The role of 5-HT_{1A} and 5-HT_{1B} receptors in MDMA self-administration

By

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Contents

List of abbreviations

5,7-DHT:	5,7-Dihydroxytryptamine
5CSRTT:	5 choice serial reaction time task
5-HIAA:	5-Hydroxyindoleacetic acid
5-HT:	5-hydroxytryptamine; serotonin
6-OH-DA	6-hydroxydopamine
CSF:	Cerebrospinal fluid
DSM:	Diagnostic and Statistical Manual of Mental Disorders
EC_{50}:	Half maximal effective concentration
EPM:	Elevated plus maze
FR:	Fixed ratio
GABA:	gamma-Aminobutyric acid
GTPγS:	guanosine 5'-O-[gamma-thio]triphosphate
K_i:	Binding affinity (amount of ligand required to bind 50% of receptors)
MDMA :	3,4-methylenedioxymethamphetamine
NAc:	Nucleus accumbens
PFC:	Prefrontal cortex
SSRI:	Selective serotonin reuptake inhibitor
SUD:	Substance use disorder
VTA:	Ventral tegmental area

List of ligands

5-MeODMT	5-$HT_{2/1A}$ receptor agonist
8-OH-DPAT	5-$HT_{1A/7}$ receptor agonist
BAY × 3702	5-HT_{1A} receptor agonist
CGS 12066	5-$HT_{1B/2}$ receptor agonist
CP 93129	5-HT_{1B} receptor agonist
CP 94253	5-HT_{1B} receptor agonist
DOI	5-$HT_{2A/2C}$ receptor agonist
F13640	5-HT_{1A} receptor agonist
F15599	5-HT_{1A} receptor agonist
Fluoxetine	Serotonin uptake inhibitor
GBR 12909	Dopamine uptake inhibitor
GR 127935	5-$HT_{1B/1D}$ receptor antagonist
Ketanserin	5-$HT_{2A/2C}$ receptor and H_1 receptor antagonist
M100907	5-HT_{2A} receptor antagonist
mCPP	5-$HT_{2/1A}$ receptor agonist
MK 212	5-HT_{2C} receptor agonist
Pindolol	β1- and β2-adrenergic receptor antagonist, 5-HT_{1A} antagonist
Propanolol	β1- and β2-adrenergic receptor antagonist, 5-$HT_{1A/1B}$ antagonist
Ro 60-175	5-HT_{2C} receptor agonist
RU 24969	5-$HT_{1B/1A}$ receptor agonist
SB 206553	5-$HT_{2C/2B}$ receptor antagonist
SB 224289	5-HT_{1B} receptor inverse agonist
SB 242084	5-HT_{2C} receptor antagonist
SDX 216-525	5-HT_{1A} receptor antagonist
Tianeptine	5-HT uptake facilitator
WAY 101405	5-HT_{1A} receptor antagonist
WAY 100635	5-HT_{1A} receptor antagonist

Abstract

Rationale: 3,4-methylenedioxymethamphetamine (MDMA) is a less efficacious reinforcer than other drugs of abuse. However, following repeated self-administration, responding increases for some animals and efficacy becomes comparable to other drugs of abuse. MDMA-stimulated serotonin (5-HT) release was negatively associated with acquisition of MDMA self-administration, and a neurotoxic 5-HT lesion reduced the latency to acquire self-administration. These findings suggest that MDMA-produced 5-HT release is an important component of self-administration. The receptor mechanisms are not, however, well understood, although it has often been suggested that the mechanism involves 5-HT-mediated inhibition of dopamine. Both 5-HT_{1A} and 5-HT_{1B} receptors are well localised to regulate dopamine release, and both have been implicated in modulating the reinforcing effects of many drugs of abuse.

Objectives: The first objective was to establish specific behavioural assays to reflect 5-HT_{1A} and 5-HT_{1B} receptor activation. Then, using the established behavioural assays, the aim was to determine the role of 5-HT_{1A} and 5-HT_{1B} receptors in the acquisition of MDMA self-administration. The impact of substantial MDMA self-administration on 5-HT_{1A} and 5-HT_{1B} receptors was also assessed.

Methods: Firstly, dose-effect relationships for the hyperactive response to the 5-HT_{1A} receptor agonist, 8-OH-DPAT (0 – 3.0 mg/kg) and the hyperactive and adipsic response to the 5-$HT_{1B/1A}$ receptor agonist, RU 24969 (0 – 3.0 mg/kg) were determined. Selectivity of these responses was determined by co-administration of the 5-HT_{1A} receptor antagonist, WAY 100635, or the 5-$HT_{1B/1D}$ receptor antagonist, GR 127935. Secondly, a pretreatment regimen of the RU 24969 (2 × 3.0 mg/kg/day, 3 days), which had been suggested to down-regulate 5-$HT_{1B/1A}$ receptors, was administered prior to self-administration testing. The effect of this manipulation on both the acquisition of MDMA self-administration, and the behavioural responses to 5-HT_{1A} and 5-HT_{1B} receptor activation, was measured. A further study measured behavioural responses to 5-HT_{1A} or 5-HT_{1B} receptor agonists prior to self-administration, to determine whether the variability in these responses would predict the variability in the latency to acquisition of MDMA self-administration. Lastly, the effect of substantial MDMA self-administration (350 mg/kg) on dose-response curves for the behavioural effects of 5-HT_{1A} or 5-HT_{1B} receptor activation was assessed.

