Latency to enter the forced-choice sample arm during the sample phase was compared between genotypes across the 25 training trials. There was no significant effect of genotype, $F_{1, 28} = .05$, $p = .82$, and no significant interaction between genotype and session, $F_{1.62, 45.37} = .26$, $p = .72$, suggesting that DAD1$^{-/-}$ and DAD1$^{+/+}$ rats entered the sample arm within a similar time frame across sessions. There was a significant effect of session, $F_{1.62, 45.37} = 10.18$, $p < .01$, which figure 17 indicates was due to the latency to enter the sample arm being slower in the first few trials than later trials. In the first session the subjects took around 10 seconds to move into the sample arm, yet by the sixth session, and thereafter, this time was reduced to 2 - 3 seconds.

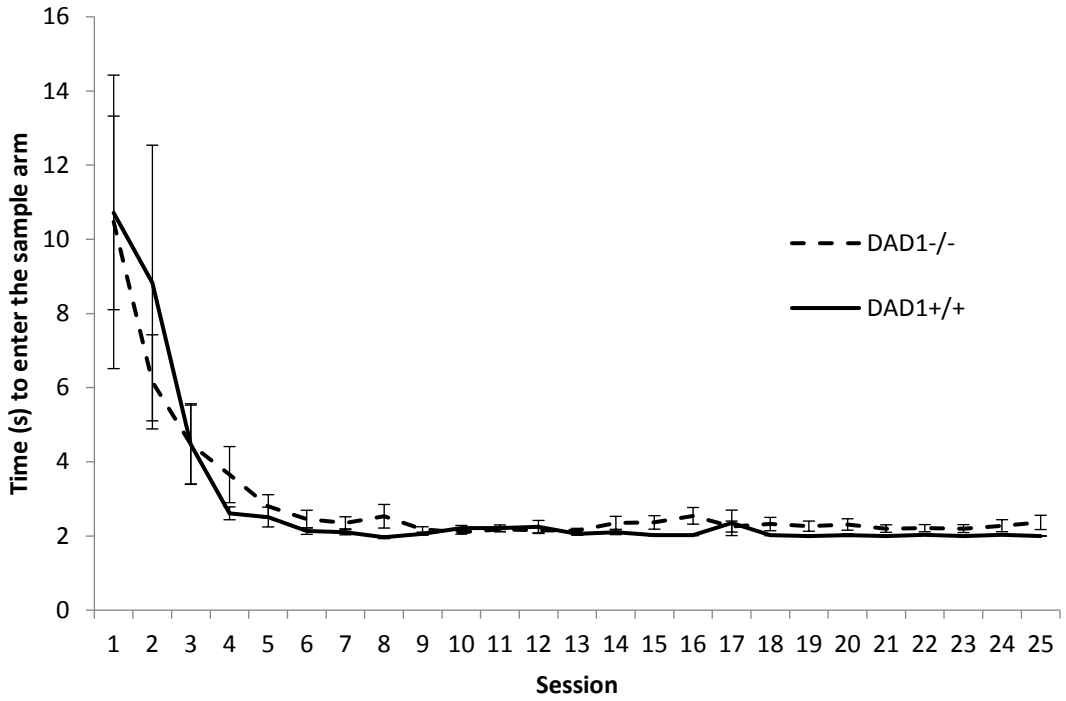

Figure 17. The time taken in seconds for DAD1$^{-/-}$ (n = 15) and DAD1$^{+/+}$ (n = 15) rats to enter the forced-choice sample arm during the sample phase on the DNMTP task.

The latency to enter an arm during the choice phase was compared between genotypes across the 15 training sessions. There was no significant effect of genotype, $F_{1, 28} = 1.63$, $p = .21$, as well as no significant interaction between genotype and session, $F_{1.98, 55.43} = .29$, $p = .75$. Interestingly, although there were no effects of genotype found, figure 18 indicates that after 12 sessions the DAD1$^{-/-}$ rats were consistently slower (taking around 3-4 seconds) than control animals (2 seconds). Again, there was an effect of session, $F_{1.98, 55.43} = .8.32$, $p < .01$, with figure 18 suggesting that this effect is due to subjects entering an arm during the choice phase slower in the first session (around 10 seconds) than in subsequent sessions (2-4 seconds). Collectively, the latency data from both the sample and choice phases suggest that the DAD1$^{-/-}$ and DAD1$^{+/+}$ rats entered the T-maze arms within a similar time frame, albeit slightly, but not significantly, slower during the choice phase, across the 25 sessions.

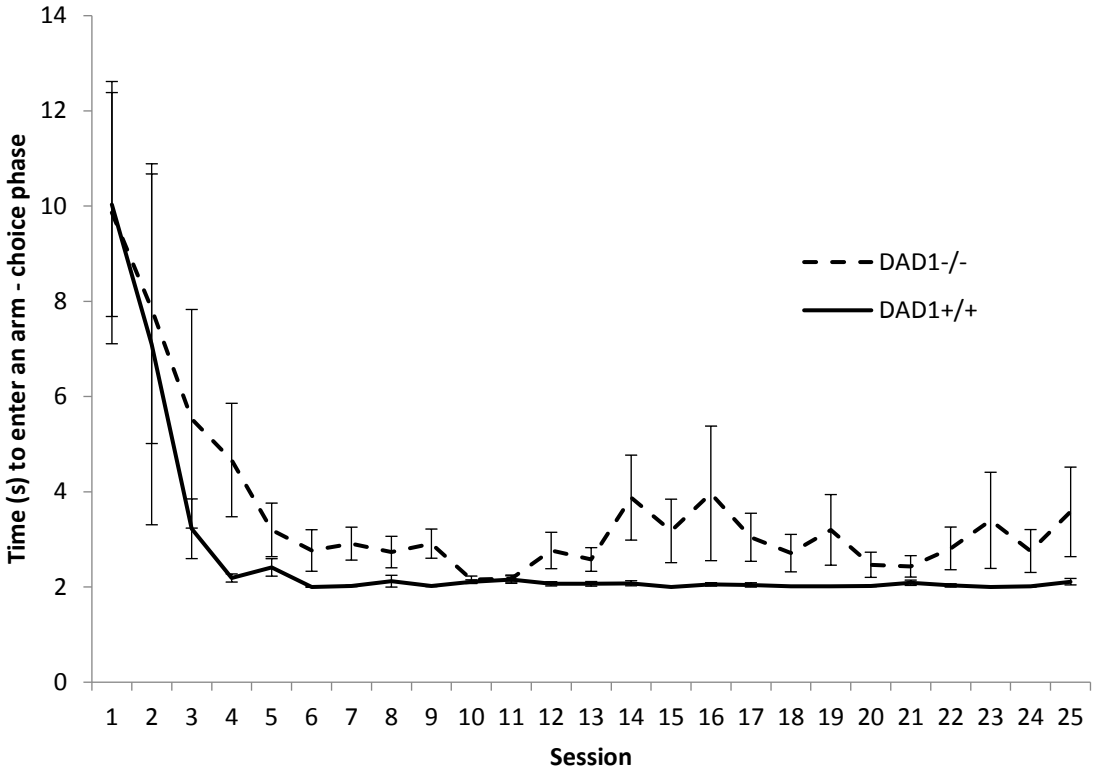

Figure 18. The time taken in seconds for DAD1$^{-/-}$ (n = 15) and DAD1$^{+/+}$ (n = 15) rats to enter an arm during the choice phase on the DNMTP task.

Discussion

This chapter explored the drug-free behavioural tendencies of DAD1$^{-/-}$ rats compared to control rats on a number of tasks that were predicted to engage DA D1 receptors. In particular, behaviours relevant for reward, movement, and memory were investigated. Although on many tasks the DAD1$^{-/-}$ rats performed similarly to controls, important differences between the DAD1$^{-/-}$ rats and controls were detected that influence the experiment selection for *chapter 5*.

Firstly, tasks that measured reward-related behaviours were conducted. In line with predictions deriving from the incentive salience hypothesis (Berridge 2007; Berridge & Robinson, 1998), DAD1$^{-/-}$ and control rats both displayed a strong preference for sucrose solution over water when they were freely available in the home cage. Both genotype groups consumed approximately 94 - 95 % of sucrose solution relative to water on the test day. This finding suggests that a genetic decrease in functional D1 receptors does not affect the hedonic impact of rewards, or reward 'liking'. Next, the time taken for rats to consume ten freely

available odourless sugar pellets was tested across three sessions. These pellets are used as reinforcement in the operant-based DMTS paradigm which is a planned experiment for *chapter 5*, as well as in the autoshaping to lever press experiment presented below. Unexpectedly, compared to control rats the DAD1$^{-/-}$ rats took significantly longer to consume the pellets during sessions two and three. Important to note is that while the DAD1$^{-/-}$ rats were slower than controls, all of the subjects from both genotypes consumed all of the pellets and by the third session the DAD1$^{-/-}$ rats took approximately 25 seconds to consume the pellets, whereas the controls took around 10 seconds. This indicates that although the DAD1$^{-/-}$ rats were comparatively slower to consume the pellets than control rats, the DAD1$^{-/-}$ rats were seemingly motivated to consume the pellets shortly after being placed in the experiment cage. While the finding that the DAD1$^{-/-}$ rats took longer to consume the pellets could reflect lower motivation to consume rewards (i.e. decreased "wanting" for rewards) there are several other possibilities which will also be briefly considered during this discussion, and at greater length in the general discussion (*chapter 6*), such as differences in movement or anxiety-like behaviours.

A third experiment relating to reward examined operant lever pressing for sugar pellets maintained by an FR 1 schedule of reinforcement. Strikingly, the DAD1$^{-/-}$ rats showed a complete attenuation of lever pressing for sugar pellets compared to controls, with virtually absent lever pressing observed in every DAD1$^{-/-}$ rat subject. Although the extent of the lever pressing impairment shown by DAD1$^{-/-}$ rats was more severe than expected, it is not surprising that the DAD1$^{-/-}$ rats displayed altered pressing relative to control rats. This finding is in line with El-Ghundi et al.'s (2003) study which found that D1 deficient mice demonstrated attenuated lever pressing maintained under an FR-1 schedule of reinforcement. However, the D1 deficient mice did engage in some lever pressing, and when food deprived these mice pressed on average 25 times per 30 minute session, with control mice pressing around 100 times per session. Similarly, although D1 receptor antagonists have been found to decrease the acquisition of lever pressing under similar conditions as those used in the current study, the extent of the decrease observed is generally less extensive (e.g. Baldwin et al., 2002; Koch, et al., 2000) compared to the decrease found here using the DAD1$^{-/-}$ rats.

There are several reasons that could account for why the DAD1$^{-/-}$ rats did not acquire operant lever pressing for sugar pellets, as well as why they were slower to consume freely available sugar pellets. Firstly, DAD1$^{-/-}$ rats might be impaired with

regard to an aspect of motor function. However, the locomotor tendencies of DAD1$^{-/-}$ rats closely matched that of controls in terms of both distance travelled and rearing. In addition these were no differences found in terms of the location or pattern of locomotor activity indicating that the DAD1$^{-/-}$ rats did not exhibit different levels of anxiety-like behaviour compared with controls in the context of the open field. In an open field, however, there is a lack of stimuli to explore in the environment and no rewards to approach. Thus it is possible that the activity counts recorded from control subjects in the open field are at floor level, which could explain why the DAD1$^{-/-}$ and DAD1$^{+/+}$ rats displayed a similar pattern of movement. Next, although the control rats did not perform the rotarod task well, the DAD1$^{-/-}$ rats displayed proficiency at this task, especially on the two slowest speeds (10 and 20 RPM). By contrast, the DAD1$^{-/-}$ rats were severely impaired at crossing the balance beam to reach their home cage. It is possible that DAD1$^{-/-}$ rats do not find the beam to be aversive, or alternatively they may not possess the motor skills required to cross the beam. This explanation is unlikely however, because the experimenter noted that DAD1$^{-/-}$ rats would often groom themselves on the beam as well as stand up on their hind legs, presumably to look around, and even turn around, yet they would not walk the full extent of the beam. Thus, it appears that on tasks that do not involve a large reward component, DAD1$^{-/-}$ rats display intact movement.

Considering that all of the tasks that the DAD1$^{-/-}$ rats displayed a deficit that involve a reward component suggests that the DAD1$^{-/-}$ rats have either altered reward processing or possess a learning deficit, rather than a motor deficit. However, a learning explanation cannot account for why the DAD1$^{-/-}$ rats performed 'normally' on the DNMTP spatial memory task. One procedural difference between the DNMTP T-maze task and operant lever pressing is that the maze task simulates foraging behaviour while lever pressing requires an operant response in order to receive the reward. If reward 'liking' is still intact in the DAD1$^{-/-}$ animals, yet 'wanting' is impaired, then perhaps their willingness to work for rewards is decreased. Speculatively, it is possible that because the T-maze task simulates foraging, whereas lever pressing could be considered more abstract to a rat, the degree of difficulty or the effort required to attain a reward differs between these tasks. To test whether the DAD1$^{-/-}$ rats are less willing to work for rewards, future research could implement a progressive ratio task using a maze, incrementally increasing the amount of arm entries required to obtain a sugar pellet. If DAD1$^{-/-}$ rats 'want' rewards

less than control rats, then these rats would be expected to reach their break point earlier than control rats.

In summary, the findings presented in this chapter suggest that the DAD1$^{-/-}$ rats are impaired with regard to behaviours relevant for reward processing, most notably in terms of operant lever pressing. In addition, the DAD1$^{-/-}$ rats moved slower or less than control rats when consuming rewards (consumption of sugar pellets) or when escaping a mildly aversive environment (balance beam). Despite these differences, the DAD1$^{-/-}$ rats demonstrated intact hedonic processing ('liking') in the sucrose preference test. Potential explanations for the behavioural differences observed between DAD1$^{-/-}$ rats and controls, and the implications of these findings for D1 receptor function, will be further explored in the general discussion (*chapter 6*). The main consequence stemming from the finding that DAD1$^{-/-}$ rats did not autoshape to press a lever is that the DNMTP paradigm conducted in a T-maze will be employed in *chapter 5* to explore the acute effects of MDMA on memory, rather than the planned operant DMTS task. Importantly, performance on the DNMTP task was comparable across DAD1$^{-/-}$ rats and controls suggesting that the DAD1$^{-/-}$ rats have intact spatial working memory function. Considering that D1-like receptors have consistently been implicated in working memory, the finding that the DAD1$^{-/-}$ rats performed the DNMTP task 'normally' suggests that the DAD1$^{-/-}$ rats possess sufficient DA transmission and D1-like receptor activation in the PFC to support effective working memory function.

Chapter 4: *C-fos* Expression and Locomotor Activity in DAD1$^{-/-}$ Rats Following Acute MDMA Administration

MDMA and other DA enhancing drugs lead to the expression of the immediate-early gene *c-fos* widely throughout the brain (Colussi-mas & Schenk, 2008; Dragunow, Logan & Laverty, 1991; Robertson, Peterson, Murphy & Robertson, 1989; Stephenson, Hunt, Topple & McGregor, 1999; Young, Porrino & Iadarola, 1991) with this effect being especially pronounced in the striatum, Nacc and in cortical areas such as the mPFC. DA D1-like receptor activation has been shown to, at least in part, mediate this response with D1-like agonist drugs increasing *c-fos* expression (Heijtz & Castellanos, 2006; Robertson et al., 1989; Young et al., 1991) and D1-like antagonists attenuating *c-fos* expression that is ordinarily caused by DA releasing agents (Young et al., 1991). In light of this, and to further validate the DAD1$^{-/-}$ rat model of down-regulated D1 receptor function, the neurochemical response of DAD1$^{-/-}$ rats to MDMA administration using *c-fos* immunohistochemistry will be investigated here. Following administration of either MDMA (3 mg/kg) or saline, *c-fos* expression will be assayed in several regions or sub-regions of the rat brain that contain rich DA innervation, namely the mPFC (cingulate (CG) cortex, prelimbic (PL) cortex and infralimbic (IL) cortex), the striatum (dorsolateral (DL) and dorsomedial (DM)), Nacc (core and shell), the VTA and substantia nigra pars compacta (SNPc). In addition, to assess whether DAD1$^{-/-}$ rats display a similar behavioural profile compared to controls in response to MDMA, locomotor activity will be recorded during the first hour post injection of either MDMA or saline.

Neuronal expression of *c-fos*, and its protein product Fos, occurs in response to a wide variety of treatments, stimuli and extracellular signals, including pharmacological manipulations and drug administration, growth factors, neurotransmitter release and induced seizures. Since the 1980s, *c-fos* has become one of the most commonly used functional anatomical markers for neuronal activity due to its expression being rapid, transient, and in relatively low levels under basal conditions (Herrera & Robertson, 1996). The two primary pathways for the induction of *c-fos* expression are 1. via increased intracellular Ca^{2+} levels or increased levels of cyclic AMP acting through a calcium response/cyclic AMP responsive element on the *c-fos* promoter, or 2. via serum response factors which activate a DNA serum response element (i.e. independent of cyclic AMP) (Hoffman & Lyo, 2002). The fact that DA D1 receptors indirectly activate

cyclic AMP likely underlies the ability of these receptors to mediate *c-fos* expression (Carlezon Jr, Duman & Nestler, 2005; Sheng, McFadden & Greenberg, 1990).

Based on the premise that MDMA leads to an increase in DA release which subsequently binds to D1 receptors, thereby increasing *c-fos* expression, it is predicted that DAD1$^{+/+}$ control rats treated with MDMA will demonstrate increased *c-fos* expression compared to DAD1$^{+/+}$ control rats treated with saline. By comparison, due to having down regulated D1 receptors it is predicted that, following MDMA, DAD1$^{-/-}$ rats will demonstrate attenuated *c-fos* expression compared to DAD1$^{+/+}$ rats. As saline administration has not been linked to DA D1 receptor activation, it is predicted that levels of Fos in response to saline will be comparable across DAD1$^{-/-}$ and DAD1$^{+/+}$ rats. The expression pattern of *c-fos* is also hypothesised to depend on the region assayed. For example, MDMA stimulates DA release in structures that contain DA neuron terminal regions which show dense expression of D1 receptors, such as in the mPFC, striatum and Nacc (Baumann et al., 2008; Fremeau et al., 1991). Thus it is predicted that the VTA and SNPc, which contain DA cell bodies rather than receiving DA afferents, will demonstrate less *c-fos* expression than in the mPFC, Nacc and DM striatum and that *c-fos* expression will be comparable in the VTA and SNPc across DAD1$^{-/-}$ and DAD1$^{+/+}$ rats. In addition, the DL striatum was previously found to express considerably less *c-fos* than in the mPFC, Nacc and DM striatum in response to 5 and 10 mg/kg of MDMA (Colussi-mas & Schenk, 2008). Thus it is predicted that the DL striatum will express lower levels of *c-fos* relative to the mPFC, Nacc and DM striatum across all groups.

Lastly, acute MDMA administration reliably produces an increase in locomotor activity (Ball et al., 2003; Bubar et al., 2004; Spanos & Yamamoto, 1989), an effect which is attenuated by SCH 23390 (Ball et al., 2003; Bubar et al., 2004). This suggests that MDMA-induced release of DA causes an increase in D1-like receptor activation, leading to hyperactivity. Thus it is predicted that the DAD1$^{+/+}$ control rats will demonstrate an MDMA-produced increase in locomotor activity relative to saline, and that the DAD1$^{-/-}$ rats will demonstrate attenuated locomotor activation in response to MDMA relative to the DAD1$^{+/+}$ rats. Lastly, in the previous chapter (*chapter 3*) it was reported that there were no baseline locomotor differences between the DAD1$^{-/-}$ and DAD1$^{+/+}$ rats. Therefore it is predicted that DAD1$^{-/-}$ and DAD1$^{+/+}$ rats treated with saline will demonstrate comparable locomotor activity.

Method

Animals

Twenty-two drug-naïve Male Wistar rats (10 DAD1$^{-/-}$ and 12 DAD1 $^{+/+}$) were used in this experiment. The subjects were handled for 5 - 10 minutes each for five consecutive days prior to the start of this experiment. See the general method section (*chapter 2*) for further information pertaining to the subjects.