Results: The hyperactive response to the 5-$HT_{1B/1A}$ receptor agonist, RU 24969, was blocked by the 5-HT_{1A} receptor antagonist, WAY 100635, but not the 5-HT_{1B} receptor

antagonist, GR127935. Similarly, the hyperactive response to the 5-HT_{1A} receptor agonist, 8-OH-DPAT, was dose-dependently blocked by WAY 100635. GR 127935, but not WAY 100635, blocked the adipsic response to RU 24969.
Repeated administration of RU 24969 produced rightward shifts in the dose-response curves for 8-OH-DPAT-produced hyperactivity and RU 24969-produced adipsia, and also greatly facilitated the acquisition of MDMA self-administration. However, there was no correlation between latency to acquire MDMA self-administration and the hyperactive response to 8-OH-DPAT or the adipsic response to RU 24969, and MDMA self-administration failed to alter these behavioural response to activation of 5-HT_{1A} or 5-HT_{1B} receptors.
Conclusions: The hyperactive response to 8-OH-DPAT and the adipsic response to RU 24969 reflect activation of 5-HT_{1A} and 5-HT_{1B} receptors, respectively. The variability in acquisition of MDMA self-administration was reduced by a treatment that also down-regulated 5-HT_{1A} and 5-HT_{1B} receptors, however there was no further indication that these receptors play a critical role in the self-administration of MDMA. Instead, it seems likely that other 5-HT receptors have a greater impact on MDMA self-administration.

General Introduction

Parts of this chapter have been adapted from:

Aronsen & Schenk (2016). MDMA self-administration fails to alter the behavioral response to 5-HT_{1A} and 5-HT_{1B} agonists. *Psychopharmacology, 233* (7), 1323-1330. DOI 10.1007/s00213-016-4226-9

Aronsen, Bukholt, & Schenk (2016). Repeated administration of the 5-$HT_{1B/1A}$ agonist, RU 24969, facilitates the acquisition of MDMA self-administration: Role of 5-HT_{1A} and 5-HT_{1B} receptor mechanisms. *Psychopharmacology, 233* (8), 1339-1347. DOI 10.1007/s00213-016-4225-x

Schenk & Aronsen (2015). Contribution of Impulsivity and Serotonin Receptor Neuroadaptations to the Development of an MDMA ("ecstasy") Substance Use Disorder, in *Current Topics in Behavioral Neuroscience: The Misuse of Licit and Illicit Drugs* in *Psychopharmacology* Susan Schenk, Suzanne Nielson, Raimondo Bruno (Eds), Springer. DOI 10.1007/7854_2015_421

Aronsen, Webster, & Schenk (2014). RU 24969-produced adipsia and hyperlocomotion: Differential role of $5HT_{1A}$ and $5HT_{1B}$ receptor mechanisms. *Pharmacology, Biochemistry and Behavior, 124*, 1-4. DOI 10.1016/j.pbb.2014.05.008

with permission from the publisher (Appendix A).

Brief history of MDMA

3,4-methylenedioxymethamphetamine (MDMA) was initially patented by the pharmaceutical company, Merck, in 1914 as a precursor for other therapeutically efficacious compounds (Green, Mechan, Elliott, O'Shea, & Colado, 2003). Some basic preclinical tests were conducted with MDMA in 1927 (Freudenmann, Öxler, & Bernschneider-Reif, 2006), and in 1953 the US Army sponsored research on the toxicity of MDMA, concluding that further study in humans should be conducted (Hardman, Haavik, & Seevers, 1973). In response to the first reports of recreational MDMA use (Gaston & Rasmussen, 1972) Alexander Shulgin published the first papers outlining the effects of MDMA in humans (Anderson, Braun, Braun, Nichols, & Shulgin, 1978; Shulgin, 1978; Shulgin & Nichols, 1978), also encouraging further human studies. Shulgin was a vocal advocate for the use of MDMA as an adjunct to psychotherapy, but it has been suggested that his public promotion of MDMA also led to increased recreational use (Benzenhöfer & Passie, 2010).

As recreational use continued to grow, there was pressure on governments to bring the use of MDMA under legislative control (Beck & Rosenbaum, 1990). MDMA was scheduled as a Class B Controlled Drug in New Zealand in 1987 (New Zealand Drug Foundation, 2015), following classification in Schedule I by the USA Drug Enforcement Agency in 1985 (Beck & Rosenbaum, 1990). The import, manufacture, supply, or administration of Class B Controlled Drugs carries a jail sentence of up to 14 years in New Zealand (Misuse of Drugs Act 1975). Nonetheless, recreational use of MDMA, in the form of the street drug, 'ecstasy', is popular in New Zealand (Wilkins, 2011; Wilkins & Sweetsur, 2008), and around the world (United Nations Office on Drugs and Crime, 2015).