Activity Recording in an Open Field

Locomotor activity was assessed using four open field activity-recording systems. The clear Plexiglas chambers (Med Associates Inc., USA; model ENV-515) measured 42 cm x 42 cm x 30 cm and were set in sound- and light-attenuated boxes. Each subject's location was tracked using four sets of 16 infra-red sensors spaced evenly on the walls of the chamber which created a lattice of beams, consisting of squares with dimensions of 25 mm x 25 mm. Distance travelled (cm) was recorded by a computer (Activity Monitor Version 5 program; Med associates Inc., St Albans, VT, USA). For analysis, the activity chamber was split into central and peripheral zones. The central zone was defined as the innermost 19 cm x 19 cm of the chamber and the residual area consisting of the peripheral zone.

While activity was being recorded, the main room lights remained on, but the lights in the sound- and light-attenuated boxes were off. A white noise generator masked extraneous noises during testing. Prior to and after each test session the inside floor and walls of the chamber were cleaned with Virkon 'S' disinfectant.

Experimental Procedures and Brain Slice Harvesting

Rats were assigned to either the drug condition (MDMA) or the control condition (saline) in a random manner, creating four independent groups: DAD1$^{-/-}$/MDMA (n = 5), DAD1$^{-/-}$/saline (n = 5), DAD1$^{+/+}$/MDMA (n = 6), DAD1$^{+/+}$/saline (n = 6). The drug condition was MDMA [(±)-MDMA hydrochloride; Institute of the Environmental Science and Research, Porirua, NZ] 3 mg/kg dissolved in 0.9 % saline solution. Both MDMA and saline solutions were delivered via i.p. injection at a volume of 1 ml/kg.

On the day of testing, rats were habituated to the experimental room for 30 minutes. They were then placed in the open field chambers to habituate to the apparatus for 30 minutes. After 30 minutes of habituation, they were then injected with their

assigned drug – either MDMA or saline – and then placed back in the activity chamber for a further 120 minutes. This time frame was selected on the basis of Fos expression peaking between 60 and 180 minutes post stimulation (Kovacs, 1998). However, only locomotor activity recorded during the first hour post-injection was used for analysis, due to MDMA-induced hyperlocomotion peaking 30 - 40 minutes after delivery of MDMA.

Once 120 minutes post-injection had elapsed, rats were deeply anesthetised with sodium pentobarbital (50 mg/kg) via i.p. injection. Intracardial perfusion was then immediately performed to administer 150 - 200 mL of saline, containing heparin to prevent blood-clotting, followed by 300 - 350 mL of newly prepared 4% paraformaldehyde solution in 0.1 M phosphate buffer (PB) (pH 7.4). The brain was then rapidly removed and placed in fresh fixative, 4 % paraformaldehyde, overnight. Next, the brain was cryoprotected in 0.1 M PB containing 30% sucrose at 4 degrees Celcius, after which it was frozen for 4 minutes in isopentane at between -35 and -40 degrees Celcius.

Coronal sections (35 microns thick) of the nine regions of interest were cut on a freezing microtome along the entire brain. For the current Fos analysis we used a one in six series of sections, with six slices per region of interest, excluding the mPFC where 3 slices were used. The slices were immersed in 10 mM PB (pH 7.4) containing 0.9% NaCl (PBS) and 0.1% sodium azide, preventing bacterial growth, and then stored at 4 degrees Celcius prior to the immunostaining.

Fos Immunohistochemistry

The sectioned slices were reacted to show neurons expressing *c-fos*, with the experimenter being blind to which of the groups (i.e. genotype and drug conditions) the rat brain slices belonged. The slices were first transferred from the wells containing 10 mM PB (pH 7.4) containing 0.9% NaCl (PBS) and 0.1% sodium azide into baskets, one basket per rat brain, that had perforated bottoms allowing for ease of transportation of the slices to and from each washing and staining solution. All of the following washes and incubations were conducted at room temperature and using mild agitation.

First, the free-floating slices were washed with PBS containing 0.3% triton X-100 (PBST) three times for 10 minutes each. They were then incubated overnight with the primary rabbit anti-Fos antibody (Ab-5; Calbiochem, EMD Biosciences, Darmstadt, Germany) diluted 1:20,000 in PBST and 1% bovine serum albumin. The anti-Fos antibody was raised against a synthetic peptide (SGFNADYEASSSRC) corresponding to

amino acids 4 - 17 of the human Fos protein. It recognizes the ~55-kDa *c-fos* and the ~62-kDa *V-Fos* proteins, and does not respond to the 39-kDa Jun protein. The slices were then washed in PBST, again three times for 10 minutes per wash. They were then incubated for 90 minutes with secondary biotinylated goat anti-rabbit antibody (Vector Laboratories, Burlingame, CA, USA) diluted in 1:1,000 in PBST. After another wash with PBST, three times for 10 minutes each, the slices were incubated for 60 minutes with freshly preformed avidinbiotinylated (ABC) horseradish peroxidase complex diluted 1:1,000 in PBST. Again, the slices were washed three times for 10 minutes each with PBST before visualization of the bound peroxidase was attained by incubating slices in a solution of 50 mM Tris-HCl (pH 7.4), containing 0.02% 3,3'-diaminobenzidine, 0.8% nickel chloride and 0.003% H_2O_2 to produce a blue-black precipitate. This reaction was stopped with three washes of PBST, 5 minutes per wash. The slices were then mounted on gelatin-coated slides, stained with neutral red and cover slipped with DePeX mounting medium (Scharlau Chemie, Barcelona, Spain).

A negative control was also conducted, with slices in this condition experiencing all iterations of the above procedure except that they were not reacted with the primary antibody. This control was run to ensure the absence of non-specific immunostaining in the tissue.

Cell Counts

The experimenter was blind to all treatment groups, with the counts being de-coded at the end of the experiment. The sections were analysed using an Olympus BX-51 microscope, with images captured at x100 magnification using a MBF Biosciences camera (CX 9000).

The brain structures were identified using the rat brain atlas of Paxinos and Watson (2005). A counting template for each structure was made based on its size and shape (adapted from Colussi-Mas & Schenk, 2008). Images of each section were overlaid with the relevant templates and cells within the templates were manually counted (only nuclei with blue-black staining were included) using the computerised image analysis system Neurolucida (MBF Biosciences, *v.* 8). See figures 19, 20 and 21 for diagrams of the regions of interest with overlaid templates and the template areas (in mm^2), with the corresponding bregma coordinates used for each structure.

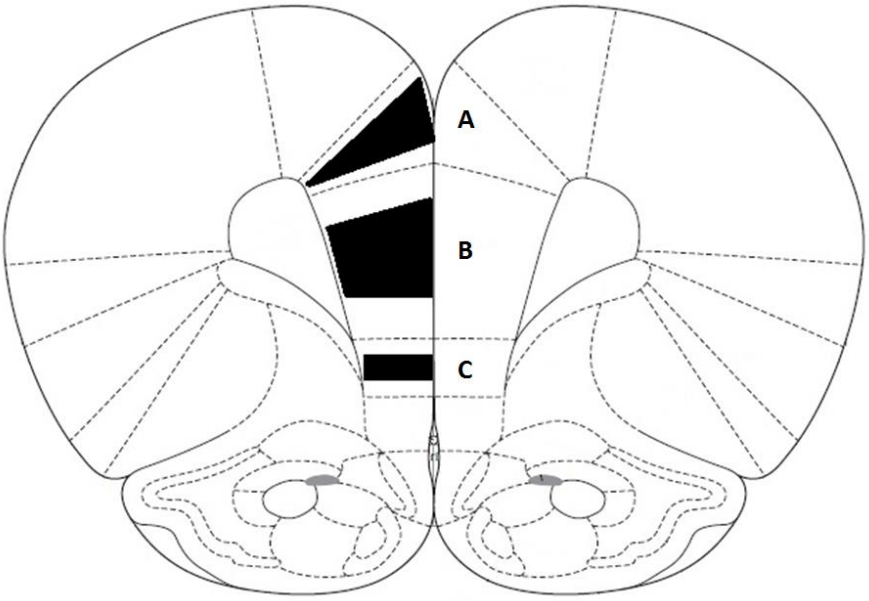

Figure 19. Diagram of the templates used to quantify Fos-immunoreative cells at bregma = 3.72 mm. Identical templates were also applied to the right hemisphere. A = cingulate cortex, area: 0.24 mm^2; B = prelimbic cortex – area: 0.50 mm^2; C = infralimbic cortex, area: 0.20 mm^2. Image adapted from Paxinos and Watson (2005).

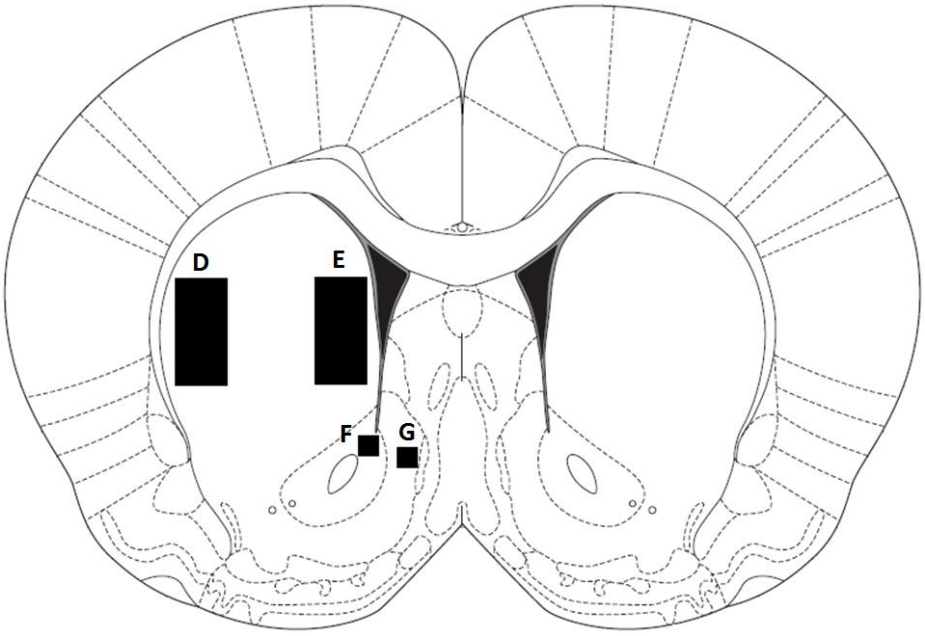

Figure 20. Diagram of the templates used to quantify Fos-immunoreative cells at bregma = 1.32 mm. Identical templates were also applied to the right hemisphere. D = DL striatum, area: 0.60 mm^2; E = DM striatum, area: 0.60 mm^2; F = Nacc core, area: 0.06 mm^2; G = Nacc shell, area: 0.06 mm^2. Image adapted from Paxinos and Watson (2005).

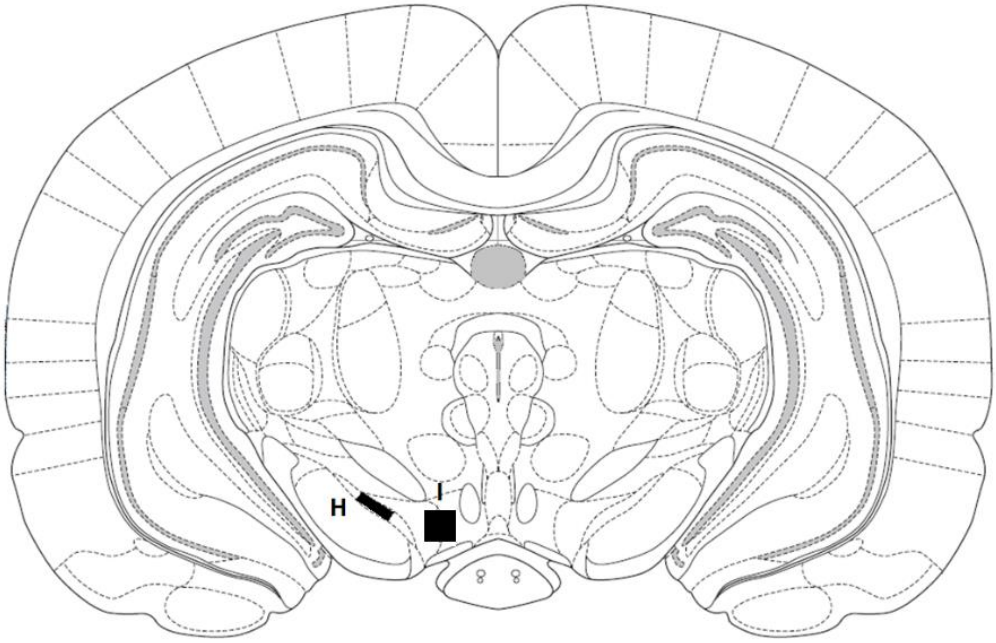

Figure 21. Diagram of the templates used to quantify Fos-immunoreative cells at bregma = -5.04 mm. Identical templates were also applied to the right hemisphere. H = SNPc, area: 0.03 mm^2; I = VTA, area: 0.07 mm^2. Image adapted from Paxinos and Watson (2005).

Data Analysis

For each region of interest, raw cell counts were averaged across the two hemispheres and across all brain slices of that region for each rat. This led to one cell count average per rat for each of the nine sub-regions. These averages were then converted to *c-fos* density measures (number of Fos-immunoreactive cells/mm^2) by dividing the areas (in mm^2) of the templates used per region. Mean *c-fos* densities, grouped by genotype and drug treatment, were then calculated for each brain structure. Group comparisons of *c-fos* densities were conducted for each of the nine brain structures using 2 (genotype) x 2 (drug treatment) between-subjects ANOVAs. Subsequently, planned comparisons were conducted to assess whether either genotype demonstrated an MDMA-induced increase in *c-fos* relative to saline in any of the regions assayed. Using two independent samples *t*-tests, the following comparisons with regard to *c-fos* density per region were conducted: $DAD1^{+/+}$/MDMA vs $DAD1^{+/+}$/saline and $DAD1^{-/-}$/MDMA vs $DAD1^{-/-}$/saline. In addition, further planned comparisons were conducted to compare each genotype's *c-fos* expression in response to saline or MDMA. Specifically, two

independent samples *t*-tests were used to compare the following groups with regard to *c-fos* density in each of the nine regions: $DAD1^{+/+}$/MDMA vs $DAD1^{-/-}$/MDMA and $DAD1^{+/+}$/saline vs $DAD1^{-/-}$/saline.

Locomotor activity was measured by quantifying the total distance travelled (in cm) in the centre and periphery of the open field for two separate time periods: 30 minutes prior to the injection (habituation period), as well as 1 hour after the injection of either saline or MDMA. First, individual subject totals were calculated separately for both the habituation period and the 1 hour post-injection period. Activity during the 30 minute habituation period, in either the centre or periphery, was then grouped by genotype, but not drug treatment as the drug treatments had not been administered at this point. Group comparison of total activity in either the centre or periphery during the 30 minute habituation period was performed using two independent samples *t*-tests, with genotype as the grouping variable. Next, subject totals of distance travelled in either the centre or periphery during the 1 hour post-injection period were averaged by genotype and drug treatment. Distance travelled in either the centre or periphery was separately analysed using two 2 (genotype) x 2 (drug treatment) between-subjects ANOVAs. Planned analyses were subsequently carried out to specifically assess whether either genotype group displayed MDMA-induced hyperactivity. Using distance travelled in the centre or periphery during the 1 hour post-injection period, independent samples *t*-tests were performed between the $DAD1^{+/+}$/MDMA and $DAD1^{+/+}$/saline rat groups, and between the $DAD1^{-/-}$/MDMA and $DAD1^{-/-}$/saline groups. Lastly, further planned comparisons were conducted to directly compare the genotype groups with regard to their locomotor response to either MDMA or saline. Specifically, activity in the centre and periphery was separately compared between the $DAD1^{+/+}$/MDMA and $DAD1^{-/-}$/MDMA groups, and between the $DAD1^{+/+}$/saline and $DAD1^{-/-}$/saline groups. All statistical analyses were conducted using IBM SPSS Statistics version 19.0 for windows. The alpha level for statistical significance was set at $p < 0.05$.

Results

C-fos density

Overall, the pattern of *c-fos* expression varied greatly across regions (figure 22). Regions with high levels of *c-fos* expression after injection of saline or MDMA were the

mPFC (CG, PL and IL corteces) and Nacc shell. There was moderate *c-fos* expression in the DM striatum and Nacc core, and low expression in the DL striatum, VTA and SNPc.

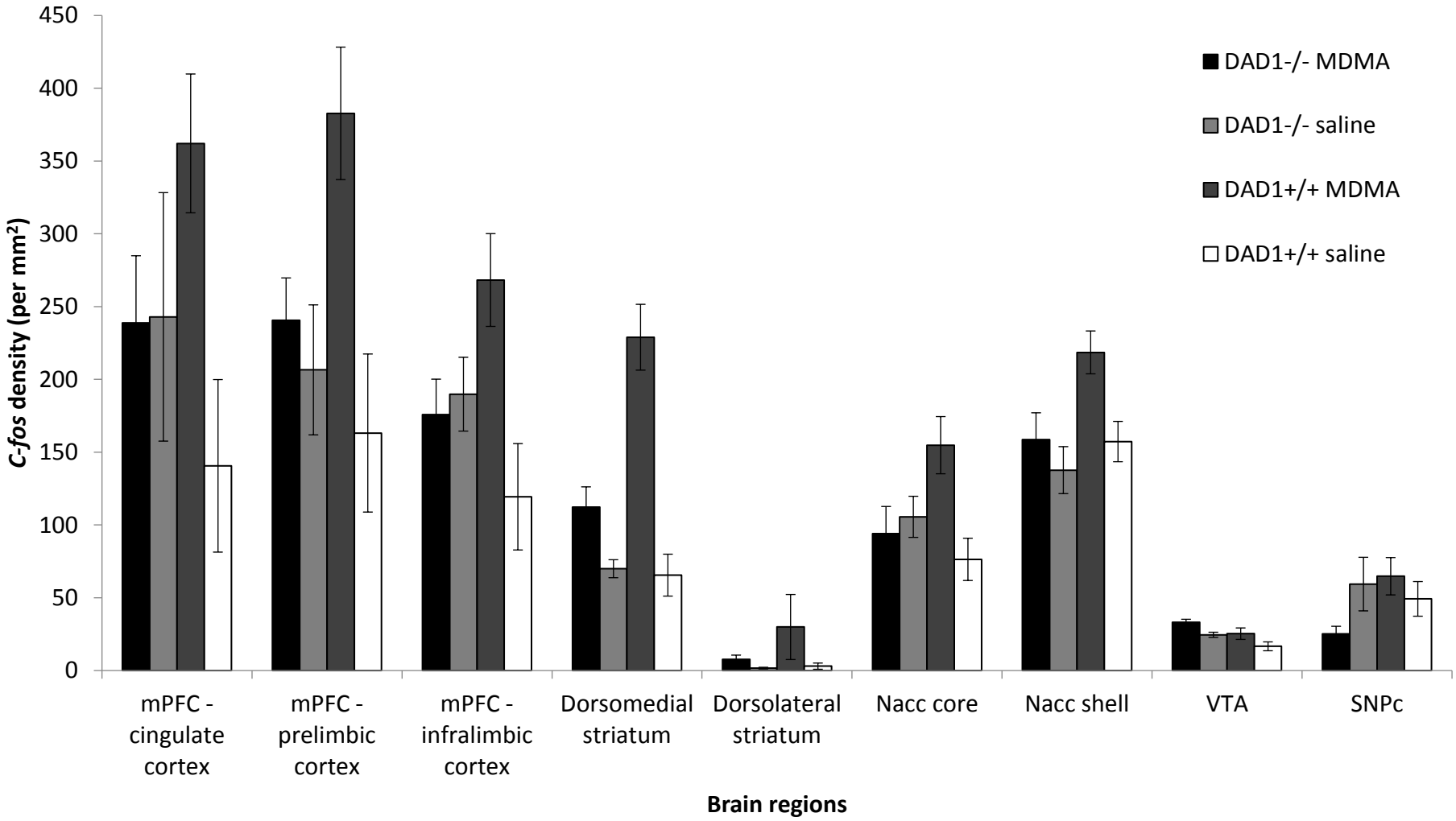

Figure 22. Comparison of *c-fos* densities quantified in nine brain regions in DAD1$^{-/-}$ (MDMA: n = 5; saline: n = 5) and DAD1$^{+/+}$ (MDMA: n = 6; saline: n = 6) rats who either received an acute injection of MDMA (3 mg/kg) or saline 2 hours prior to an intracardial perfusion of a tissue fixative.

See table 4 for the *F* statistics and *p*-values generated from the factorial ANOVA analyses of *c-fos* densities by region, and tables 5 and 6 for breakdowns of the means and standard deviations of *c-fos* densities by region. In addition, tables 7 and 8 display the *t*-test results of the planned comparisons.

MDMA-induced *c-fos* expression and genotype effects. As expected, MDMA (3 mg/kg) significantly increased *c-fos* expression, measured using *c-fos* density, compared to saline across several brain regions. Specifically, a significant main effect of MDMA was found in the PL cortex, IL cortex, DM striatum, Nacc shell, and VTA (table 4).

There were also significant effects of genotype found whereby the $DAD1^{+/+}$ expressed significantly more *c-fos* than the $DAD1^{-/-}$ rats in the DM striatum and the Nacc shell, yet $DAD1^{-/-}$ rats expressed significantly more *c-fos* than the $DAD1^{+/+}$ rats in the VTA. However, the difference in *c-fos* expression between $DAD1^{+/+}$ and $DAD1^{-/-}$ rats in the VTA is slight given the very low levels of *c-fos* expression found in this region.

Attenuated MDMA-induced c-fos expression in $DAD1^{-/-}$ rats. *C-fos* expression interacted significantly between genotype and drug treatment in the IL cortex, DM striatum and Nacc core. The planned comparisons reveal that these interactions are likely due to the MDMA-induced increase in *c-fos* expression being more pronounced in $DAD1^{+/+}$ rats than in $DAD1^{-/-}$ rats. Compared to $DAD1^{+/+}$ rats treated with saline, $DAD1^{+/+}$ rats treated with MDMA displayed marked increases in *c-fos* expression that reached the level for significance in the CG cortex, PL cortex, IL cortex, DM striatum, Nacc core and Nacc shell (table 7). By contrast, $DAD1^{-/-}$ rats only displayed an MDMA-induced increase in *c-fos* expression in the DM striatum and VTA, with the increase observed in the $DAD1^{-/-}$ rats in the DM striatum being of a lesser magnitude than demonstrated by the $DAD1^{+/+}$ rats in this region. Furthermore, table 8 shows that $DAD1^{+/+}$ rats treated with MDMA displayed significantly more *c-fos* expression than $DAD1^{-/-}$ rats treated with MDMA in the PL cortex, IL cortex, DM striatum, Nacc shell and SNPc, yet significantly less *c-fos* expression than $DAD1^{-/-}$ rats treated with MDMA in the VTA. Again, *c-fos* levels in the VTA are comparatively low, meaning that the difference between the genotypes in this region is very small.

Table 4

Results of the 2 (genotype) x 2 (drug treatment) between subject ANOVAs conducted to compare c-fos densities in the nine brain regions assayed

	Drug treatment	Genotype	Drug x genotype
mPFC**			
Cingulate cortex	$F_{1,17} = 3.37$, $p = .08$	$F_{1,17} < .01$, $p = .93$	$F_{1,17} = 3.24$, $p = .09$
Prelimbic cortex	**$F_{1,17} = 8.49$, $p = .01$***	$F_{1,17} = 1.25$, $p = .28$	$F_{1,17} = 3.63$, $p = .07$
Infralimbic cortex	**$F_{1,17} = 7.13$, $p = .02$***	$F_{1,17} = .50$, $p = .49$	**$F_{1,17} = 7.22$, $p = .02$***
Striatum			
Dorsomedial	**$F_{1,18} = 39.7$, $p < .01$***	**$F_{1,18} = 11.8$, $p < .01$***	**$F_{1,18} = 13.7$, $p < .01$***
Dorsolateral	$F_{1,18} = 1.73$, $p = .21$	$F_{1,18} = .90$, $p = .36$	$F_{1,18} = .71$, $p = .41$
Nacc			
Core	$F_{1,18} = 3.8$, $p = .07$	$F_{1,18} = .85$, $p = .37$	**$F_{1,18} = 6.89$, $p = .02$***
Shell	**$F_{1,18} = 6.90$, $p = .02$***	**$F_{1,18} = 6.41$, $p = .02$***	$F_{1,18} = 1.64$, $p = .22$
VTA	**$F_{1,18} = 8.30$, $p = .01$***	**$F_{1,18} = 6.88$, $p = .02$***	$F_{1,18} < .01$, $p = .99$
SNPc	$F_{1,18} = .52$, $p = .48$	$F_{1,18} = 1.30$, $p = .27$	$F_{1,18} = 3.69$, $p = .07$

Bold typeface and * is used when p < .05. ** For the mPFC analysis $n = 21$ - one rat in the DAD1$^{+/+}$/saline group was removed due to damaged tissue. For all other regions, $n = 22$.

Table 5

Means and standard deviations of c-fos densities in nine brain regions grouped separately by drug treatment and genotype

	Drug treatment		Genotype	
	MDMA	Saline	$DAD1^{+/+}$	$DAD1^{-/-}$
mPFC				
Cingulate cortex	*M* = 306.06	*M* = 191.79	*M* = 261.44	*M* = 240.88
	SD = 123.39	*SD* = 164.03	*SD* = 165.01	*SD* = 144.65
Prelimbic cortex	***M* = 318.13***	***M* = 184.86***	*M* = 282.97	*M* = 223.54
	SD = 115.90	*SD* = 107.2	*SD* = 158.92	*SD* = 81.50
Infralimbic cortex	***M* = 226.23***	***M* = 154.62***	*M* = 200.61	*M* = 182.81
	SD = 81.08	*SD* = 76.01	*SD* = 108.47	*SD* = 53.00
Striatum				
Dorsomedial	***M* = 175.94***	***M* = 67.55***	***M* = 147.26***	***M* = 91.13***
	SD = 75.06	*SD* = 26.53	*SD* = 96.16	*SD* = 31.79
Dorsolateral	*M* = 19.78	*M* = 2.41	*M* = 16.46	*M* = 4.66
	SD = 40.61	*SD* = 3.84	*SD* = 39.62	*SD* = 5.45
Nacc				
Core	*M* = 127.19	*M* = 89.66	*M* = 115.60	*M* = 99.82
	SD = 53.51	*SD* = 35.55	*SD* = 57.49	*SD* = 35.42
Shell	***M* = 191.37***	***M* = 148.38***	***M* = 187.93***	***M* = 148.21***
	SD = 47.93	*SD* = 34.65	*SD* = 46.24	*SD* = 38.03
VTA	***M* = 28.91***	***M* = 20.23***	***M* = 20.98***	***M* = 28.88***
	SD = 8.46	*SD* = 7.16	*SD* = 9.40	*SD* = 6.06
SNPc	*M* = 46.79	*M* = 53.84	*M* = 57.01	*M* = 42.29
	SD = 31.23	*SD* = 33.63	*SD* = 30.00	*SD* = 33.75

Bold typeface and * used when $p < .05$.

Table 6

Means and standard deviations of c-fos densities grouped by DAD1$^{-/-}$ and DAD1$^{+/+}$ rats treated with either MDMA or saline

	DAD1$^{+/+}$ MDMA *n* = 6	DAD1$^{+/+}$ Saline *n* = 6*	DAD1$^{-/-}$ MDMA *n* = 5	DAD1$^{-/-}$ Saline *n* = 5
mPFC				
Cingulate cortex	*M* = 358.95	*M* = 141.36	*M* = 246.02	*M* = 243.98
	SD = 115.05	*SD* = 131.95	*SD* = 95.80	*SD* = 190.47
Prelimbic cortex	*M* = 389.95	*M* = 167.92	*M* = 250.68	*M* = 204.16
	SD = 114.96	*SD* = 122.60	*SD* = 71.94	*SD* = 101.90
Infralimbic	*M* = 270.60	*M* = 111.12	*M* = 169.66	*M* = 170.16
cortex	*SD* = 75.87	*SD* = 81.29	*SD* = 54.43	*SD* = 53.82
Striatum				
Dorsomedial	*M* = 228.98	*M* = 65.54	*M* = 112.30	*M* = 69.95
	SD = 55.41	*SD* = 35.27	*SD* = 31.02	*SD* = 13.82
Dorsolateral	*M* = 29.91	*M* = 3.01	*M* = 7.62	*M* = 1.70
	SD = 54.70	*SD* = 5.22	*SD* = 6.59	*SD* = 1.24
Nacc				
Core	*M* = 154.82	*M* = 76.38	*M* = 94.03	*M* = 105.60
	SD = 48.13	*SD* = 35.56	*SD* = 41.76	*SD* = 31.56
Shell	*M* = 218.56	*M* = 157.30	*M* = 158.74	*M* = 137.68
	SD = 36.06	*SD* = 33.92	*SD* = 40.95	*SD* = 36.06
VTA	*M* = 25.33	*M* = 16.64	*M* = 33.2	*M* = 24.55
	SD = 9.66	*SD* = 7.47	*SD* = 4.50	*SD* = 3.96
SNPc	*M* = 64.80	*M* = 49.22	*M* = 25.19	*M* = 59.39
	SD = 31.39	*SD* = 29.14	*SD* = 11.68	*SD* = 41.18

* For the mPFC regions *n* = 5 due to one rat in the DAD1$^{+/+}$/saline group being removed due to damaged tissue.

Table 7

Results of the planned independent samples t-tests comparing c-fos densities in nine brain regions between DAD1$^{+/+}$/MDMA vs DAD1$^{+/+}$/saline and DAD1$^{-/-}$/MDMA vs DAD1$^{-/-}$/saline subject groups to assess for MDMA-induced increases in c-fos expression

	DAD1$^{+/+}$/MDMA vs DAD1$^{+/+}$/saline	DAD1$^{-/-}$/MDMA vs DAD1$^{-/-}$/saline
mPFC**		
Cingulate cortex	**t_9 = 2.93, p = .02***	t_8 = .02, p = .98
Prelimbic cortex	**t_9 = 3.1, p = .01***	t_8 = .83, p = .43
Infralimbic cortex	**t_9 = 3.36, p < .01***	t_8 = -.02, p = .99
Striatum		
Dorsomedial	**t_{10} = 6.1, p < .01***	**t_8 = 2.79, p = .04***
Dorsolateral	t_{10} = 1.2, p = .26	t_8 = 1.97, p = .12
Nacc		
Core	**t_{10} = 3.21, p < .01***	t_8 = -.49, p = .64
Shell	**t_{10} = 3.03, p = .01***	t_8 = .86, p = .41
VTA	t_{10} = 1.74, p = .11	**t_8 = 3.23, p = .01***
SNPc	t_{10} = .89, p = .39	t_8 = -1.79, p = .11

Bold typeface and * is used to when p < 0.05, indicating that subjects in the MDMA group had significantly greater *c-fos* expression compared to subjects in the like-genotype saline group in the respective brain region. ** For the mPFC regions n = 5 due to one rat in the DAD1$^{+/+}$/saline group being removed due to damaged tissue.

Table 8

Results of the planned independent samples t-tests comparing c-fos densities in nine brain regions between DAD1$^{+/+}$/MDMA vs DAD1$^{-/-}$/MDMA and DAD1$^{+/+}$/saline vs DAD1$^{-/-}$ /saline subject groups

	DAD1$^{+/+}$/MDMA vs DAD1$^{-/-}$/MDMA	DAD1$^{+/+}$/saline vs DAD1$^{-/-}$/saline
mPFC		
Cingulate cortex	t_9 = -1.74, p = .12	t_8 = .99, p = .35
Prelimbic cortex	**t_9 = -2.34, p = .04***	t_8 = .51, p = .63
Infralimbic cortex	**t_9 = -2.48, p = .04***	t_8 = 1.35, p = .21
Striatum		
Dorsomedial	**t_9 = -4.17, p < .01***	t_9 = .28, p = .79
Dorsolateral	t_9 = -.9, p = .39	t_9 = -.55, p = .60
Nacc		
Core	t_9 = -2.21, p = .05	t_9 = 1.43, p = .19
Shell	**t_9 = -2.58, p = .03***	t_9 = -.93, p = .38
VTA	**t_9 = 1.67, p = .13***	t_9 = 2.24, p = .06
SNPc	**t_9 = -2.86, p = .03***	t_9 = .48, p = .64

Bold typeface and * is used to when p < 0.05, indicating that the MDMA treated DAD1$^{+/+}$ rats had significantly more *c-fos* expression compared to MDMA treated DAD1$^{-/-}$ rats in the respective regions except in the VTA in which MDMA treated DAD1$^{-/-}$ rats demonstrated significantly more *c-fos* expression than MDMA treated DAD1$^{+/+}$ rats.

Locomotor activity

Figure 23 displays the time course of the distance travelled across the 90 minutes of recorded activity for each of the four groups – DAD1$^{+/+}$/MDMA, DAD1$^{+/+}$/saline, DAD1$^{-/-}$/MDMA and DAD1$^{-/-}$/saline, with activity being collapsed across the periphery and centre of the open field chamber. During the 1 hour post-injection period, the DAD1$^{+/+}$ rats treated with MDMA demonstrate a pronounced increase in activity compared to all other groups. While the DAD1$^{-/-}$ rats treated with MDMA also display an increase in activity compared to saline groups, the magnitude of the increase is much smaller than seen in the control animals.

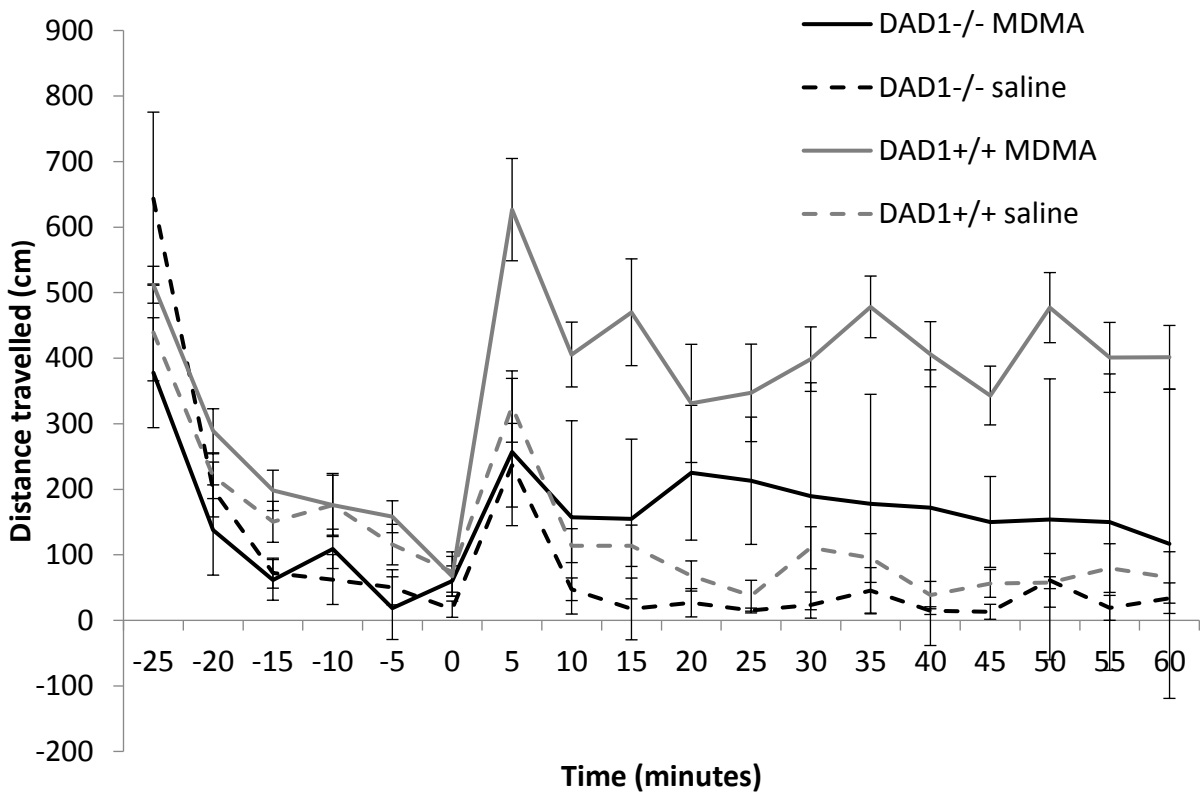

Figure 23. Time course of the distance travelled across the 90 minutes of recorded activity by each of the four groups: DAD1$^{+/+}$/MDMA (n = 6), DAD1$^{+/+}$/saline (n = 6), DAD1$^{-/-}$/MDMA (n = 5) and DAD1$^{-/-}$/saline (n = 5). The first 30 minutes constitutes the pre-injection habituation period. Injections of either saline or MDMA were administered after 30 minutes (represented by '0' on the x axis), and the subsequent 60 minutes constitutes the post-injection period.

30 minute habituation (pre-injection) period. Distance travelled during the 30-minute habituation period was analysed to investigate whether the activity patterns of DAD1$^{-/-}$ rats differed from DAD1$^{+/+}$ rats prior to injection of either MDMA or saline (figure 24). Because the injections had not been administered at this point, the drug groups were collapsed within genotypes. In contrast to findings presented in the previous data chapter (*chapter 3*), distance travelled in the periphery of the chamber by DAD1$^{-/-}$ rats (M =586.37, SD = 295.4) was significantly less than DAD1$^{+/+}$ rats (M = 851.94, SD = 180.47), t_{20} = -2.59, p = .02. However, there was no difference between the genotype groups in terms of distance travelled in the centre of the chamber, t_{20} = -1.34, p = .20.

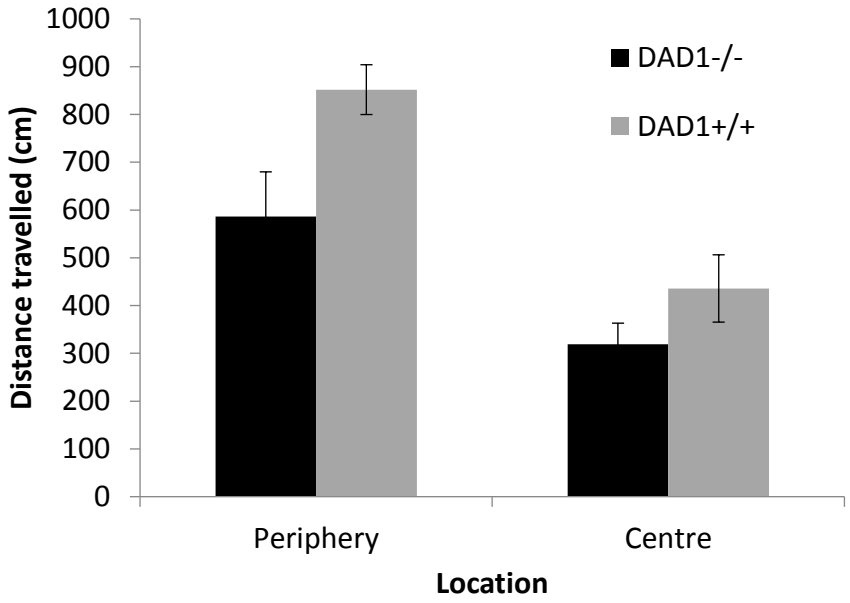

Figure 24. Comparison of DAD1$^{-/-}$ (n = 10) and DAD1$^{+/+}$ (n = 11) rat's total distance travelled (cm), in either the periphery or centre of the activity chamber, during the 30-minute habituation period. * $p < .05$.

Post-injection period. Group comparisons of the total distance travelled in either the periphery (figure 25) or centre (figure 26) during the 1 hour period post-injection of either MDMA or saline were conducted. Consistent with the habituation period, in the periphery of the chamber the DAD1$^{-/-}$ rats (M = 1090.09, SD = 1122.53) moved less overall than the DAD1$^{+/+}$ rats (M = 2420.85, SD = 2064.02), $F_{1,18} = 5.78$, $p = .03$. As expected, there was a significant effect of drug, $F_{1,18} = 14.08$, $p < .01$, with MDMA-treated rats (M = 2887.57, SD = 2029.42) moving more than saline treated rats (M = 744.34, SD = 375.9). However, there was no significant interaction between genotype and drug treatment found, $F_{1,18} = 1.70$, $p = .21$.