Recently, there has been a revival in the push to harness the subjective effects of MDMA in the treatment of psychiatric disorders. Some therapists claim that MDMA helps patients talk openly, and fosters an atmosphere of trust (Kupferschmidt, 2014). Clinical trials are currently underway assessing the utility of MDMA as a therapeutic adjunct in the treatment of post-traumatic stress disorder, and anxiety associated with a life-threatening illness (National Institutes of Health, 2015).

MDMA use

MDMA is generally consumed as the primary psychoactive component of the popular street drug, ecstasy (also known as E, Molly, pingers, pills, disco biscuits). Ecstasy is most commonly available in tablet form, and tablets are usually either consumed orally or crushed for intranasal administration (De La Garza, Fabrizio, & Gupta, 2007; Parrott, 2013a; Solowij, Hall, & Lee, 1992). In recent years recreationally used ecstasy tablets have been shown to contain a wide range of psychoactive substances, including significant quantities of methamphetamine, ketamine, caffeine, meta-Chlorophenylpiperazine (mCPP) and mephedrone, and have sometimes contained no MDMA whatsoever (Brunt, Koeter, Niesink, & van den Brink, 2012; Morefield, Keane, Felgate, White, & Irvine, 2011; Togni, Lanaro, Resende, & Costa, 2015; Vogels et al., 2009). Therefore, throughout this thesis, the term 'ecstasy' will be used to refer to the street drug that generally contains MDMA, while 'MDMA' will be used to refer specifically to the psychoactive substance.

Ecstasy became popular in the underground dance party scene of the 1980s, in part because it increases energy levels, heightens sensual awareness, and facilitates bonding (McDowell & Kleber, 1994; Schwartz & Miller, 1997). In the 1990s and early

2000s ecstasy use became more mainstream, becoming a popular recreational drug among young adults. A recent study reported worldwide prevalence of ecstasy use to be the second highest of all illicit drugs (Global Drug Survey, 2014). Recently, popularity of ecstasy has been facilitated by a 're-branding' of ecstasy as 'Molly' in the mainstream media. 'Ecstasy' has associations with the old dance parties of the 1980s, electronic music, and un-masculine displays of affection, misaligning it with the modern zeitgeist which is heavily influenced by pop and hip-hop culture. On the other hand, 'Molly' has been embraced by the hip-hop and pop communities, providing a 'new' drug that youth can associate with (Carter, 2016).

Although ecstasy use is common, patterns of use differ widely. A recent study showed that, of 109 subjects who had recently used ecstasy for the first time, 43 did not take ecstasy again in the following 12 months, while 23 consumed more than 10 ecstasy pills in that time period (Wagner, Becker, Koester, Gouzoulis-Mayfrank, & Daumann, 2013), illustrating that some will use ecstasy very infrequently, while others will use ecstasy regularly. Furthermore, recent surveys have found a significant proportion of regular ecstasy users met Diagnostic and Statistical Manual of Mental Disorders (DSM) -IV-based criteria for dependence (Cottler, Leung, & Abdallah, 2009; Cottler, Womack, Compton, & Ben-Abdallah, 2001; Uosukainen, Tacke, & Winstock, 2015). The more recent DSM 5 provides diagnostic criteria for 'substance use disorders' (SUDs) rather than 'dependence' (American Psychiatric Association, 2013). Although there is no specific ecstasy SUD, some ecstasy users met a number of SUD criteria, including using more drug than intended (Cottler et al., 2009; Cottler et al., 2001) unsuccessful efforts to cut down on use (Jansen, 1999), craving (A. K. Davis & Rosenberg, 2014; Hopper et al., 2006), neglecting activities other than acquiring and taking drug (Cottler et al., 2009; Cottler et al., 2001; Jansen, 1999; Yen & Hsu, 2007), use in spite of known negative consequences (Cottler et al., 2009; Cottler et al., 2001; Jansen, 1999; Schifano & Magni, 1994; Yen & Hsu, 2007), tolerance (Cottler et al., 2001; Jansen, 1999; Kirkpatrick et al., 2014; Parrott, 2005; Peroutka, Newman, & Harris, 1988; Yen & Hsu, 2007), and withdrawal (Cottler et al., 2009; Cottler et al., 2001; Jansen, 1999; Peroutka et al., 1988). Thus, while some ecstasy users take ecstasy relatively infrequently, a subpopulation of users show regular use, and some show signs of an SUD.

Harms associated with MDMA use

The regular use of ecstasy in some users is of concern, not only because of the potential to develop an SUD, but also because ecstasy use has been associated with a number of cognitive, behavioural, and neurochemical deficits. Ecstasy users showed deficits in learning (Wagner et al., 2013), and in attention and memory (McCann, Mertl, Eligulashvili, & Ricaurte, 1999) compared to ecstasy-naïve controls or those with limited ecstasy use. Ecstasy users reported higher levels of depression, impulsiveness, and sleep disturbances than poly-drug users who did not use ecstasy (Soar, Turner, & Parrott, 2006; Taurah, Chandler, & Sanders, 2014). These cognitive and behavioural deficits were persistent, suggesting that regular ecstasy use may cause long-lasting neuroadaptations (Parrott, 2013a, 2013b; Schifano & Magni, 1994). With increased experience some heavy ecstasy users report persistent problematic behaviour, including paranoid delusions (Schifano & Magni, 1994), severe weight loss (Jansen, 1999; Schifano & Magni, 1994), and suicidal thought (Jansen, 1999; Schifano & Magni, 1994).