In the centre of the chamber, there was no difference between the genotypes observed, $F_{1,18} = 3.22$, $p = .09$ and no interaction between genotype and drug treatment, $F_{1,18} = 3.23$, $p = .09$. Again there was an effect of drug treatment, with MDMA treated rats (M = 849.19, SD = 929.29) moving more than saline treated rats (M = 141.67, SD = 85.87), $F_{1,18} = 6.79$, $p = .02$.

Despite a lack of drug x genotype interactions found in the factorial analysis, planned comparisons were conducted to more finely dissect the locomotor responses of $DAD1^{+/+}$ and $DAD1^{-/-}$ rats after treatment with either MDMA or saline. The results demonstrate that in the periphery of the open field, $DAD1^{+/+}$ rats treated with MDMA (M = 3820.17, SD = 2145.57) moved significantly more than $DAD1^{+/+}$ rats treated with saline (M = 1021.52, SD = 263.64), $t_{5.15} = 3.17$, $p = .02$, indicating that MDMA produced peripheral hyperactivity in the control animals. Regarding $DAD1^{-/-}$ rats, there was an approaching significant difference between the MDMA and saline treated groups, $t_{4.06} = 2.34$, $p = .08$, suggesting that the MDMA-treated (M = 1768.46, SD = 1292) $DAD1^{-/-}$ rats moved more than the saline treated (M = 411.72, SD = 113.27) $DAD1^{-/-}$ rats in the periphery. Although this finding should be interpreted with caution due to being shy of statistical significance, it suggests that MDMA produced peripheral hyperactivity in the $DAD1^{-/-}$ rats.

Regarding activity in the centre of the open field, there was an approaching significant differences between $DAD1^{+/+}$ rats treated with MDMA versus saline, $t_{5.05} = 2.49$, $p = .06$, indicating that the $DAD1^{+/+}$ rats treated with MDMA (M = 1266.22, SD = 1103.23) moved more than those treated with saline (M = 141.28, SD = 76.77). Again, although this finding may indicate that the $DAD1^{+/+}$ rats demonstrated MDMA-induced hyperactivity in the centre of the open field, this finding should be interpreted with caution due to not reaching statistical significance. There was no significant difference found between $DAD1^{-/-}$ rats treated with MDMA compared to saline, $t_{5.35} = 1.69$, $p = .15$, indicating that MDMA did not produce central hyperactivity in the $DAD1^{-/-}$ rats.

Comparison of the groups treated with MDMA revealed no differences between $DAD1^{-/-}$ and $DAD1^{+/+}$ rats in either the periphery, $t_9 = -1.87$, $p = .10$ or centre, $t_{5.62} = -1.81$, $p = .10$, of the open field. These analyses suggest that $DAD1^{-/-}$ and $DAD1^{+/+}$ rats treated with MDMA moved a comparable amount. However, the high degree of variability present in the data and small sample sizes could also explain the lack of significant differences observed between these groups. Lastly, analyses of distance travelled in the periphery by the saline treated groups revealed a significant difference between the genotypes, suggesting that $DAD1^{-/-}$ rats (M = 411.72, SD = 113.27) moved less than the $DAD1^{+/+}$ rats (M = 1021.52, SD = 263.64), $t_{7.03} = -5.13$, $p < .01$. Thus in line with data from the 30 minute habituation period, at baseline the $DAD1^{-/-}$ rats moved less

in the periphery than the DAD1$^{+/+}$ rats. By contrast, there was no difference in activity found between saline treated DAD1$^{-/-}$ and DAD1$^{+/+}$ rats in the centre of the open field.

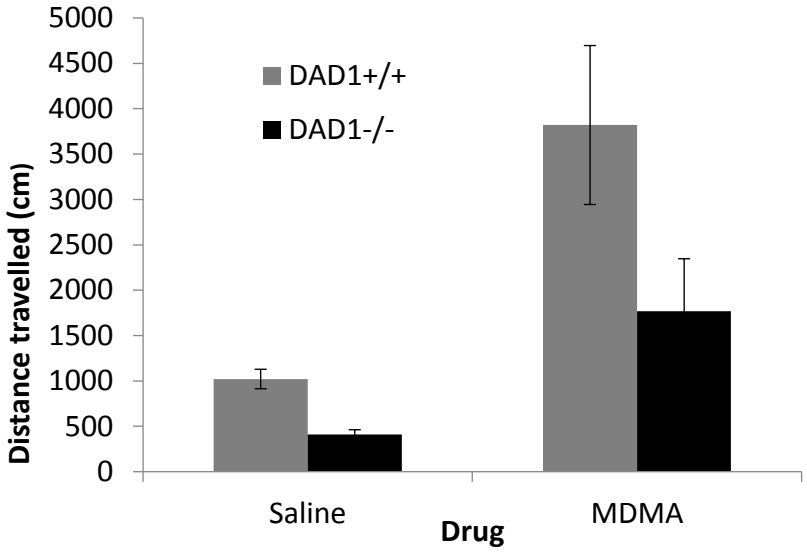

Figure 25. Total distance travelled (cm) in the periphery during the 1 hour post injection period. The graph compares DAD1$^{+/+}$ (MDMA: *n* = 6; saline: *n* = 6) and DAD1$^{-/-}$ (MDMA: *n* = 5; saline: *n* = 5) rats after treatment with either MDMA (3 mg/kg) or saline.

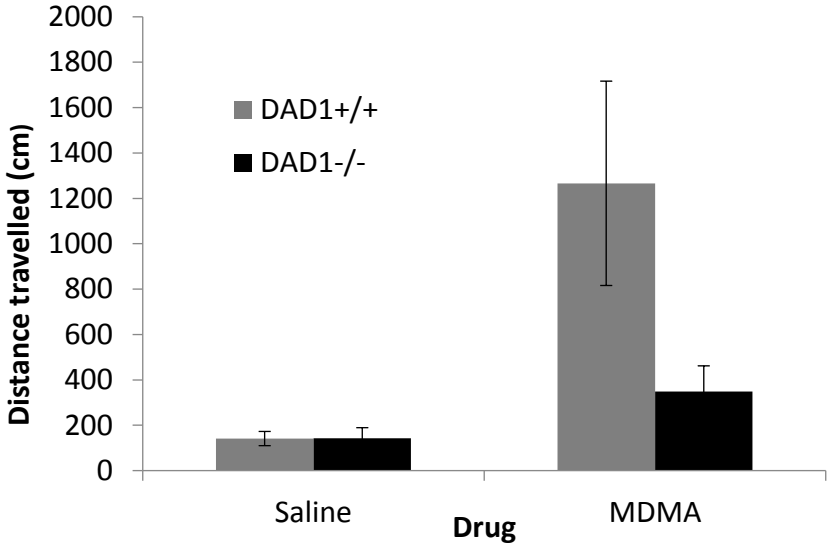

Figure 26. Total distance travelled (cm) in the centre during the 1 hour post injection period. The graph compares DAD1$^{+/+}$ (MDMA: *n* = 6; saline: *n* = 6) and DAD1$^{-/-}$ (MDMA: *n* = 5; saline: *n* = 5) rats after treatment with either MDMA (3 mg/kg) or saline.

Discussion

This chapter compared *c-fos* expression and locomotor activity in DAD1$^{-/-}$ and DAD1$^{+/+}$ rats in response to an acute dose of MDMA (3 mg/kg) or saline (i.p). *C-fos* expression was quantified in the mPFC (CG, PL and IL corteces), striatum (DL and DM), Nacc (core and shell), VTA and SNPc.

As predicted, MDMA-treated DAD1$^{+/+}$ control rats demonstrated an increase in *c-fos* expression compared to saline-treated DAD1$^{+/+}$ rats in the CG cortex, PL cortex, IL cortex, DM striatum and Nacc core and shell. In these control rats, MDMA did not appear to increase *c-fos* expression relative to saline in the two regions that contain DA cell bodies, namely the VTA and SNPc. On the contrary, DAD1$^{-/-}$ rats demonstrated attenuated *c-fos* expression in response to MDMA relative to the MDMA-treated DAD1$^{+/+}$ rats. Specifically, increased *c-fos* expression in MDMA-treated DAD1$^{-/-}$ rats, compared to saline treated DAD1$^{-/-}$ rats, was only observed in the DM striatum and VTA, with the increase in the VTA being of a very small magnitude. Furthermore, MDMA-treated DAD1$^{+/+}$ rats demonstrated increased *c-fos* expression compared to MDMA-treated DAD1$^{-/-}$ rats in the PL cortex, IL cortex, DM striatum, Nacc shell, VTA and SNPc. Finally, by contrast to the differences observed between MDMA-treated DAD1$^{+/+}$ and DAD1$^{-/-}$ rats, saline-treated DAD1$^{+/+}$ and DAD1$^{-/-}$ rats demonstrated comparable levels of *c-fos* expression across all regions, as predicted.

The observation that DAD1$^{-/-}$ rats displayed reduced levels of *c-fos* expression compared to DAD1$^{+/+}$ rats in response to acute MDMA suggests that MDMA acts to stimulate DA release which subsequently binds to D1 receptors thereby inducing *c-fos* expression. If decreased MDMA-induced *c-fos* expression in the DAD1$^{-/-}$ rats is attributable to less functional D1 receptors, then the attenuation of MDMA-induced *c-fos* expression in DAD1$^{-/-}$ rats across most regions assayed suggests that the down-regulation of D1 receptors in DAD1$^{-/-}$ rats is fairly widespread throughout DA's terminal regions, rather than simply being localised to a particular area of the brain. In particular, that DAD1$^{-/-}$ rats demonstrated decreased MDMA-induced *c-fos* expression compared to control rats in the mPFC can be interpreted as being in line with the inverted-U theory of DA transmission in the PFC. This idea will be considered in the general discussion (*chapter 6*) in order to take into account the effects of MDMA on memory performance in the DAD1$^{-/-}$ rats which is the focus of the following chapter (*chapter 5*).

In contrast to findings from the previous data chapter (*chapter 3*), DAD1$^{-/-}$ rats moved less at baseline than DAD1$^{+/+}$ rats in the 30 minute habituation period as well as after treatment with saline during the post-injection period. In *chapter 3* the DAD1$^{-/-}$ and DAD1$^{+/+}$ rats displayed almost identical levels of locomotor activity across three 30 minute sessions, which closely aligns to the procedure used in the current chapter, albeit there was only one 30 minute session of activity recorded. Because the groups of animals used for these separate experiments had starkly different experimental histories prior to the open field experiments, it is possible that the different histories of the rat groups contributed to these contrasting findings. The subjects in the open field experiment presented in *chapter 3* had previously experienced 3 and a half months of extensive handling during a maze experiment (presented in *chapters 3* and *5*) in which subjects were trained to find sugar pellets using the DNMTP paradigm. In comparison, the subjects employed for the current chapter had not been exposed to previous experiments or experienced extensive handling prior to the start of the open field/*c-fos* experiment. Perhaps as a result of exploring a maze for an extended period of time, as well as having exploration paired with the receipt of reward, the DAD1$^{-/-}$ subjects used in *chapter 3* were more explorative in novel environments compared to the rats used in the current chapter. In light of this view, the data from the current chapter suggests that a down-regulation of D1 receptors leads to decreased spontaneous locomotor activity under basal conditions.

Lastly, in line with predictions the DAD1$^{+/+}$ rats demonstrated pronounced MDMA^{-}induced hyperactivity in the periphery of the chamber. However, the DAD1$^{-/-}$ rats demonstrated this pattern of activity in a less conclusive manner. On average, the MDMA^{-}treated DAD1$^{-/-}$ rats did display a large increase in activity compared to saline-treated DAD1$^{-/-}$ rats in the periphery of the chamber, yet means testing revealed this difference as an approaching significant difference (p = .08). Considering the low baseline levels of activity in the DAD1$^{-/-}$ rats, as well as the high variability present in the data and low sample size (n = 5), it is possible that MDMA-treated DAD1$^{-/-}$ rats do display MDMA-induced hyperactivity, yet a lack of statistical power has obscured this finding. Nevertheless, since D1-like receptors have been implicated in MDMA-induced hyperactivity, it is likely that the DAD1$^{-/-}$ rats demonstrate an attenuated locomotor response to MDMA, in a similar fashion to MDMA-induced *c-fos* expression. It would be beneficial for future research to further investigate the behavioural and neural responses of DAD1$^{-/-}$ rats to MDMA in order to characterise the neurochemical effects of MDMA.

Chapter 5: Acute MDMA-induced memory deficits and the role of the DA D1 receptor

Acute MDMA exposure reliably impairs memory performance in human and rat subjects (e.g. humans: Kuypers & Ramaekers, 2005; 2007; Ramaekers, Kuypers, Wingen, Heinecke & Formisano, 2009; rats: Braida et al., 2002; Galizio et al., 2009; 2014; Harper, 2013; Harper et al., 2005; 2006; 2011; Hawkey et al., 2014; Kay et al., 2010; Moyano et al., 2004; 2005; Young et al., 2005), yet the neurological mechanisms by which acute MDMA disrupts memory function are unclear. In light of recent research implicating the D1-like receptor in acute MDMA-induced memory deficits in rats (Harper, 2013; Harper et al., 2011; Rozas et al., 2012), and given that DA is an important neurotransmitter for memory function (Goldman-Rakic, 1995; Seamans et al.,1995; 1998; Williams & Goldman-Rakic, 1995; Zahrt et al., 2007), this chapter investigates the role of D1 receptors in MDMA's acute effects on memory performance using DAD1$^{-/-}$ rats.

There are two main research questions under consideration. The first question investigates whether acute MDMA administration increases memory errors by MDMA-induced DA release causing over-stimulation of DA D1 receptors. Harper (2013) found that the DA D1-like receptor antagonist SCH 23390, when applied concurrently with MDMA, attenuated MDMA-induced memory deficits in a delayed matching-to-sample (DMTS) operant working memory task. The current chapter provides a partial replication of Harper's (2013) study by examining MDMA's acute effects on DNMTP memory performance using DAD1$^{-/-}$ rats. Importantly, although the DMTS and DNMTP tasks differ procedurally in many respects the parameters of the DNMTP task closely match the DMTS task used by Harper (2013).

During the DMTS paradigm subjects are presented with a 'sample' lever (either the left or right lever) and required to press this lever. Following a short delay, the subject is then required to press this same lever during the 'choice' phase in order to receive reinforcement. A similar format is used with DNMTP in that the subject is allowed two arm visits, their first arm visit being the equivalent to the sample lever presentation, and the second arm visit corresponding to the choice phase. However, in DMTS the rat is required to respond on the same lever during both the sample and choice phases, for the purpose of DNMTP, accurate responding during the choice phase will require the subject to select the opposite arm from the one they visited during the sample phase. Secondly,

the DMTS and DMNTP both have two response options available to the subject (two levers in DMTS vs two arms in a T-maze). Thirdly, the intertrial interval will be matched across the tasks. Fourthly, the delay period between the sample and choice phases in DNMTP will approximately correspond to that used in Harper (2013). Lastly, on both the DMTS and DNMTP, perseverative errors can be isolated from other memory errors for separate analysis.

A benefit of the current study relates to the use of DAD1$^{-/-}$ rats rather than D1-like receptor antagonists to model decreased D1 receptor function. Employing DAD1$^{-/-}$ rats to investigate MDMA's impact on memory eliminates the confounding, non-selective effects (e.g. activation of D5 receptors) associated with D1-like receptor antagonists. Based on the hypothesis that acute MDMA administration impairs memory via over-stimulation of D1 receptors, it is predicted that MDMA-treated DAD1$^{-/-}$ rats will perform more accurately on the DNMTP task compared to MDMA-treated DAD1$^{+/+}$ rats. Using a range of behaviourally relevant doses of MDMA (1.5, 2.25 and 3 mg/kg), it is expected that the 3 mg/kg dose will impair accuracy to a greater extent than the lower doses. Stemming from the findings presented in *chapter 3* illustrating that DAD1$^{-/-}$ and DAD1$^{+/+}$ rats performed comparably during 25 training sessions of DNMTP, it is predicted that there will be no group differences following treatment with saline. Lastly, to further explore the hypothesis that MDMA impairs memory by way of over-stimulation of D1 receptors, performance on DNMTP was also assessed following acute treatment with a range of doses (0.5, 1, 1.5, 3 and 4.5 mg/kg) of the D1-like receptor agonist SKF 81297. Based on the idea that MDMA impairs memory via over-stimulation of D1 receptors, it is similarly predicted that over-stimulation of D1 receptors induced by SKF 81297 will impair accuracy in a dose-dependent manner in the control rats, compared to saline. On the contrary, DAD1$^{-/-}$ rats are not expected to demonstrate reduced accuracy following treatment with SKF 81297.

The second research question addressed in the current study relates to the nature of the memory errors observed following acute MDMA exposure. Specifically, if acute MDMA does impair memory on the DNMTP task, do the MDMA-induced impairments reflect a *direct* disruption of memory function, such as compromised working memory processes, or do the impairments reflect an *indirect* disruption of memory such as by way of proactive interference? Proactive interference on tests of memory can be thought of as when a subject's current response is influenced by previous trial information or stimuli.

Acute MDMA has been found to increase proactive interference during operant based tasks, leading to a perseverative pattern of responding. Specifically, subjects treated with MDMA tended to repeat the response they made on the immediately preceding trial, rather than perform the current trial's required response (Harper and colleagues, 2005; 2006; 2013). Furthermore, the alleviation of MDMA-induced memory errors via concurrent administration of SCH 23390, outlined above, was found to correspond to a reduction in proactive interference (Harper, 2013).

To explore whether MDMA increases proactive interference, and whether the D1 receptor is involved, accuracy on trials that require the same response from the previous trial ('previous same trials') will be compared to accuracy on trials that require a different response from the previous trial ('previous different trials'). Lowered accuracy during 'previous different' trials is indicative of perseverative responding or proactive interference. This is because during 'previous different' trials, in order to execute the correct response the subject is required to perform the opposite response that it emitted on the previous trial. On the contrary, during 'previous same' trials, the required response or arm entry is the same as on the previous trial, meaning that if the subjects were influenced by proactive interference on these trials, their response would still be deemed accurate. Therefore, by separating the trials on the basis of whether they are 'previous same' or previous different' allows for examination of whether rats are influenced by proactive interference in certain conditions, such as following drug administration.

Based on the hypothesis that MDMA interferes with memory performance by increasing proactive interference via over-activation of D1 receptor, it is predicted that MDMA-treated DAD1$^{+/+}$ rats will demonstrate increased errors on previous different trials compared to their accuracy on previous same trials. Furthermore, the 3 mg/kg dose of MDMA is expected to produce the greatest difference in accuracy between trial types compared to the 1.5 and 2.25 mg/kg doses of MDMA. Applying the same logic as used for MDMA, SKF 81297-treated DAD1$^{+/+}$ rats are expected to display increased errors on previous different trials compared to previous same trials. However, following treatment with saline DAD1$^{+/+}$ rats are expected to demonstrate comparable accuracy scores irrespective of whether the trial was a previous same or previous different trial. In contrast to DAD1$^{+/+}$ rats, it is predicted that MDMA- and SKF 81297-treated DAD1$^{-/-}$ rats will not demonstrate a decrease in accuracy on previous same trials compared to previous different trials due to possessing down-regulated D1 receptor function. Similarly,

following saline the DAD1$^{-/-}$ rats are not expected to demonstrate a difference between accuracy on previous same and previous different trials.

Method

Subjects

Thirty adult male Wistar rats (15 DAD1$^{-/-}$ and 15 DAD1$^{+/+}$ - groups two and three from cohort three) were used in this experiment. See the general method section (*chapter 2*) for information pertaining to the generation of the DAD1$^{-/-}$ rats. Water and wood shavings were available *ad libitum*, and their weights were maintained at 85% of their free feeding weights with food pellets delivered to their home cages 7 days a week and after experimentation had been completed for the day. Data from these rats on the acquisition of this DNMTP were presented in *chapter 3.* These rats were drug naïve, and were prior subjects in the sucrose preference test described in *chapter 3*.

Apparatus

This paradigm was conducted using a wooden T-maze which had metal inners inserted into it to form the walls of the maze. This was done in order to create taller walls and shorter arm and stem lengths than provided by the wooden maze. The metal inserts were 30 cm high, the two arms were 30 cm long and the stem was 45 cm long. The width of the maze throughout was 9 cm. The stem was painted grey and the arms were painted black. Two pieces of black plexi-glas that were the same height as the maze walls were used to block the maze arms. These "doors" were attached at the top of the maze, and acted as sliding doors that were controlled manually when they were needed to block off either of the arms. Where the doors crossed the arms to block entry were defined as the entry lines to the arms.

White odourless sugar pellets (45 mg Bio-serv®) were used as reinforcement. The sugar pellets were delivered at the end of each T-maze arm, in round white plastic containers (3 cm across) which were secured to the floor of the maze using adhesive.

Procedure

The subjects were habituated to the apparatus for four days and trained on the DNMTP procedure over 25 sessions as presented in *chapter 3*. The trial structure during training, described in *chapter 3*, was identical to that used here during the drug phase of

this experiment. Briefly, there were six trials per session with each trial being separated by a 5 minute inter- trial interval. Rats experienced 4 – 5 sessions per week (no more than 1 per day) with a training session conducted between each drug session in order to ensure subjects remained at baseline accuracy. Each trial involved a sample presentation phase, a delay and a choice phase. During sample presentation either the left or right arm was baited with one sugar pellet and entry to this arm was unimpeded. Entry to the opposite arm was blocked using the Plexiglas door thus only the sample arm was able to be accessed by the rat. The rat was placed at the end of the stem of the maze, facing the junction between the stem and the arms, and was given 2 minutes to consume the pellet. Once the rat entered the baited arm (deemed the sample arm) defined as when all four feet had crossed the entry line, the door was slid closed to confine the subject to this arm. On the contrary, if the subject did not enter the sample arm within the allotted 2 minutes, the rat was removed from the maze and the trial was terminated. Once the rat had consumed the pellet, it was removed from the arm and placed in a holding cage for 10 seconds which was situated on a table behind the stem end of the maze. During this brief delay both doors were slid open and both arms appeared to be re-baited. In reality, only the previously blocked arm was baited and the sample arm was unbaited. Thus in order to retrieve the reward the rat was required to visit the opposite arm to the sample arm. The choice phase began after the delay period. The rat was placed in the stem of the maze and given 2 minutes to explore. If the rat entered one of the arms, the door to that arm was slid closed and the rat was confined to that arm. In the event that the arm entered was the previously baited arm (i.e. the sample arm) which was now unbaited, the rat was confined to this arm for 2 minutes. If the rat alternated their arm entry and entered the baited arm, the rat was allowed to consume the pellet and then removed from the maze and placed back into their home cage. If the rat did not enter either of the arms during the 2 minutes given then the rat was removed from the maze and the trial was terminated. Lastly, the experimenter monitored the consumption of the sugar pellets and in the event that a rat entered an arm but did not consume an available sugar pellet within 1 minute, the trial was terminated.

Whether the baited arm during the sample phase was on the left or right side was pseudo-randomly selected and counterbalanced across both groups of rats. Of the six trials conducted per session per rat, three of the sample arms were on the left, and three

were on the right. Between each trial the maze was wiped out thoroughly with Virkon 'S' disinfectant.

Drug Administration

All drugs were dissolved in 0.9% saline solution, which also served as the control condition, and were administered 15 minutes prior to the start of a session via i.p. injection at a volume of 1ml/kg. Three doses of MDMA [(±)-MDMA hydrochloride; Institute of the Environmental Science and Research, Porirua, NZ] (1.5 mg/kg, 2.25 mg/kg and 3 mg/kg) and five doses of SKF 81297 (0.5, 1, 1.5, 3 and 4.5) were administered. Each rat was administered saline and all doses of MDMA (1.5, 2.25 and 3 mg/kg) 3 times each and all doses of SKF (0.5, 1, 1.5, 3, 4.5 mg/kg) once each. Drugs were administered in four blocks, with a block being defined as all doses of given drug (either MDMA + saline or SKF + saline). To illustrate, all doses of MDMA (1.5, 2.25 and 3.0 mg/kg + saline) were administered in a counterbalanced manner (i.e. block one) before moving on to the next block. Firstly, rats experienced an MDMA block, then secondly the SKF block, thirdly another MDMA block, and lastly the final MDMA block.

Performance Measures

Three performance measures were collected:

1. whether the arm entered during the choice phase matched the sample arm (incorrect response) or did not match the sample arm (correct response);
2. latency in seconds to enter the forced choice arm during the sample presentation phase;
3. latency in seconds to enter an arm during the choice phase.

Data Analysis

Accuracy. Firstly, each rat's accuracy following each of the drug sessions was separately determined. Accuracy scores were then averaged by genotype for each of the drug sessions. To assess the effect of each drug dose on accuracy for the genotype groups, for each of the four drug blocks (MDMA 1, MDMA 2, MDMA 3 and SKF) group comparisons of mean accuracy scores were conducted using four mixed-measures ANOVAs with genotype as a between-subjects variable and drug dose as a within-subjects variable. Follow-up *t*-tests will be conducted where necessary to explore any effects found.

Secondly, in the event that any of the drug block comparisons reveal significant effects of drug or genotype x drug interactions, the following analyses will be conducted using group accuracies from the respective drug blocks. To explore the prediction that MDMA-treated DAD1$^{+/+}$ rats will demonstrate increased errors on previous different trials compared to their accuracy on previous same trials, whereas MDMA-treated DAD1$^{-/-}$ rats will show no difference in accuracy across previous same or previous different trial types, individual accuracy scores for each drug session will be averaged across trial types. Previous same and previous different trials are determined by identifying whether the correct response on the current trial was the same or different, respectively, from the response made by the subject on the immediately previous trial. Lowered accuracy during previous different trials is indicative of perseverative responding. Individual averages for previous same and previous different trials for each of the drug sessions will then be averaged by genotype. Split by genotype, accuracy will be analysed using two repeated measures ANOVAs per drug block assessed, with drug dose and trial type (previous same or previous different) as within-subject variables. *Post hoc* paired-samples *t*-tests will be conducted where necessary.

Lastly, in between each drug session a training session (no injection) was run to ensure that the subjects maintained accurate baseline responding, with 18 maintenance sessions occurring in total. Accuracy over these sessions was averaged as a function of genotype and compared using a 2 (genotype) x 18 (session) mixed-measures ANOVA with genotype as a between-subjects factor and session as a within subjects factor.

Latency. Each rat's latency on sample and choice phases were averaged within each drug session. Individual latencies were then averaged as a function of genotype. For each of the four drug blocks, a group comparison was conducted using mixed-measures ANOVAs with run (sample or choice) and drug dose as within-subject variable, and genotype as a between subject variable. Follow-up *t*-tests will be conducted where necessary to explore any effects found. All statistical analyses were conducted using IBM SPSS Statistics version 19.0 for windows. The alpha level for statistical significance was set at $p < 0.05$. Greenhouse-Geisser corrections were used in the event of Mauchly's test of sphericity being violated.

Results

Accuracy

Baseline responding during maintenance sessions. Accuracy across the maintenance sessions (no injection) was compared by genotype to ensure that baseline responding in between the drug sessions remained stable (figure 27). There were no significant effects of genotype, $F_{1, 22} = .23$, $p = .635$, or session, $F_{17,374} = .91$, $p = .56$, and no genotype x session interaction observed, $F_{17,374} = .91$, $p = .56$. These results demonstrate that DAD1$^{-/-}$ and DAD1$^{+/+}$ rats performed with comparable accuracy during the maintenance sessions. Across genotype, accuracy during the maintenance sessions always remained at or above 80%, and was 86% on average, suggesting that accuracy did not alter over the course of the drug sessions.

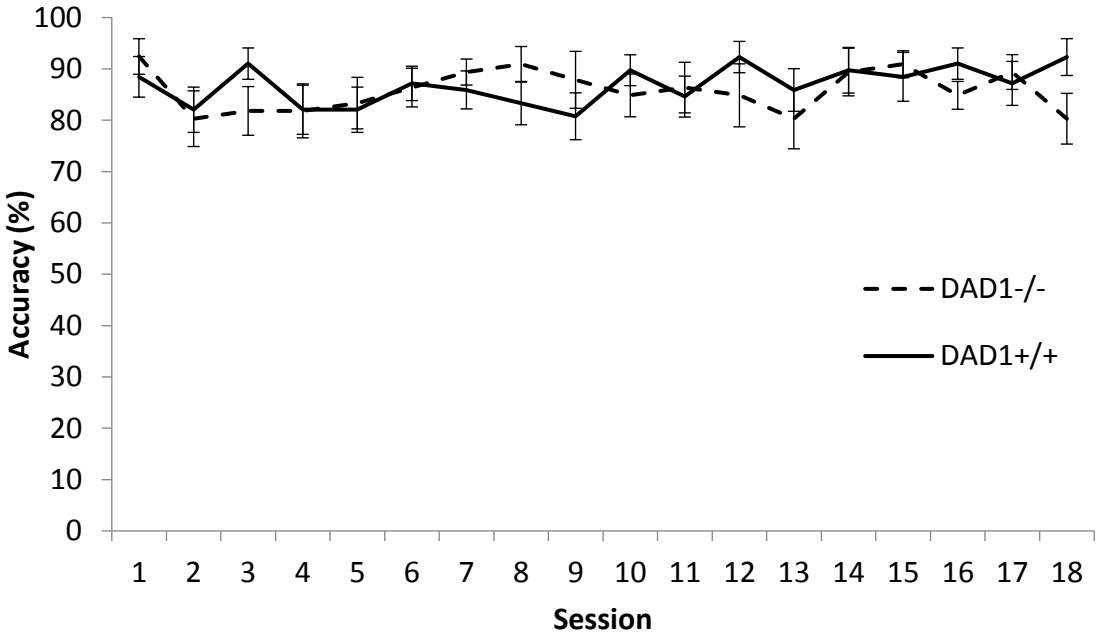

Figure 27. Accuracy of DAD1$^{-/-}$ (n = 11) and DAD1$^{+/+}$ (n = 13) rats across 18 baseline maintenance sessions that occurred in between each drug session. Each session contained six trials, with an accurate response defined as when subjects entered the opposite arm during the choice phase to the arm entered during the sample phase. No drugs were administered during these sessions.

Drug block one: MDMA. Figure 28 displays group means for accuracy following saline and the three MDMA doses tested. There were high levels of attrition during this drug block, especially among the DAD1$^{+/+}$ group. Only two out of thirteen (15%) DAD1$^{+/+}$ and six out of eleven (55%) DAD1$^{-/-}$ rats responded on at least four out of six trials for each dose. With these small sample sizes in mind, there was no main effect of drug, $F_{3,18} = 2.58$, $p = .09$, or genotype, $F_{1,6} = .87$, $p = .39$, and no interaction between drug and genotype observed, $F_{3,18} = .66$, $p = .59$.

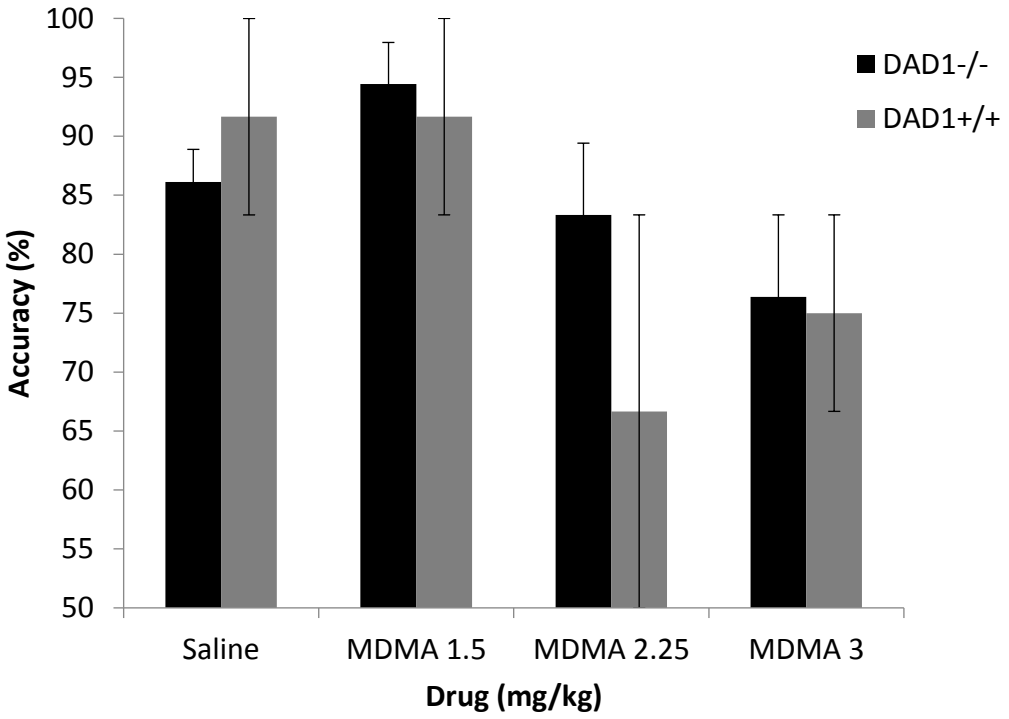

Figure 28. Accuracy of DAD1$^{-/-}$ (n = 6 (55%)) and DAD1$^{+/+}$ (n = 2 (15%)) rats on the DNMTP task during the first block of MDMA and saline doses. Each session contained six trials, with an accurate response defined as when subjects entered the opposite arm during the choice phase to the arm entered during the sample phase. Drugs were administered via i.p. injection 15 minutes prior to the start of a session.

Drug block two: MDMA. Figure 29 displays group means for accuracy following saline and the three MDMA doses tested. Less attrition was observed during this drug block than the 1st block, with eight out of thirteen (62%) DAD1$^{+/+}$ and ten out of eleven (91%) DAD1$^{-/-}$ rats responding on at least four out of six trials for each dose.

Again, there was no main effect of drug, $F_{3,48} = 2.06$, $p = .12$, or genotype, $F_{1,16} = .01$, $p = .91$, found. There was an approaching significant interaction between drug and genotype observed, $F_{3,18} = 2.65$, $p = .06$, yet follow-up *t*-tests revealed that there were no differences between genotypes at any of the drug doses including saline.

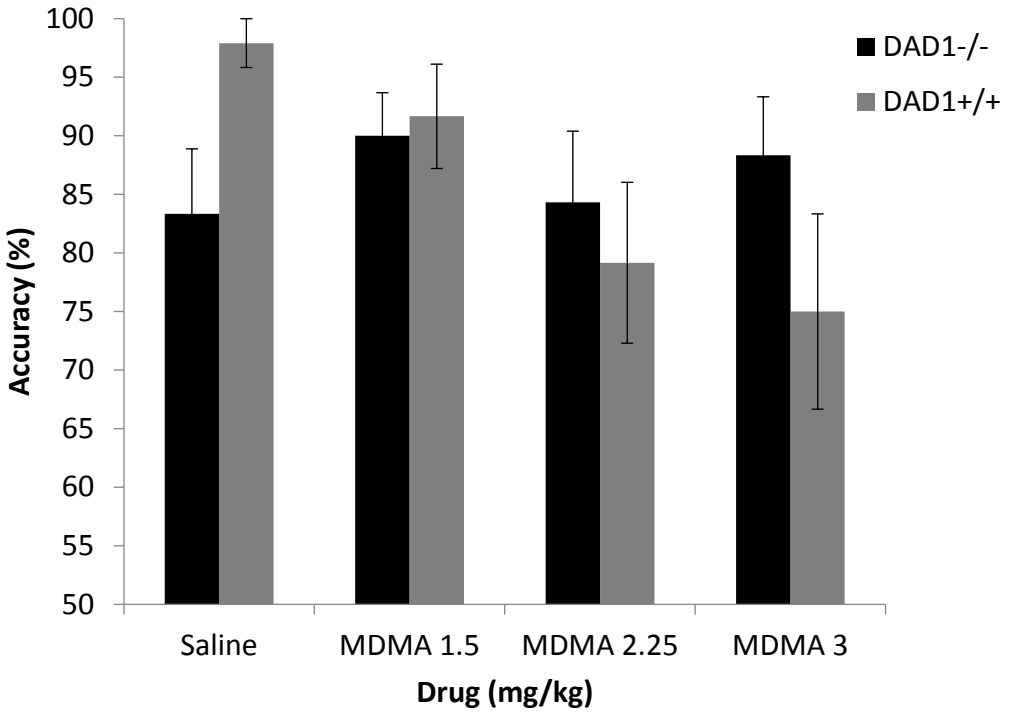

Figure 29. Accuracy of DAD1$^{-/-}$ (n = 10 (91%)) and DAD1$^{+/+}$ (n = 8 (62%)) rats on the DNMTP task during the second block of MDMA and saline doses. Each session contained six trials, with an accurate response defined as when subjects entered the opposite arm during the choice phase to the arm entered during the sample phase. Drugs were administered via i.p. injection 15 minutes prior to the start of a session.

Drug block 3: MDMA. Figure 30 displays group means for accuracy following saline and the three MDMA doses tested. Attrition was minimal during this final drug block, with 12 out of 13 (92%) DAD1$^{+/+}$ and 11 out of 11 (100%) of the DAD1$^{-/-}$ rats responding on at least 4 out of 6 trials for each dose. There was an approaching significant effect of genotype, $F_{1,21} = 4.12$, $p = .06$, with DAD1$^{-/-}$ rats tending to be more accurate across doses ($M = 89.92$, $SD = 11.95$) than DAD1$^{+/+}$ rats ($M = 83.26$, $SD = 17.20$). There was a main effect of drug dose found, $F_{3,63} = 5.03$, $p < .01$, suggesting that

accuracy varied as a function of the drug dose administered. However, this effect should be interpreted in light of the significant interaction between drug and genotype that was observed, $F_{3,63} = 3.05$, $p = .04$. Follow-up independent sample *t*-tests comparing genotype accuracies for each drug dose suggest that the two highest doses of MDMA (2.25 and 3 mg/kg) significantly decreased accuracy for the DAD1$^{+/+}$ rats (2.25 mg/kg: $M = 73.61$, $SD = 16.60$; 3 mg/kg: $M = 76.11$, $SD = 19.32$) compared to the DAD1$^{-/-}$ rats (2.25 mg/kg: $M = 87.88$, $SD = 13.10$; 3 mg/kg: $M = 91.51$, $SD = 13.28$), 2.25 mg/kg: $t_{21} = 2.27$, $p = .03$; 3 mg/kg: $t_{21} = 2.21$, $p = .04$, whilst no differences between saline, $t_{21} = -.48$, $p = .64$, or MDMA 1.5 mg/kg, $t_{21} = -.22$, $p = .83$, were found. The finding that the two highest doses of MDMA decreased the accuracy of DAD1$^{+/+}$ rats, but not DAD1$^{-/-}$ rats, during the third block of MDMA doses is in line with predictions and suggests that the D1 receptor is involved in acute MDMA's impact on memory performance.