Ecstasy use has also been associated with deficits in the neurotransmitter, serotonin (5-HT). Ecstasy users had decreased 5-HT transporter binding (Kish et al., 2010; McCann, Szabo, Scheffel, Dannals, & Ricaurte, 1998), reduced levels of the 5-HT metabolite, 5-hydroxyindoleacetic acid (5-HIAA), in cerebrospinal fluid (McCann, Mertl, et al., 1999), reduced 5-HT synthesis in frontal and parietal regions (Booij et al., 2014), and autopsied striatal tissue from a heavy ecstasy user indicated decreased 5-HT and 5-HIAA levels (Kish, Furukawa, Ang, Vorce, & Kalasinsky, 2000). These markers of reduced 5-HT function correlate with lifetime ecstasy use (Kish et al., 2010; McCann et al., 1998) and levels of behavioural impairment (Kish et al., 2010). Therefore, it is possible that at least some of these adaptations underlie the long term behavioural problems seen after repeated ecstasy use. The mechanisms by which MDMA might produce these effects is not clear.

Problems associated with studying the harmful effects of MDMA

Given the global popularity of ecstasy, and the deficits associated with regular use, it becomes important to investigate potential treatments to reduce intake, and to reverse harmful neuroadaptations. However, there are a number of potential confounds associated with studies that use human subjects to determine the effects of MDMA use on the brain and/or behaviour. Firstly, results from studies on ecstasy users can be

limited by a number of factors. For example, the accuracy of subjects' reported use and the range of other drugs the subject also uses may confound results. This concept is illustrated in the abovementioned report by Jansen (1999) describing the effects of ecstasy use in three regular users, in which total lifetime ecstasy exposure was determined by self-report for periods of over two years. This method for determining drug intake relies on memory for drug taking episodes even though ecstasy use is associated with memory impairments. Furthermore, the study by Jansen highlights the poly-drug use typical of regular ecstasy users (Cottler et al., 2009; Cottler et al., 2001) – the first patient reported regular amphetamine use of 1g/day, the second was dependent on benzodiazepines, while the third consumed roughly 1 bottle of spirits every night. Regular use of other drugs makes it more difficult to isolate the effects of MDMA.

A second potential issue with human studies is that varied individual histories of ecstasy users can limit the conclusions that can be drawn. For example, while symptoms of depression and anxiety are widely reported after regular ecstasy use (Rogers et al., 2009), a causal link cannot be drawn between ecstasy use and psychological deficits, given that pre-existing problems such as anxiety and depression might predispose an individual to regular ecstasy consumption as a form of self-medication (Parrott, 2006, 2013a). Without random allocation and an appropriate control group, causal links between drug use and its effects cannot be drawn. In an effort to overcome this limitation a small number of studies have randomly assigned participants to receive acute administrations of MDMA, but, as the authors of one study lament, ethical constraints on dosing regimens severely limit the ecological validity and scope of such studies (Peiró et al., 2013).

Some researchers have gone to great lengths to minimise the impact of such confounds on the results of their studies. For example, a recent study investigated current ecstasy users and compared results to a control group of poly-drug users that have never used ecstasy. Thus, any differences should be attributable to ecstasy use. The results showed that ecstasy users had higher levels of cognitive and behavioural disturbances than non-ecstasy poly-drug users (Taurah et al., 2014). Such results strengthen claims that MDMA use is harmful and help to illustrate the nature of these harms. However, because of ethical constraints that restrict the doses of MDMA that can be administered to humans, investigations into the mechanisms behind these effects of MDMA cannot be readily conducted.

Animal studies

For these reasons, animal models are often turned to in order to obtain information regarding the effects of exposure to MDMA. The real value of animal laboratory studies is that they allow experimenters some control over the histories of subjects, the drugs administered, and environmental factors. Furthermore, a wider range of doses can be administered to animals than is ethically viable with humans. There is some loss of ecological validity when animal models are employed, particularly as they necessarily ignore the complex environment in which ecstasy is consumed, but such studies can be incredibly helpful in evaluating properties of MDMA that cannot be determined in humans.

A number of studies have replicated the findings of human studies after administering MDMA to animals. Typically, high doses of MDMA are administered repeatedly, after which some behavioural or neurochemical measures are made. For example, exposure to high doses of experimenter-administered MDMA decreased tissue 5-HT levels (Battaglia, Yeh, & De Souza, 1988; Commins et al., 1987; McGregor et al., 2003), damaged 5-HT cells (Commins et al., 1987; Jensen et al., 1993), and reduced 5-HT transporter binding (Battaglia, Yeh, et al., 1988; McGregor et al., 2003). In behavioural tests, repeated administration of MDMA increased anxiety-like behaviour in adult (McGregor et al., 2003) and adolescent rats (Bull, Hutson, & Fone, 2003; Bull, Hutson, & Fone, 2004; Cox et al., 2014), and impaired novel object discrimination, a measure of recognition memory (Shortall et al., 2013). Although this method of experimenter-administered, high dose MDMA is useful for determining the harmful effects of MDMA, these studies have been criticised for employing a physiologically irrelevant dosing regimen (Baumann & Rothman, 2009; Cole & Sumnall, 2003; De La Garza et al., 2007; Meyer, Piper, & Vancollie, 2008), given that this high level of exposure is rarely, if ever, experienced by ecstasy users (D. Hansen, Maycock, & Lower, 2001; Parrott, 2005; Verheyden, Henry, & Curran, 2003).

One alternative to an experimenter administered drug regimen is to give the animal control over the delivery of drug, in a manner similar to how humans control their drug intake. This is the basis of the popular self-administration paradigm, in which an animal performs some operant (e.g. nose poke, lever press) in order to obtain a dose of drug. Often, the route of drug administration is intravenous, meaning the animal requires a surgically implanted indwelling venous catheter. After recovery from

this surgery, the animal is placed in an operant chamber and the catheter is connected via tubing to a syringe encased in a mechanical syringe pump. The operant activates the syringe pump, resulting in a predetermined intravenous dose of the drug being investigated. Drug infusions are generally paired with a stimulus (e.g. light, tone). Usually there is a second manipulandum (e.g. nose poke hole, lever) for which the operant has no programmed consequence, but responses are recorded as a measure of non-specific responding.

Human drug taking is a complex behaviour that is influenced by an interaction of social, economic, and personal factors, and as such it cannot be modelled in a single animal paradigm. Furthermore, as with all animal models, ecological validity is lost in order to gain experimental control and practicality. For example, self-administration studies generally allow an animal to self-administer only the drug of interest (with no adulterants), in order to draw causal conclusions about this drug. In contrast, human drug users tend to use a range of drugs, and drugs procured on the street tend not to be pure. Thus, the self-administration paradigm trades ecological validity for experimental control (De La Garza et al., 2007). While experimental design can help to minimise the loss of validity, no self-administration model can perfectly replicate human drug taking. Nonetheless, as will be explained below, the self-administration paradigm is an excellent paradigm for MDMA administration, and also allows for studies in which drug taking is the dependent measure.

A particular strength of the self-administration paradigm is that the animal has control over their drug intake. Firstly, this reduces concerns over the administration of irrelevantly large drug doses. Figure 1.1 presents data adapted from Schenk, Gittings, Johnstone, and Daniela (2003) showing the number of infusions of MDMA that were self-administered in a session, for different doses of MDMA. It is clear that MDMA self-administration behaviour adjusts as dose changes, illustrating that the animal utilises control over responding to regulate total drug intake. Thus, it is less likely that physiologically irrelevant doses will be administered, as has been suggested for studies using experimenter-administered MDMA.

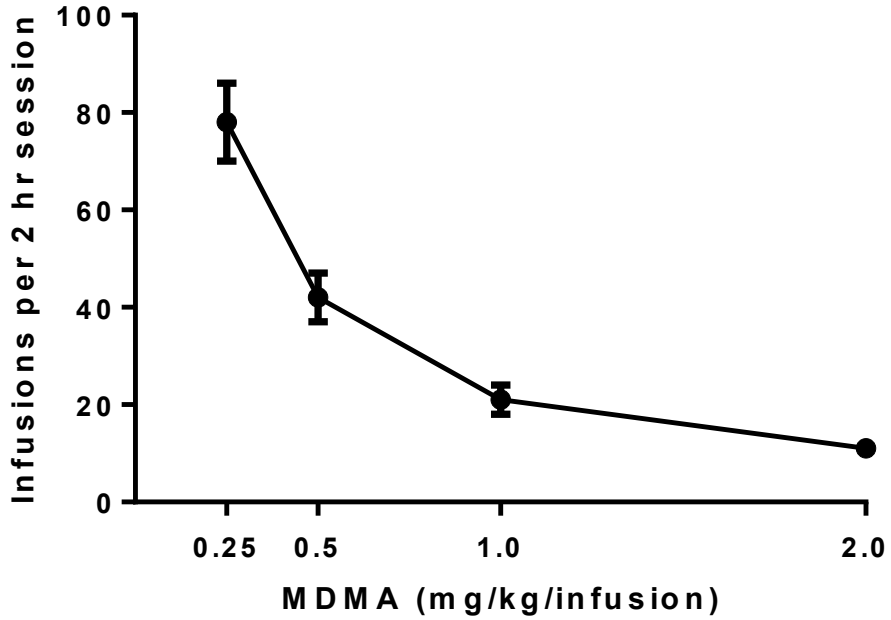


Fig. 1.1 Dose response curve for MDMA self-administration. Adapted from Schenk et al. (2003).

Secondly, self-administered drug produces neuroadaptations that are not solely due to the action of the drug. For example, self-administered cocaine produced significantly greater changes in dopamine transporter binding than the same doses administered non-contingently, suggesting that the stimulus-response associations learned in self-administration contribute to the neuroadaptations produced by drugs of abuse (Miguéns et al., 2008). Because human users also have control over their drug intake, and because the neuroadaptations produced by drugs may be dependent on this control, self-administered MDMA is probably a better model of human drug administration than experimenter-administered MDMA.