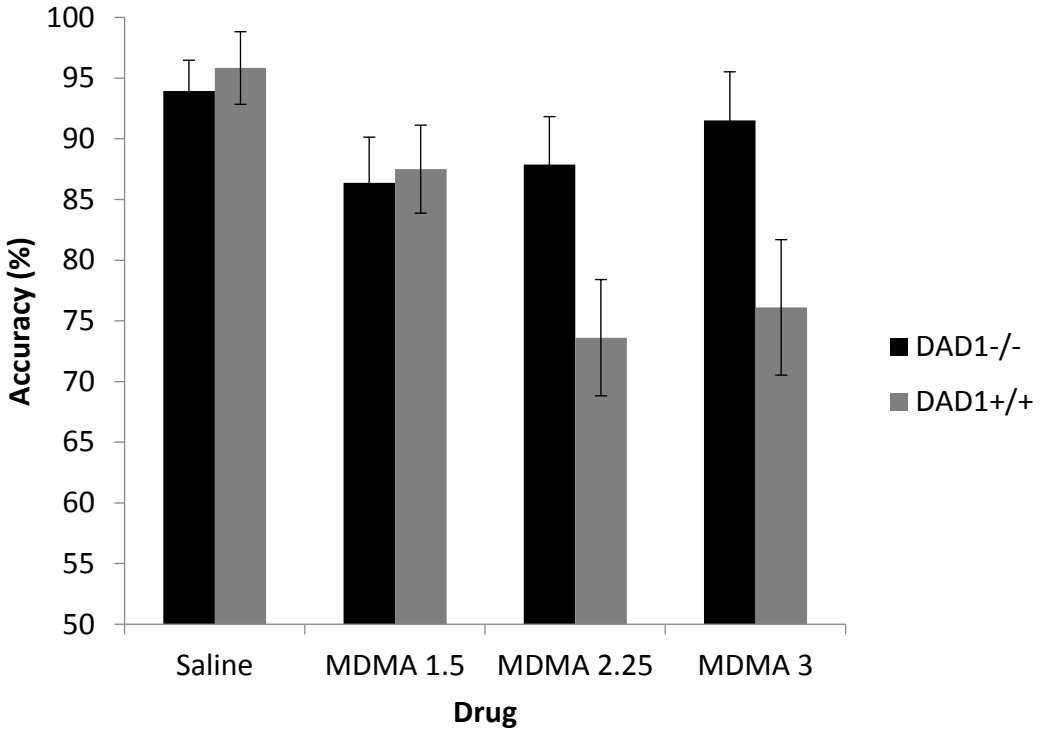

Figure 30. Accuracy of DAD1$^{-/-}$ (n = 11 (100%)) and DAD1$^{+/+}$ (n = 12 (92%)) rats on the DNMTP task during the third block of MDMA and saline doses. Each session contained six trials, with an accurate response defined as when subjects entered the opposite arm during the choice phase to the arm entered during the sample phase. Drugs were administered via i.p. injection 15 minutes prior to the start of a session.

Drug block 4: SKF 81297. Figure 31 displays group means for accuracy following saline and the five doses of SKF 81297 tested. Group comparison revealed no significant effects of genotype, $F_{1,14} = .60$, $p = .45$, or drug dose, $F_{5,70} = 1.00$, $p = .42$, as well as no significant interaction between drug dose and genotype, $F_{5,70} = .53$, $p = .75$. The lack of differences found suggest that the D1-like receptor agonist did not affect performance on the DNMTP task. However, the fact that only five out of thirteen (~38%) $DAD1^{+/+}$ rats completed four or more trials at the highest dose (4.5 mg/kg) of SKF 81297, compared to eleven out of eleven (100%) $DAD1^{-/-}$ rats suggests that the genotypes did respond differently to this drug. The high rates of attrition among $DAD1^{+/+}$ rats may have contributed to the lack of effects found. Furthermore, figure 31 indicates that the 3 mg/kg dose of SKF 81297 appeared to enhance the performance of $DAD1^{-/-}$ rats compared to $DAD1^{+/+}$ rats. As such, exploratory independent samples *t*-tests were conducted to further investigate the effects of SKF 81297 on accuracy across the genotypes. No differences between groups were observed for saline or the 0.5, 1, 1.5 and 4.5 mg/kg doses of SKF 81297 ($p = .12, .68, .78, .33,$ & $.33$, respectively). However, following 3 mg/kg the $DAD1^{+/+}$ rats ($M = 66.90$, $SD = 22.98$) were found to be significantly less accurate than $DAD1^{-/-}$ rats ($M = 89.39$, $SD = 17.11$), $t_{16} = 2.38$, $p = .03$. However, as this analysis was exploratory, it should be interpreted with caution.

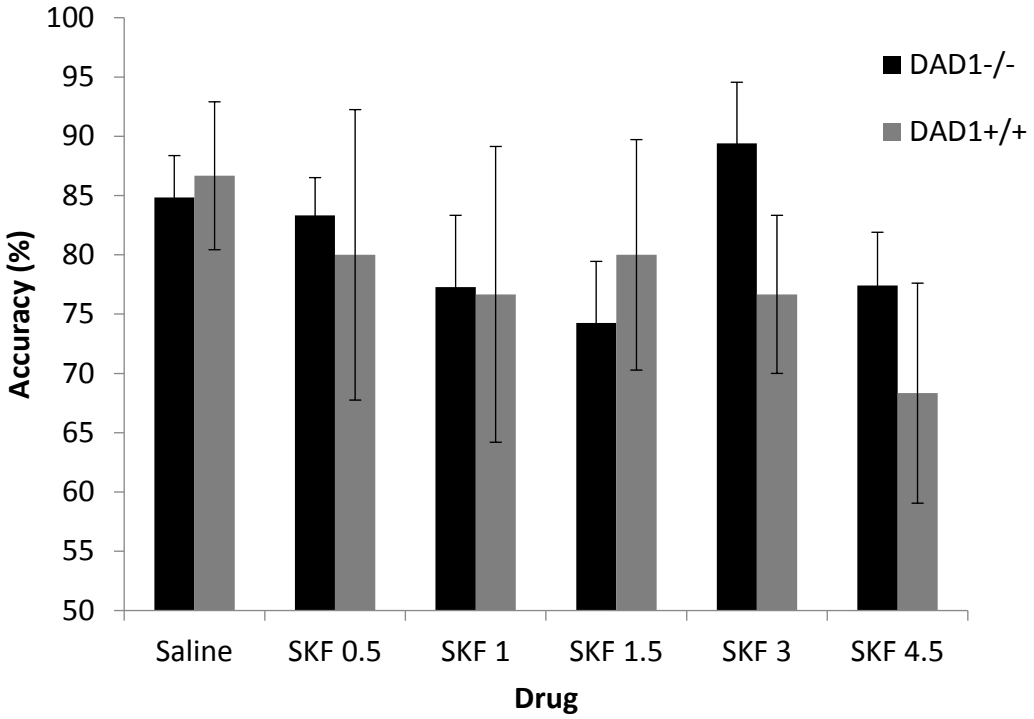

Figure 31. Accuracy of DAD1$^{-/-}$ (n = 11 (100%)) and DAD1$^{+/+}$ (n = 5 (38%)) rats on the DNMTP task during the block of SKF 81297 and saline doses. Each session contained six trials, with an accurate response defined as when subjects entered the opposite arm during the choice phase to the arm entered during the sample phase. Drugs were administered via i.p. injection 15 minutes prior to the start of a session.

MDMA-induced proactive interference in DAD1$^{+/+}$ rats. On the basis that the third block of MDMA administration revealed a significant drug dose x genotype interaction, these data were submitted to further analyses to assess whether trial type (either previous same or previous different) influenced accuracy. Figure 32 displays the accuracy of DAD1$^{-/-}$ rats, split by trial type, over the drug doses tested. The analysis revealed no significant differences in accuracy as a function of drug dose, $F_{3, 27} = 1.10$, $p = .37$, or trial type, $F_{1, 9} = .49$, $p = .50$, as well as no interaction between drug dose and trial type, $F_{3, 27} = .80$, $p = .51$. These results suggest that trial type did not affect accuracy in DAD1$^{-/-}$ rats on any of the MDMA doses tested.

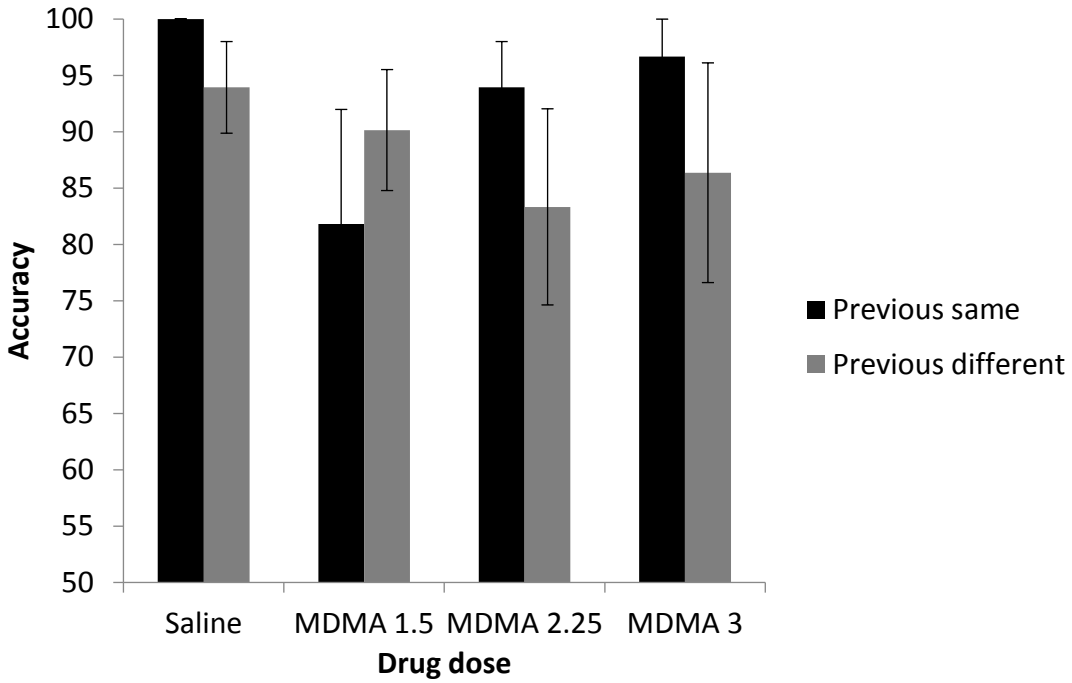

Figure 32. Accuracy, as a function of trial type, as demonstrated by DAD1$^{-/-}$ rats (n = 11) on the third drug block of MDMA administration. Trials were categorised as being either 'previous same' or 'previous different' trials on the basis that the accurate response on the current trial was either the same or different, respectively, as the required response on the immediately preceding trial.

Figure 33 displays the accuracy of DAD1$^{+/+}$ rats, split by trial type, over the drug doses tested during the third block of MDMA administration. In contrast to DAD1$^{-/-}$ rats, the DAD1$^{+/+}$ rats demonstrated a significant effect of trial type, $F_{1, 11} = 4.83$, $p = .05$, suggesting that DAD1+/+ rats were more accurate on previous same trials (M = 88.62, SD = 8.53) than previous different trials (M = 78.06, SD = 19.42) across drug doses. Furthermore there was a significant effect of drug dose, $F_{3, 33} = 3.44$, $p = .03$, and a significant interaction between drug dose and trial type, $F_{3, 33} = 5.24$, $p = < .01$. Paired-sample t-tests revealed that DAD1$^{+/+}$ rats displayed no difference in accuracy on previous same or previous different trials following saline, $t_{12} = .19$, $p = .85$ or MDMA 1.5 mg/kg, $t_{11} = -1.78$, $p = .10$. However, there were significant differences found between accuracy on previous same and previous difference trials following administration of the two highest doses of MDMA. Specifically, and in line with predictions, following MDMA 2.25 mg/kg DAD1$^{+/+}$ rats were significantly more accurate on previous same trials (M = 91.67, SD = 15.07) than previous different trials (M = 57.64, SD = 38.67), $t_{11} = 2.96$, $p =$

.01. In a similar fashion, following MDMA 3 mg/kg, DAD1$^{+/+}$ rats were significantly more accurate during previous same trials (M = 90.28, SD = 22.98) than previous different trials (M = 65.28, SD = 28.61), $t_{11} = 2.25$, $p = .05$.

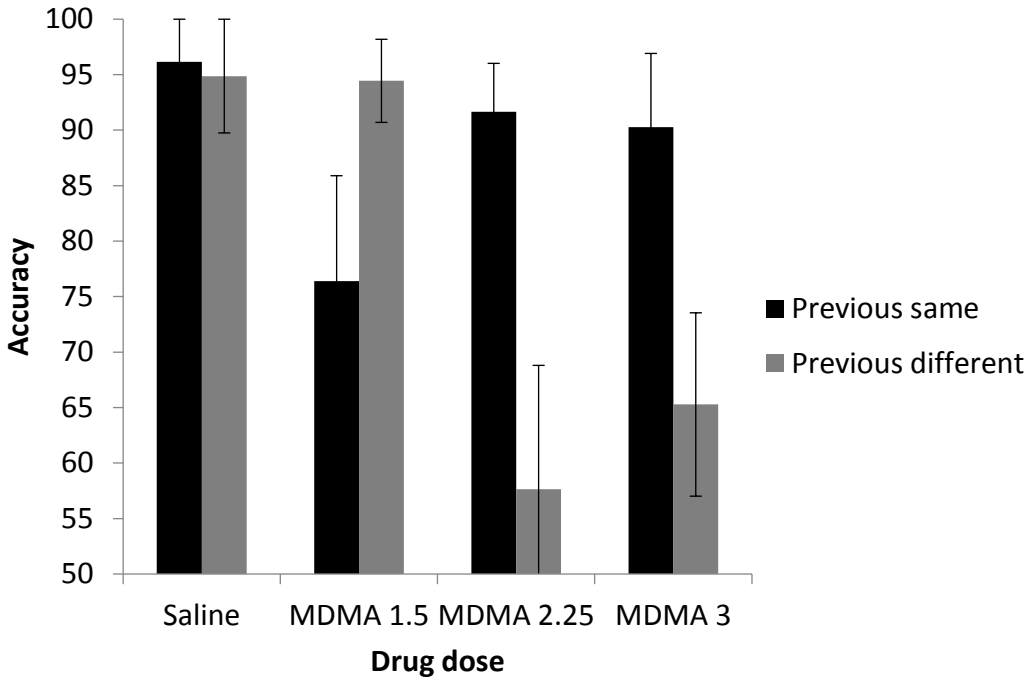

Figure 33. Accuracy, as a function of trial type, as demonstrated by DAD1$^{+/+}$ rats (n = 12) on the third drug block of MDMA administration. Trials were categorised as being either 'previous same' or 'previous different' trials on the basis that the accurate response on the current trial was either the same or different, respectively, as the required response on the immediately preceding trial.

Latency

Drug block one: MDMA. A comparison of the times taken by DAD1$^{-/-}$ and DAD1$^{+/+}$ rats during the sample or choice phases following the first block of MDMA doses is displayed in figure 34. There were no significant effects of drug, $F_{3, 66} = .19$, $p = .91$, trial stage, $F_{1, 22} = .16$, $p = .69$, or genotype, $F_{1, 22} = 1.79$, $p = .20$. Furthermore, no interactions were observed between drug and genotype, $F_{3, 66} = 1.43$, $p = .24$, trial phase and genotype, $F_{1, 22} = .02$, $p = .90$, drug and trial phase, $F_{3, 66} = 1.15$, $p = .34$, or drug, trial phase and genotype, $F_{3, 66} = 1.14$, $p = .34$. These results indicate that DAD1$^{-/-}$ and

DAD1$^{+/+}$ rats moved into the arms of the T-maze within similar time frames across the first block of MDMA doses.

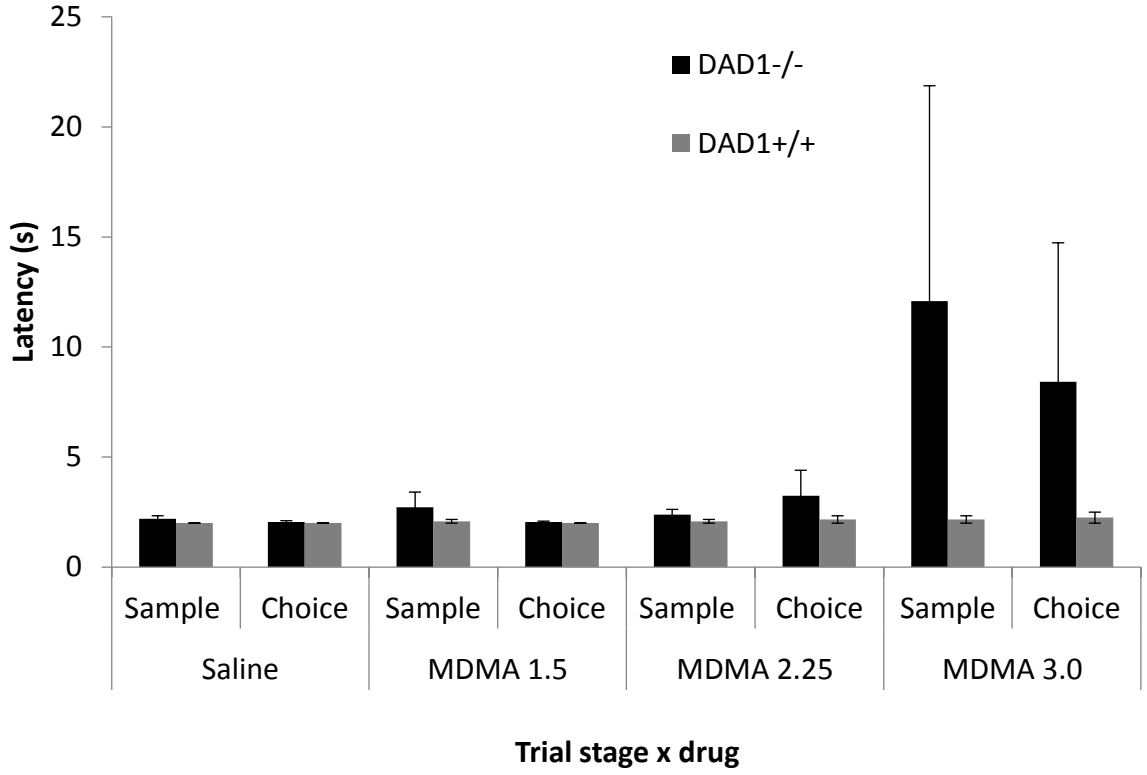

Figure 34. The time taken in seconds for DAD1$^{-/-}$ (n = 6 (55%)) and DAD1$^{+/+}$ (n = 2 (15%)) rats to enter an arm on the sample or the choice phase during the first drug block of the DNMTP task. Fifteen minutes prior to the start of each DNMTP session the subjects were administered either saline, 1.5, 2.25 or 3 mg/kg of MDMA.

Drug block two: MDMA. A comparison of the times taken by DAD1$^{-/-}$ and DAD1$^{+/+}$ rats during the sample or choice phases following the second block of MDMA doses is displayed in figure 35. There was an approaching significant effect of drug, $F_{1.1, 18.6}$ = .3.64, p = .07, which as displayed by figure 35 is likely due to rats taking longer to enter both the sample and choice arms following MDMA 3 mg/kg compared to the other drug doses. There were no effects of trial stage, $F_{1, 17}$ = 1.83, p = .19, or genotype, $F_{1, 17}$ = .14, p = .72 found. Furthermore, no interactions were observed between drug and genotype, $F_{3, 51}$ = .03, p = .99, trial phase and genotype, $F_{1, 17}$ = .08, p = .78, drug and trial phase, $F_{1.15, 19.59}$ = 1.66, p = .22, or drug, trial phase and genotype, $F_{1.15, 19.69}$ = .67, p = .44.

These results indicate that DAD1$^{-/-}$ and DAD1$^{+/+}$ rats moved into the arms of the T-maze within similar time frames across the second block of MDMA doses.