A third strength of the self-administration paradigm, and of particular relevance to MDMA, is that the overall pattern of drug taking is similar in animals and humans. On their first exposure to MDMA human users generally consume ½ - 1 ecstasy tablet (D. Hansen et al., 2001) with drug use being intermittent, but with experience some users may consume upwards of 20 pills in a session (Parrott, 2005; Verheyden et al., 2003). A similar pattern of low, intermittent initial intake followed by increased intake in some subjects is seen in MDMA self-administration in rats and monkeys (Banks et al., 2008; Beardsley, Balster, & Harris, 1986; De La Garza et al., 2007; Schenk, Colussi-Mas, Do, & Bird, 2012). It is important that, in both animals and humans, initial exposure to MDMA is low and intermittent, because intermittent or low dose

exposure to MDMA was neuroprotective against the neuroadaptations produced by subsequent high dose administrations (Bhide, Lipton, Cunningham, Yamamoto, & Gudelsky, 2009; Piper, Ali, Daniels, & Meyer, 2010). Indeed, self-administered MDMA produced smaller deficits in tissue levels of 5-HT compared to high dose experimenter-administered MDMA (Do & Schenk, 2011; Scanzello, Hatzidimitriou, Martello, Katz, & Ricaurte, 1993; Schenk et al., 2007), even though the total amount self-administered (165-350 mg/kg over 20-30 days of testing) was greater than is generally administered to produce extensive neurotoxicity (20-80 mg/kg in a single day). Given that the neuroadaptations produced by MDMA are dependent on the pattern of prior MDMA exposure, self-administered MDMA likely produces neuroadaptations more similar to the human condition than those produced by experimenter-administration.

Furthermore, the self-administration paradigm allows for the behaviour of drug taking to be studied, which can be useful when investigating how a certain manipulation might affect drug taking behaviour. In this manner, self-administration has been a valuable pre-clinical tool in determining the efficacy of purported treatments for reducing drug consumption. For example, self-administration of a range of drugs is reduced by vaccines that use the body's immune system to block drugs from crossing the blood/brain barrier (Fox et al., 1996; Kantak, 2003; Skolnick, 2015). Based in part on the results of self-administration studies, a number of these vaccines have progressed to clinical trials, representing an exciting new potential rehabilitative tool for reducing drug taking (Heidbreder & Hagan, 2005; Skolnick, 2015).

Overall, the self-administration model allows for direct assessment of drug taking behaviour, and reduces some of the confounds associated with investigating the effects of experimenter-administered MDMA on animals (De La Garza et al., 2007; Fantegrossi, 2007). Furthermore, self-administration of MDMA produces different neuroadaptations to experimenter administration, and these neuroadaptations are probably more similar to those produced by regular recreational ecstasy use.

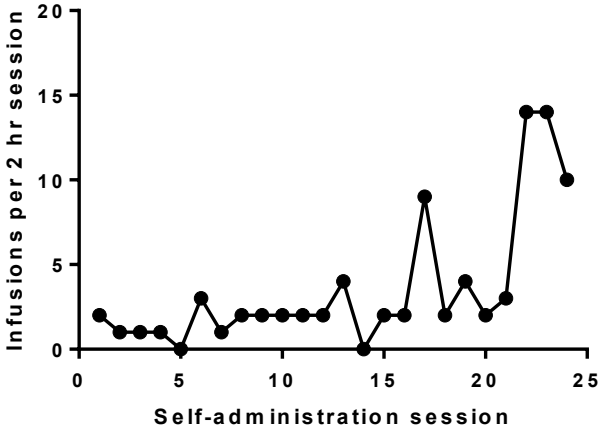
Profile of MDMA self-administration

Just as humans show tremendous variability in their patterns of ecstasy use, there is considerable variability in the self-administration of MDMA in animals. More specifically, some individuals are more vulnerable to the reinforcing effects of MDMA, and the reinforcing efficacy of MDMA appears to increase with repeated

exposure. To investigate this variability our laboratory has defined an acquisition criterion and measured latency to acquisition of MDMA self-administration. To meet the acquisition criterion a rat must self-administer a total of 90 infusions of MDMA (1.0 mg/kg/infusion) within 25 self-administration sessions. Our laboratory has shown that roughly 50% of subjects will acquire MDMA self-administration under these conditions (Colussi-Mas, Wise, Howard, & Schenk, 2010; Schenk et al., 2012; Schenk et al., 2003; Schenk et al., 2007). Figure 1.2 presents raw data collected for this thesis that help to illustrate the variability in acquisition of MDMA self-administration. The top panel shows the number of MDMA infusions self-administered within the 25 session for a subject (Kererū) that did not meet acquisition criteria. Responding across the 25 sessions is consistently low, although up to 3 infusions were self-administered within a session. It should be noted that this rate of self-administration is lower than that supported by the saline vehicle, which supports roughly 5-7 infusions per session. The middle panel shows the same data for a subject (Black Stilt) that was slow to acquire (24 sessions), while the bottom panel shows a subject (Kea) that acquired relatively quickly (13 sessions). As is typical in these self-administration studies, initial intake is low in all 3 subjects, but the subjects that did acquire show a sudden increase in intake. That Kea required less self-administration experience before increasing intake than Black Stilt shows the underlying variability in the reinforcing effects of MDMA between these subjects, and this variability is reflected in their latency to acquisition. The decrease in Kea's responding in session 7 is typical after the first exposure to a high dose of MDMA, and can be seen to a lesser extent in the data from Black Stilt.

Subject: Kererū

Subject: Black Stilt



Subject: Kea

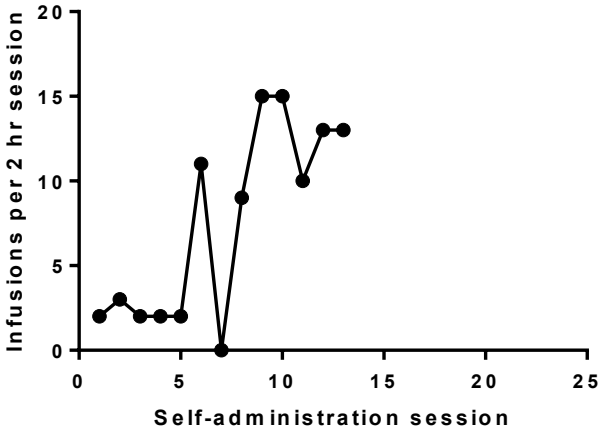


Fig. 1.2 Number of MDMA infusions (1.0 mg/kg/infusion) self-administered over a 25 day acquisition period. Top panel: a subject that did not acquire. Middle panel: A subject that was slow to acquire (24 sessions). Bottom panel: A subject that was relatively quick to acquire (13 sessions).

Of the subjects that do acquire MDMA self-administration, some self-administer more reliably than others. In our laboratory once a subject meets the acquisition criterion the dose of MDMA is halved, and we expect that responding will compensate accordingly. Often, we will further increase the FR schedule so that more responses are required to obtain an infusion of drug. Some subjects will not increase responding as the FR schedule is increased, while in others responding will compensate for increases in FR (see Chapter 7). Figure 1.3 illustrates these compensatory increases in responding with data collected for this thesis from a rat that shows reliable self-administration. The top panel shows that responding compensated for the decrease in dose (from section A to section B), and for increases in FR values (sections C and D). The bottom panel shows that total intake becomes consistent over time, although initially there is some variability, particularly after the first high dose of MDMA was self-administered.

These patterns of self-administration behaviour have been a focus of our laboratory for some time. In particular, we find it interesting that some subjects will increase responding for MDMA after relatively low MDMA intake, while others will show similar behaviour after relatively high MDMA intake, and others still will not increase responding for MDMA within our 25 day cut-off period. We have suggested that this behavioural profile might reflect the pharmacodynamic profile of MDMA.