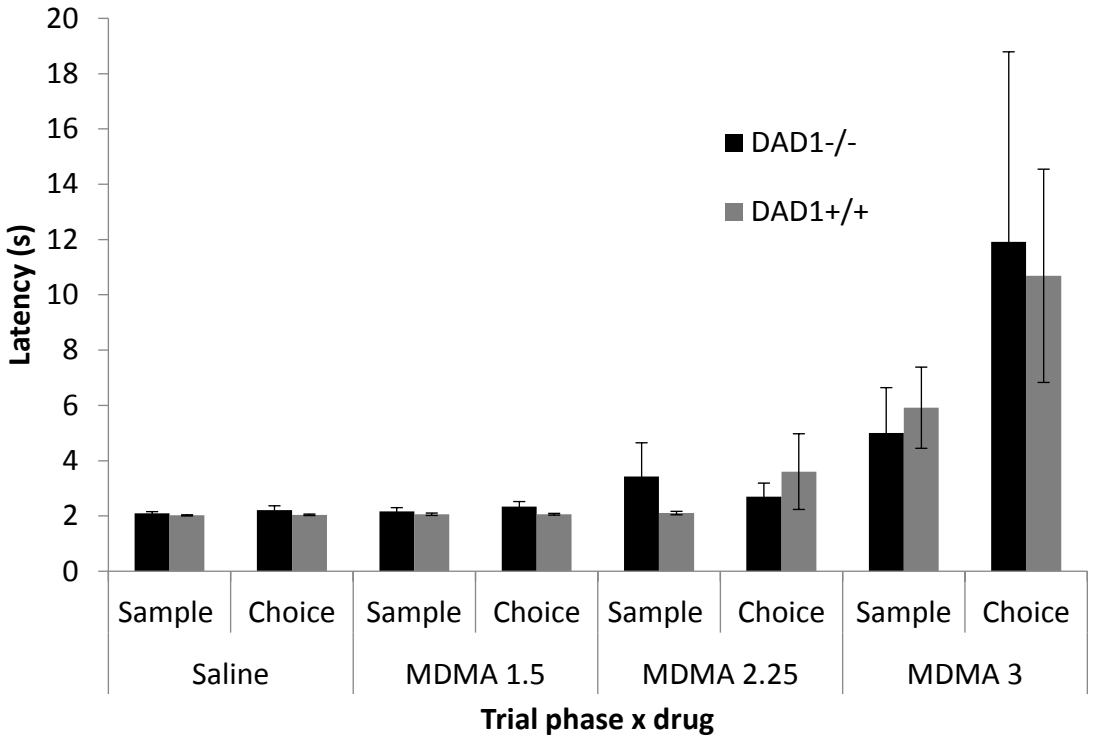

Figure 35. The time taken in seconds for DAD1$^{-/-}$ (n = 10 (91%)) and DAD1$^{+/+}$ (n = 8 (62%)) rats to enter an arm on the sample or the choice phase during the second drug block of the DNMTP task. Fifteen minutes prior to the start of each DNMTP session the subjects were administered either saline, 1.5, 2.25 or 3 mg/kg of MDMA.

Drug block three: MDMA. A comparison of the times taken by DAD1$^{-/-}$ and DAD1$^{+/+}$ rats during the sample or choice phases following the third block of MDMA doses is displayed in figure 36. There was a significant effect of drug, $F_{1.03,\ 21.67} = 6.23$, $p = .02$, an effect which figure 36 suggests is driven by rats taking longer to enter either a sample or choice arm following MDMA 3 mg/kg compared to the other drug doses. There were no effects of trial stage, $F_{1,\ 21} = .01$, $p = .97$, or genotype, $F_{1,\ 21} = .35$, $p = .56$. Furthermore, no interactions were observed between drug and genotype, $F_{3,\ 63} = .44$, $p = .73$, trial phase and genotype, $F_{1,\ 21} = .2.11$, $p = .16$, drug and trial phase, $F_{1.04,\ 21.93} = .19$, $p = .67$, or drug, trial phase and genotype, $F_{1.04,\ 21.93} = .2.40$, $p = .14$. However, as shown on

figure 36, DAD1$^{-/-}$ and DAD1$^{+/+}$ latencies to enter a choice arm following MDMA 3 mg/kg seem contrasting, with DAD1$^{-/-}$ rats appearing to move faster than DAD1$^{+/+}$ rats. Given that accuracy at this dose during this drug block was decreased in DAD1$^{+/+}$ compared to DAD1$^{-/-}$ rats, an exploratory independent samples *t*-test was carried out to further examine this difference further. Nonetheless, the result of this test indicated that the difference observed was not significant, $t_{12.04} = -1.49$, $p = .16$. In sum, these results indicate that DAD1$^{-/-}$ and DAD1$^{+/+}$ rats moved into the arms of the T-maze within similar time frames across the third block of MDMA doses, yet that there was a trend for DAD1$^{+/+}$ rats to be slower than DAD1$^{-/-}$ rats during the choice phase following MDMA 3 mg/kg.

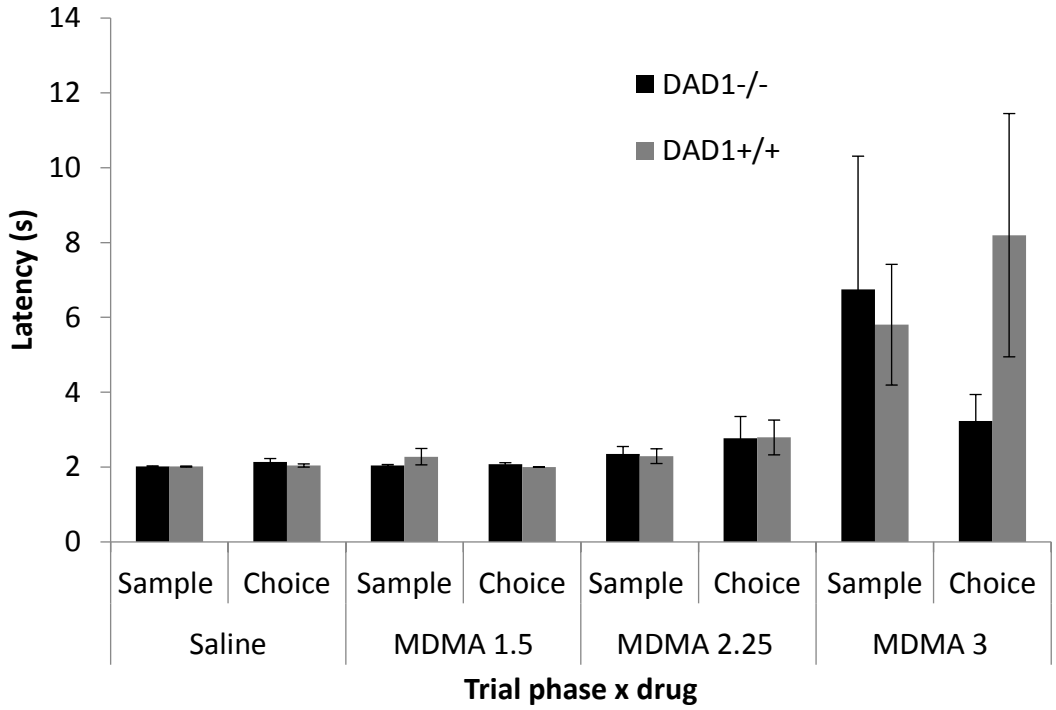

Figure 36. The time taken in seconds for DAD1$^{-/-}$ (*n* = 11 (100%)) and DAD1$^{+/+}$ (*n* = 12 (92%)) rats to enter an arm on the sample or the choice phase during the third drug block of the DNMTP task. Fifteen minutes prior to the start of each DNMTP session the subjects were administered either saline, 1.5, 2.25 or 3 mg/kg of MDMA.

Drug block four: SKF 81297 (D1-like receptor agonist). A comparison of the times taken by DAD1$^{-/-}$ and DAD1$^{+/+}$ rats during the sample or choice phases following

the block of SKF 81297 doses is displayed in figure 37. There were no significant effects of drug, $F_{1.46, 30.58} = 1.02$, $p = .35$, trial stage, $F_{1, 21} = .06$, $p = .81$, or genotype, $F_{1, 21} = .49$, $p = .49$. Furthermore, no interactions were observed between drug and genotype, $F_{5, 105} = 1.01$, $p = .42$, trial phase and genotype, $F_{1, 21} = .64$, $p = .43$, drug and trial phase, $F_{1.19, 25.02} = 1.02$, $p = .34$, or drug, trial phase and genotype, $F_{1.19, 25.02} = 1.44$, $p = .25$. These results indicate that DAD1$^{-/-}$ and DAD1$^{+/+}$ rats moved into the arms of the T-maze within similar time frames across the block of SKF 81297 doses.

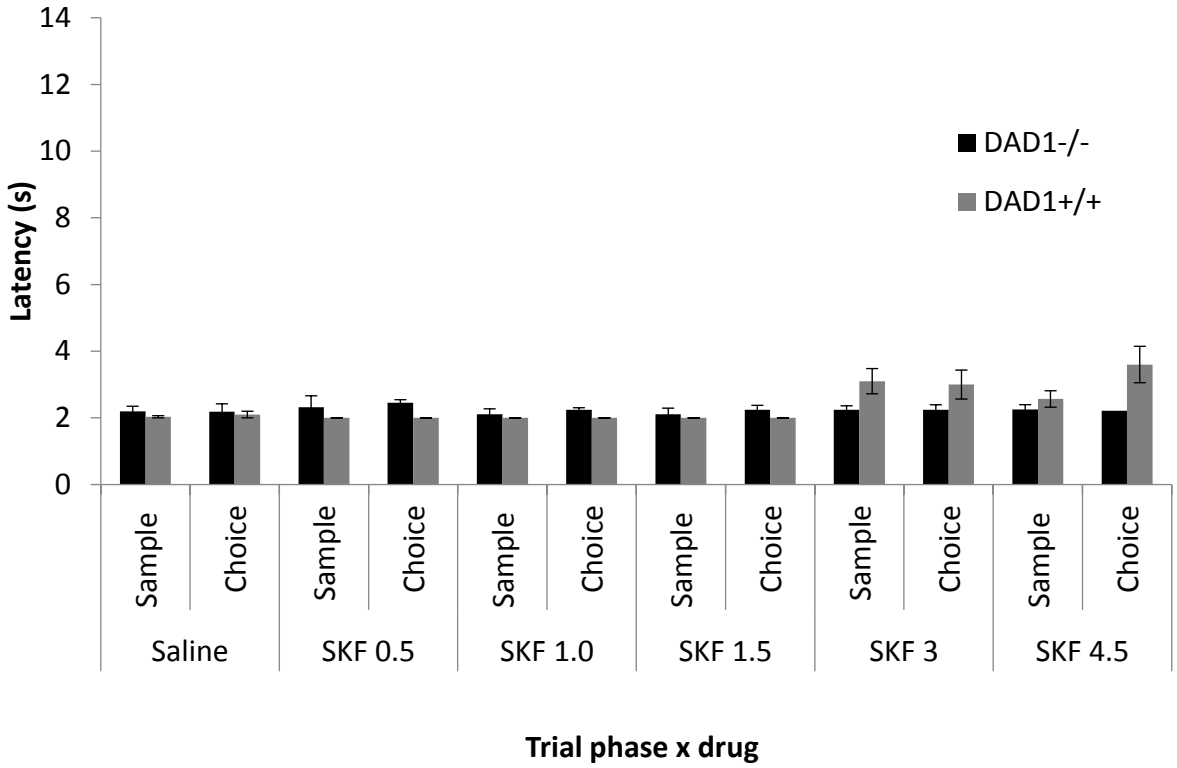

Figure 37. The time taken in seconds for DAD1$^{-/-}$ (n = 11 (100%)) and DAD1$^{+/+}$ (n = 5 (38%)) rats to enter an arm on the sample or the choice phase during the SKF 81297 drug block of the DNMTP task. Fifteen minutes prior to the start of each DNMTP session the subjects were administered either saline, 0.5, 1, 1.5, 3 or 4 mg/kg of SKF 81297.

Discussion

This chapter explored the hypothesis that acute MDMA exposure acts to interfere with memory performance in rats due to an MDMA-induced DA release causing the

over-stimulation of D1 receptors. Within this framework, developed by Harper and colleagues, the over-stimulation of D1 receptors is posited to induce proactive interference, or perseverative responding, whereby subjects tend to repeat the previous trial's required response, rather than performing the current trial's required response. To test this hypothesis, memory performances of $DAD1^{-/-}$ rats, that possess down-regulated D1 receptors, and $DAD1^{+/+}$ rats were assessed using the DNMTP paradigm, conducted in a T-maze. Once the subjects were trained on the DNMTP task, a range of acute doses of MDMA, as well as saline, were administered to explore the impact of acute MDMA exposure on task performance. It was predicted that $DAD1^{+/+}$ rats would display decreased accuracy following the highest dose of acute MDMA, specifically 3 mg/kg, and that this decrease in accuracy would be due to a greater number of errors on 'previous different' trials (indicative of proactive interference). In contrast to $DAD1^{+/+}$ rats, it was predicted that $DAD1^{-/-}$ rats would be relatively protected from acute MDMA-induced memory deficits, due to having less D1 receptor function, and that this group would not display more errors during 'previous different' compared to 'previous same' trials during acute MDMA exposure. As a further control, memory performance was also assessed following administration of the D1-like receptor agonist SKF 81297. The rationale applied was that if MDMA interferes with memory performance via over-stimulation of D1 receptors, then application of a D1-like agonist should similarly interfere with memory by increasing perseverative errors in the $DAD1^{+/+}$, but not the $DAD1^{-/-}$ animals.

Overall, the two central hypotheses regarding the impact of acute MDMA exposure on the memory performances of $DAD1^{+/+}$ and $DAD1^{-/-}$ rats were supported by the data. During the third block of MDMA doses, the $DAD1^{+/+}$ rats demonstrated significant decreases in accuracy following administration of the 2.25 and 3 mg/kg doses of MDMA. Furthermore, the magnitude of decrease in accuracy induced by the two highest doses of MDMA is comparable to that observed in previous studies using the DMTS paradigm (Harper and colleagues, 2005; 2006; 2013). Importantly, the decrease in accuracy at these doses observed in the control rats appears to be directly attributable to an increase in proactive interference. Specifically, accuracy at these doses was significantly decreased during 'previous different' trials compared to 'previous same' trials, yet accuracy following saline and MDMA 1.5 mg/kg was comparable during 'previous same' and 'previous different' trials. This means that when exposed to the highest doses of MDMA, $DAD1^{+/+}$ rats were tending to enter the maze arm that they

entered on the immediately preceding trial, rather than enter the correct arm required for the current trial. In direct comparison, on the third block of MDMA trials DAD1$^{-/-}$ rats did not display a significant decrease in accuracy following any of the doses of MDMA tested. What is more, DAD1$^{-/-}$ rats did not display an increase in errors on 'previous different' trials at these doses, indicating that MDMA did not increase proactive interference in these rats.

Although it was expected that MDMA 3 mg/kg would interfere with memory in the DAD1$^{+/+}$ rats, it was not expected that the magnitude of the impairment would be matched following administration of the 2.25 mg/kg dose. However, the difference between these doses is relatively small, thus is possible that past research has not explored the behavioural effects of the 2.25 mg/kg dose as extensively as 3 mg/kg. If MDMA 2.25 mg/kg were to impair memory in a similar fashion using the DMTS task, then future research investigating the acute effects on memory may like to employ the 2.25 mg/kg dose for the practical reason of utilising less MDMA. However, if memory is relatively preserved during DMTS following MDMA 2.25 mg/kg, then this may suggest that the DNMTP maze task is more sensitive to perseverative errors than the DMTS operant task. Furthermore, although performance deficits were observed in the third block of MDMA doses, accuracy was unimpaired during the first and second blocks of MDMA doses. However, it is possible that due to high levels of attrition in the DAD1$^{+/+}$ rats during these two blocks that statistical differences between the genotypes were obscured.

Despite the finding that accuracy in the DAD1$^{+/+}$ rats was impaired following the two highest doses of MDMA, the time taken for DAD1$^{-/-}$ and DAD1$^{+/+}$ rats to enter the arms during the sample and choice phases were similar, and did not differ significantly. However, in the third drug block, following MDMA 3 mg/kg, there was an apparent trend evident in which the DAD1$^{+/+}$ rats were slower to enter the choice arm than DAD1$^{-/-}$ rats. It is possible that the motor capabilities of DAD1$^{+/+}$ rats were more greatly affected following this dose compared to DAD1$^{-/-}$ rats. Nonetheless, since the latencies observed for the 2.25 mg/kg dose of MDMA, which also procured accuracy impairments in DAD1$^{+/+}$ rats but not DAD1$^{-/-}$ rats, during the third MDMA block were highly comparable, it is unlikely that motor deficits contributed to the decreased accuracy in DAD1$^{+/+}$ rats at the 3 mg/kg dose.

The results from the SKF 81297 investigation did not completely support predictions. It was predicted that SKF 81297 administration in DAD1$^{+/+}$ rats would lead to decreased accuracy in comparison to saline, yet that DAD1$^{-/-}$ rats would not demonstrate a decrease in accuracy following SKF 81297. Importantly, the DAD1$^{+/+}$ rats demonstrated high levels of attrition, with only six control rats (46%) completing four out of six trials across all doses of SKF 81297. By comparison, none of the DAD1$^{-/-}$ rats demonstrated a general impairment following SKF 81297 administration, and all of these rats were included in the analysis. This suggests that the higher doses of SKF 81297 produced a general performance impairment in the DAD1$^{+/+}$ rats due to over-stimulation at D1 receptors. Although there was a large degree of variation present in these data, which is especially pronounced in the DAD1$^{+/+}$ rats likely due to high attrition, the non-significant trend observed suggests that both genotype groups were similarly affected by SKF 81297, with accuracy lowering slightly as the dose increased, except at the 3 mg/kg dose. Following administration of SKF 81297 3 mg/kg, the DAD1$^{-/-}$ rats displayed increased accuracy compared to the DAD1$^{+/+}$ rats. This finding raises the possibility that at 3 mg/kg, DAD1$^{-/-}$ rats may benefit from enhanced memory performance due to an increase in D1-like receptor stimulation. However, considering SKF 81297 is not completely selective for D1 receptors, non-selective effects cannot be ruled out.

Lastly, based on research demonstrating that enhanced activation of D1-like receptors in the PFC impairs memory function in rats (Vijayraghavan et al., 2007; Zahrt et al. 1997), it is surprising that the SKF-treated DAD1$^{+/+}$ rats that did manage to perform 4 out of 6 trials across doses did not display a larger decrease in accuracy than observed. However, studies that have examined memory function following activation of D1-like receptors have not always provided consistent results. For example, although low doses of D1-like receptor agonists have been shown to improve memory function, higher doses do not necessarily impair memory function relative to saline (Cai & Arnsten, 1997). A further study demonstrated that excessive DA release in the PFC in response to ketamine administration impaired memory in rats via D2-like receptor mechanisms, as opposed to D1-like receptors mechanisms (Verma & Moghaddam, 1996). In the face of such discrepant findings, it has been posited that the effects of D1-like receptor agonists on memory function depend on the endogenous levels of D1 activation prior to administration of a D1-like receptor ligand (e.g. Vijayraghavan et al., 2007). Thus, perhaps the DAD1$^{+/+}$ subjects did not demonstrate an impairment following

administration of SKF 81297 due to having low baseline levels of PFC D1 receptor activation during the DNMTP task. Such individual differences in baseline levels of D1 activation could also provide an explanation for why the majority of DAD1$^{+/+}$ rats displayed a general impairment following administration of the higher doses of SKF 81297 and stopped responding altogether.

In summary, the results presented in this chapter suggest that acute MDMA administration acts to interfere with memory due to the over-stimulation of D1 receptors. The wider implications of these findings, with regard to recent research suggesting that MDMA-induced memory deficits are a product of indirect memory interference rather than direct working memory impairment will be discussed in the general discussion (*chapter 6*).

Chapter 6: General Discussion

This project employed the DAD1$^{-/-}$ rat model of down-regulated D1 receptor function to investigate the behavioural and neurological mechanisms by which acute MDMA impairs memory performance. Memory deficits observed following MDMA administration are often ascribed to a direct working memory impairment caused by 5-HT dysfunction (e.g. Braida et al., 2002; Kuypers & Ramaekers, 2005). However, there is evidence to suggest that, instead, MDMA-induced release of DA may indirectly interfere with memory performance (Harper, 2013; Harper et al., 2011; Rozas et al., 2012). Using rat subjects, Harper (2013) found that MDMA-induced impairments on a DMTS working memory task were ameliorated by concurrent administration of a D1-like receptor antagonist, SCH 23390. Specifically, SCH 23390 decreased MDMA-induced proactive interference, demonstrated by a reduction in perseverative responding. Accordingly, the central hypothesis of the current research was that MDMA-induced release of DA may lead to the over-stimulation of D1 receptors, thereby causing an increased susceptibility to proactive interference. Firstly, in control rats, proactive interference was posited to result in perseverative responding during the DNMTP maze task. In this regard it was predicted that control rats would demonstrate an MDMA-induced increase in errors during 'different' trials relative to 'same' trials. Secondly, if MDMA impairs memory via over-stimulation of D1 receptors then it was predicted that DAD1$^{-/-}$ rats would be protected from an MDMA-induced deficit on the DNMTP task compared to control rats. Key findings presented in *chapter 3* support this hypothesis and expand on previous research (e.g. Harper, 2013) by employing a model of down-regulated D1 receptor function that isolates D1 receptor function from D5 receptor function.

Prior to testing the effects of acute MDMA using DAD1$^{-/-}$ rats, a drug-free behavioural investigation was conducted. Because the DAD1$^{-/-}$ model is a novel animal model of down-regulated D1 receptor function, a behavioural assessment was vital to evaluate the performance of these subjects on tasks that are thought to involve intact D1 receptor function, namely basic tests of reward, movement and memory. The rationale behind this assessment was that behaviours that are required for performance on memory tests, conducted in *chapter 3*, might be altered in the DAD1$^{-/-}$ rats due to possessing down-regulated D1 receptor function. The DAD1$^{-/-}$ rats were impaired on some behavioural tests, yet demonstrated intact performance on others. Regarding behaviours that measured hedonic liking of rewards, the DAD1$^{-/-}$ rats behaved in line with control

rats in that they readily discriminated and consumed palatable foods when they were freely available. This finding supports predictions stemming from the incentive salience hypothesis which holds that mesocorticolimbic DA systems mediate *wanting*, but not *liking*, of rewards (Berridge 2007; Berridge & Robinson, 1998). Intact hedonic impact of palatable rewards in DAD1$^{-/-}$ rats suggests that these animals can detect and prefer rewards, for example DAD1$^{-/-}$ rats demonstrated a preference for sucrose solution over water. However, when we attempted to autoshape DAD1$^{-/-}$ rats to press a lever for sugar pellets their responding was completely abolished compared to controls rats. Such a fundamental impairment in lever pressing by the DAD1$^{-/-}$ rats was unexpected, especially when compared to a study undertaken by El-Ghundi et al. (2003), who found an attenuation in lever pressing by DA D1 deficient mice, but not a complete abolishment in their responding. Perhaps this discrepancy highlights species specific differences in DA D1 receptor function regarding food reinforcement.

The finding that DAD1$^{-/-}$ rats did not autoshape to press a lever for reinforcement could reflect impairment of one or a combination of several behavioural mechanisms. It is possible that DAD1$^{-/-}$ rats simply move less than control rats, resulting in less exploration within the operant chamber, meaning that the DAD1$^{-/-}$ rats failed to discover the contingency between lever pressing and the receipt of a sugar pellet. Considering antagonism of D1-like receptors reduces spontaneous movement (Hoffman & Beninger, 1985; Meyer et al., 1993), this account is certainly plausible. If reduced movement in DAD1$^{-/-}$ rats resulted in the observed lever pressing deficits, we would expect that DAD1$^{-/-}$ rats moved or reared less than controls in the open field experiments presented in *chapters 1* and *2*. Interestingly, during the behavioural assessment in *chapter 1*, DAD1$^{-/-}$ rats that had experienced extensive handling and exposure to the DNMTP task prior to the open field experiment displayed similar locomotor tendencies to controls in the open field. On the contrary, experimentally-naïve DAD1$^{-/-}$ rats moved less than control rats during open field testing presented in *chapter 2*. Given that the autoshaping experiment subjects were similarly experimentally-naïve, it is possible that decreased exploration contributed to the finding that DAD1$^{-/-}$ rats displayed abolished lever pressing.

What is more, DAD1$^{-/-}$ rats demonstrated an impaired ability to cross the balance beam compared to control rats, which may further illustrate decreased movement in naïve DAD1$^{-/-}$ rats. However, motivation to cross and move off of the beam may not only require intact movement, but also the perception that being atop a narrow, elevated beam

is aversive. Since D1-like receptors have been found necessary for the acquisition of conditioned taste aversion and conditioned place aversion (Fenu, Bassareo & Di Chiara, 2001; Hoffman & Beninger, 1988), it is possible that the DAD1$^{-/-}$ rats are impaired with regard to learning about and responding to aversive stimuli. Further evidence for this idea derives from the performance of the DAD1$^{-/-}$ rats on the rotarod task. The data suggest that the DAD1$^{-/-}$ rats may have found rotarod task to be less aversive than the control rats. On the rotarod task, in which subjects are compelled to move due to being placed on an already rotating rod, the DAD1$^{-/-}$ rats out-performed the control rats, with the two groups of control rats (minimally handled versus extensively handled) appearing to find the rotarod procedure as highly aversive and rarely voluntarily stepped on to the rotarod. Not only does the ability of DAD1$^{-/-}$ rats to perform on the rotarod task suggest that their lack of beam crossings reflect less aversion to the beam compared to controls, their performance on the rotarod also strengthens the argument that these rats have intact movement abilities required for the tests conducted here.

Furthermore, during training on the DNMTP task, the time taken by DAD1$^{-/-}$ and DAD1$^{+/+}$ rats to enter an arm during the sample and choice phases were identical and the DAD1$^{-/-}$ rats had no trouble learning this task. Taken together, these findings suggest that although the DAD1$^{-/-}$ rats may have moved less during the autoshaping experiment, resulting in fewer lever presses, it is also likely that the DAD1$^{-/-}$ rats possess a further deficit that contributed to the abolished lever pressing, such as with regard to translating motivation into action (i.e. *wanting* rewards). As argued in *chapter 3*, the autoshaping task could be considered more complex, and less naturalistic from a rat's perspective, than the DNMTP test. This difference may account for the finding that DAD1$^{-/-}$ rats performed poorly during autoshaping, but normally during DNMTP. In order to test this, future research could conduct a progressive ratio maze task to compare the breakpoints of DAD1$^{-/-}$ and control rats. Alternatively, future research could increase the amount of reinforcement obtained during autoshaping. Perhaps if lever pressing were paired with a more potent reinforcer, such as cocaine, then DAD1$^{-/-}$ rats would be relatively more willing to perform and acquire lever pressing. Using self-administration to assess *wanting* in DAD1$^{-/-}$ rats could also provide insight into the neural mechanisms that mediate drug dependence.

Although discussion of the behavioural functions of the D1 receptor is valuable, the finding that the DAD1$^{-/-}$ rats would not lever press was crucial because it meant we

could not employ the planned DMTS operant task to assess memory function in DAD1$^{-/-}$ rats during acute MDMA exposure. On the basis that DAD1$^{-/-}$ rats demonstrated intact memory performance during training on the DNMTP T-maze task, this task was subsequently selected to assess memory function in DAD1$^{-/-}$ rats during acute MDMA exposure. Although the DNMTP and DMTS tasks are procedurally different, effort was made to align the two procedures as closely as possible in order to extend previous research from our laboratory that investigated the acute effects of MDMA on performance using the DMTS task.

Acute MDMA administration has been found to decrease accuracy on the DMTS task (Harper and colleagues, 2005; 2006; 2013). Rather than reflecting an impairment of working memory, MDMA's effects on DMTS performance are characterised by an increased susceptibility to proactive interference. Following acute MDMA administration, rat subjects demonstrate a tendency to repeat a response that they performed on the directly previous trial, rather than the required response for the current trial (Harper and colleagues, 2005; 2006; 2013). This MDMA-induced proactive interference was ameliorated by a D1-like receptor antagonist that was applied concurrently with MDMA (Harper, 2013). This finding suggests that MDMA interferes with performance on the DMTS task by increasing perseverative responding via over-stimulating D1-like receptors. Using DAD1$^{-/-}$ rats to test this idea in the current project, Harper's (2013) finding was supported using the DNMTP task.

During the third block of MDMA administration presented in *chapter 5*, control rats demonstrated decreased accuracy on the DMNTP task at both the 2.25 and 3 mg/kg doses. The decreases in accuracy at these two doses of MDMA were of comparable magnitude, and, moreover, were of similar scale to the MDMA-induced deficits observed in previous research (Harper and colleagues, 2005; 2006; 2013). On the contrary, DAD1$^{-/-}$ rats were not impaired on the DNMTP task following acute MDMA at any of the doses tested. In itself, this genotype difference provides strong evidence for the notion that D1 receptors are implicated in MDMA's detrimental effects on memory performance on the DNMTP task. In order to test whether the deficit observed following MDMA in the control rats was due to increased proactive interference, accuracy during 'previous different' and 'previous same' trials was compared. Following MDMA (2.25 and 3 mg/kg) the control rats displayed decreased accuracy during 'previous different' trials, compared to 'previous same' trials, whereas following saline and the low dose of MDMA

(1.5 mg/kg) they displayed comparable levels of accuracy during 'previous different' and 'previous same' trials. This pattern of errors was expected, and indicates that the two highest doses of MDMA impaired memory by way of increased proactive interference in the control rats. Very clearly, this pattern of errors reflects perseverative responding, with the subjects repeating their response that they emitted on the previous trial. By comparison, DAD1$^{-/-}$ rats did not demonstrate any differences in accuracy on 'previous same' and 'previous different' trials following saline or any dose of MDMA. That DAD1$^{-/-}$ rats were protected from an MDMA-induced impairment on the DNMTP task and did not demonstrate MDMA-induced proactive interference is consistent with previous research suggesting that the D1 receptor is a neural substrate through which acute MDMA impairs memory (Harper, 2013). Furthermore, Harper's (2013) study employed a non-selective D1-like receptor antagonist (SCH 23390) to decrease D1 receptor function in order to explore MDMA's effect on memory. Thus, a question posed during this thesis was whether the amelioration of MDMA's detrimental effect on memory by SCH 23390 was in part due to D5 receptor signalling. Although the role played by D5 receptors cannot be completely ruled out, the data presented provides direct evidence for the involvement of D1 receptors in MDMA's acute effects on memory performance.

Currently, the conditions under which MDMA produces perseverative responding are unclear. It is possible that during the current DNMTP task as well as the DMTS task employed by Harper and colleagues (2005; 2006; 2013), perseverative errors following MDMA administration occurred due to motoric perseveration. Motoric perseveration would result in the subject being compelled to repeat the same motor output across trials. Alternatively, perseveration might have arisen due to the fact that the subject's previous response was reinforced, with the subject performing the response again in order to receive reinforcement once more. Although this issue is not directly investigated in the current thesis, Harper and Hunt (2011) and Lie, Macaskill and Harper (in press) explored whether acute MDMA may cause disruptions to stimulus control which could account for the impairments observed following acute MDMA across a range of conditional discrimination tasks. Harper and Hunt (2011) and Lie et al., (in press) employed the concurrent choice procedure devised by Davison and Baum (2000) to investigate whether MDMA altered subjects' sensitivity to different rates of reinforcement offered across concurrently available response options (levers). These studies found that following the highest dose of acute MDMA administered (2 mg/kg), rats displayed increased sensitivity

to reinforcement. That is, following a response on a lever that led to reinforcement, the rats were more likely to respond on that lever again immediately following receipt of reward. This perseverative pattern of responding, dubbed 'preference pulses', resulted in the lever that was currently associated with the richest reinforcement schedule being responded on more than the alternative lever (Harper & Hunt, 2011, Lie et al., in press). These studies suggest that the perseverative responding observed during the current investigation following acute MDMA in control rats may be related to their behaviour being directed by the pursuit of reward.

As well as appearing to increase a subject's susceptibility to proactive interference, acute (as well as sub-chronic) MDMA administration increases impulsivity (Bird & Schenk, 2013; Dalley et al., 2007; van Wel et al., 2012). Impulsivity has traditionally been thought of as a disorder of frontal 'executive' systems. More recently, the actions of DA, 5-HT and NE have all been implicated in varying aspects behavioural inhibition, with the PFC, striatum and Nacc considered vital for action inhibition (Eagle & Robbins, 2003; Eagle, Bari & Robbins, 2008). In behavioural terms, action inhibition could refer to either 'action restraint' (inhibition of an action before the response has begun) or 'action cancellation' (inhibition of motor output during the response) (Schachar et al., 2007). Although impulsivity and proactive interference have been operationalised as distinct phenomena in the current project, it is possible that they in fact encompass the same underlying behavioural components. Essentially, proactive interference is referring to the inability of a subject to engage in 'action restraint', with MDMA appearing to decrease a subject's ability to inhibit a prepotent behavioural response. Similarly, the opposing processes (i.e. 'action' vs 'action inhibition') required for accurate performance on behavioural memory tasks are reliant on intact working and reference memory traces. Given the extensive overlap between impulsivity, susceptibility to proactive interference, and inaccurate memory performance, it is possible that aspects of these behavioural disruptions reflect a common neurological or behavioural change. Thus, to further understand the basis of the memory alterations observed following acute MDMA, future studies could focus on the relationship between memory performance and impulse control.

In contrast to MDMA's acute effects, chronic exposure to MDMA has not been found to increase impulsivity in rats. On the contrary, subjects that exhibit trait impulsivity are predisposed to becoming MDMA-dependent (Bird & Schenk, 2012).

Despite chronic MDMA exposure not leading to increased impulsivity, chronic MDMA is associated with memory impairments, yet there is some debate over whether these impairments are permanent. Interestingly, both acute and chronic MDMA-induced memory impairments are characterised by an increase in reference memory errors, as opposed to working memory errors (Able et al., 2005; Braida et al., 2002; Harper et al., 2011; 2013; Kay et al., 2010; 2011; Piper & Meyer, 2004; Skelton et al., 2008; Sprague et al., 2003; Vorhees et al, 2004). This suggests that there may be common neurological changes associated with both acute and chronic MDMA exposure that lead to altered reference memory performance. Future research may like to employ the DAD1$^{-/-}$ rat model to test whether these short and long-term cognitive changes caused by MDMA reflect common, or distinct, neurological mechanisms. That DAD1$^{-/-}$ rats were protected from acute MDMA-induced memory impairments is argued to be due to the inability for MDMA to over-stimulate D1 receptors in these rats. If acute and chronic regimes of MDMA impair memory via distinct mechanisms, then it follows that the DAD1$^{-/-}$ rats may not be protected from chronic memory impairments associated with MDMA exposure. On the contrary, if common mechanisms underlie these changes, then DAD1$^{-/-}$ rats may similarly display intact memory following chronic regimens of MDMA.

Further evidence consistent with the hypothesis that MDMA impairs memory through the over-activation of D1 receptors can be drawn from the analysis of *c-fos* expression presented in *chapter 4*. DAD1$^{-/-}$ rats expressed significantly less MDMA-induced *c-fos* expression in the mPFC compared to control rats. Intact DA signalling in the PFC has been associated with effective performance on tests of working memory. Specifically, the inverted-U hypothesis of DA function in the PFC (e.g. Williams and Goldman-Rakic, 1995; Zahrt et al., 1997) posits that too much or too little DA transmission in the PFC leads to detrimental effects on working memory performance. In particular, stimulation of D1-like receptors by DA has been implicated in this effect (Zahrt et al., 1997). Perhaps, in a drug-free state, DAD1$^{-/-}$ and DAD1$^{+/+}$ rats both exhibit levels of PFC DA transmission that fall within the 'optimum' range required for the normal execution of DA-mediated behaviours, such as working memory. However, during MDMA exposure, DAD1$^{+/+}$ rats have a significant increase in DA release causing excess D1 receptor stimulation, leading to impaired memory performance. On the contrary, it is possible that during acute MDMA exposure, DAD1$^{-/-}$ rats experience less MDMA-induced D1 receptor activation (brain-wide, including the PFC), resulting in less

PFC DA transmission compared to controls. A reduction in PFC DA transmission following MDMA exposure, compared to controls, could mean that DAD1$^{-/-}$ rats remain within the 'optimum' range of DA transmission required for memory function and are protected from memory interference caused by stark increases in DA release associated with drugs of abuse (Baumann et al., 2008).

That DAD1$^{-/-}$ rats expressed less *c-fos* in the mPFC in response to 3 mg/mg MDMA, compared to control rats, provides an indirect neural marker for the supposition that DAD1$^{-/-}$ rats exhibit less DA transmission in the PFC during acute MDMA exposure, compared to controls. However, it would be interesting to further characterise the neural responses of DAD1$^{-/-}$ rats to MDMA, and other DA exerting drugs, using direct measures. As such, future studies may like to conduct micro-dialysis in the DAD1$^{-/-}$ rats to quantify neurochemical release in these rats in response to a range of drugs. It is possible that a genetic down-regulation of D1 receptors in DAD1$^{-/-}$ rats will result in lowered DA transmission in response to DA agonist drugs. Alternatively, it is possible that DAD1$^{-/-}$ rats will display similar levels of DA release in response to DA exerting drugs, yet that DA's effects are dampened due to less functional D1 receptors available for DA to bind to.

While the use of *c-fos* expression as a marker for neural activation is certainly a widely used and useful approach, it is also important to recognise the shortcomings of this technique. Firstly, *c-fos* expression and neuron depolarization do not have a 1 : 1 relationship in that it is possible to observe neuronal depolarisation without *c-fos* expression and vice versa (Hoffman & Lyo, 2002). Secondly, *c-fos* expression is modulated by numerous brain receptors, not simply DA receptors. As outlined in *chapter 1*, acute MDMA preferentially stimulates the release of 5-HT, in addition to increasing the release of NE and DA. Importantly, the brain regions selected for the analysis of *c-fos* expression presented in *chapter 4* receive rich 5-HT input (e.g. Bonsi et al., 2007; Herve, Pickel, Joh & Beaudet, 1987; Martin-Ruiz et al., 2001), with various 5-HT receptors known to modulate *c-fos* expression (Leslie, Moorman, Coulson, & Grahame-Smith, 1993; Mitsikostas, del Rio, Moskowitz & Waeber, 1999). Given that we do not know the developmental or neurological consequences of having a genetic down-regulation of functional D1 receptors means that there are potential compensatory mechanisms unaccounted for in this study. It is possible that the decrease in *c-fos* expression observed in DAD1$^{-/-}$ rats following MDMA was in part due to a compensatory change in 5-HT

receptors in DAD1$^{-/-}$ rats. In order to test this, future researchers could administer a drug that primarily exerts its effects on DA (i.e. amphetamine) to DAD1$^{-/-}$ rats to investigate whether the reduction in *c-fos* expression is of the same magnitude as found here. Alternatively, if decreased D1 receptor function can account for the pattern of *c-fos* expression reported in the current study, then systemic antagonism of 5-HT receptors in DAD1$^{-/-}$ rats prior to the administration of MDMA should still result in a comparable pattern of *c-fos* expression to that reported here.

Lastly, alternative explanations that may account for why acute MDMA administration impaired memory performance in control rats, yet not the DAD1$^{-/-}$ rats, should also be considered. One possibility is that acute MDMA administration could slow down response selection in control rats, thereby increasing the delay between the sample and choice runs, leading to poorer memory performance. However, analysis of response latencies on the DNMTP task did not reveal any systematic, or significant, differences between DAD1$^{-/-}$ and DAD1$^{+/+}$ rats, indicating that this explanation cannot account for our findings. Another possibility is that during acute MDMA exposure control subjects were distracted from the task by visual distortions or other subjective effects. Although it is difficult to assess the subjective effects of drugs using non-human animal models, as pointed out by Kay et al. (2010), if visual distortions or other subjective effects were behind acute MDMA's disruptive effects on memory performance then it would be expected that during MDMA exposure all types of memory errors would increase (in this case errors during both 'previous same' and 'previous different' trials). On the contrary, we found that in the control animals, acute MDMA exposure at the two highest doses administered (2.25 and 3.0 mg/kg) selectively increased errors during 'previous difference' trials compared to 'previous same' trials.

Conclusions

In contrary to the commonly held view that MDMA's acute impacts on memory are due to dysfunctional 5-HT mechanisms, the current research highlights the importance of DA signalling in MDMA's acute effects on memory. Using the DNMTP spatial memory paradigm conducted in a T-maze, control rats displayed decreased accuracy following acute MDMA administration (2.25 and 3 mg/kg i.p) that was due to a selective increase in perseverative errors. This MDMA-induced perseverative pattern of responding in control rats is argued to reflect an increased susceptibility to proactive interference. On

the contrary, DAD1$^{-/-}$ rats that possess down-regulated D1 receptor function were protected from MDMA-induced memory impairments, and did not demonstrate an increased susceptibility to proactive interference. Thus, the current project provided further evidence for the hypothesis that acute MDMA exposure impairs memory performance in rats via over-stimulation of D1 receptors.

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