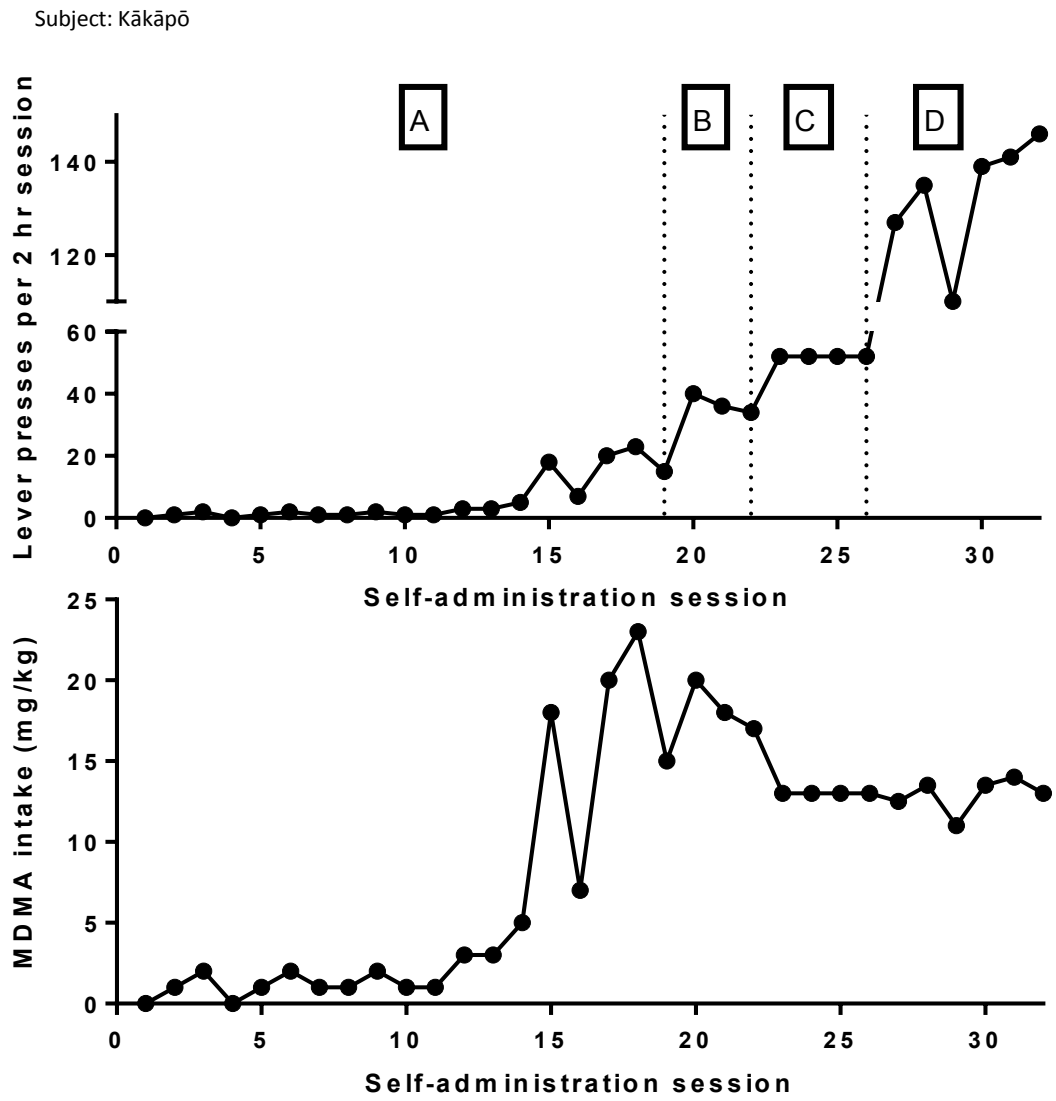


Fig. 1.3 Top panel: lever presses reinforced by MDMA per session across different MDMA doses and FR schedules. Section A: 1.0 mg/kg/infusion, FR 1. Section B: 0.5 mg/kg/infusion, FR 1. Section C: 0.5 mg/kg/infusion, FR 2. Section D, 0.5 mg/kg/infusion, FR 5.
Bottom panel: Total MDMA intake over the same self-administration sessions.

Pharmacodynamics of MDMA

MDMA has a diverse pharmacodynamic profile. Battaglia, Brooks, Kulsakdinun, and De Souza (1988) categorised the 5-HT transporter, 5-HT_2 receptors, α2 adrenergic receptors, and M-1 muscarinic receptors as targets for which MDMA has high affinity (0-10μM). Moderate affinity (10μM-100μM) targets included the norepinephrine and dopamine transporters, and 5-HT_1 receptors, and low affinity (>100μM) targets included dopamine-D1 and -D2 receptors and the choline transporter. A small number of studies have shown that MDMA produces modest increases in extracellular levels of glutamate (Anneken & Gudelsky, 2012; Nash & Yamamoto, 1992) and acetylcholine (Acquas et al., 2001; Nair & Gudelsky, 2006a, 2006b), but there is limited evidence for effects on extracellular norepinephrine (Starr, Page, & Waterhouse, 2012) or GABA (Bankson & Yamamoto, 2004; Yamamoto, Nash, & Gudelsky, 1995). In contrast, a great deal of research on MDMA has focused on 5-HT and dopamine mechanisms.

MDMA preferentially releases 5-HT via reverse transport (Gu & Azmitia, 1993; Gudelsky & Nash, 1996; Hekmatpanah & Peroutka, 1990). Although MDMA has moderate affinity for the norepinephrine and dopamine transporters, MDMA is more potent at releasing 5-HT (EC_{50}=74.3 nM) than norepinephrine (EC_{50}=136 nM) or dopamine (EC_{50}=278 nM) (Baumann, Wang, & Rothman, 2007). MDMA also inhibits the 5-HT transporter (Berger, Gu, & Azmitia, 1992; Rothman & Baumann, 2003), vesicular monoamine transporter 2 (Bogen, Haug, Myhre, & Fonnum, 2003; Erickson, Schafer, Bonner, Eiden, & Weihe, 1996; Pifl, Reither, & Hornykiewicz, 2015) and activity of monoamine oxidase A and B (Leonardi & Azmitia, 1994; Matsumoto et al., 2014; Scorza et al., 1997). Thus, MDMA enhances extracellular 5-HT levels by inhibiting the reuptake of 5-HT, directly releasing 5-HT from terminals, inhibiting the packaging of 5-HT into vesicles, and inhibiting the degradation of 5-HT. Results from *in vivo* microdialysis studies reliably show that MDMA preferentially increases extracellular 5-HT levels (For review see Schenk (2011)). Following acute administration of MDMA there was an immediate (15 min) and prolonged (2 week) decrease in tryptophan hydroxylase activity, as measured by a $^{14}CO_2$-trapping procedure (Schmidt & Taylor, 1987; Stone, Hanson, & Gibb, 1987; Stone, Johnson, Hanson, & Gibb, 1988; Stone, Merchant, Hanson, & Gibb, 1987), indicating that MDMA also inhibits the further production of 5-HT.

MDMA produces minor and transient reductions in dopamine transporter function, as measured in *ex vivo* synaptosomes, but failed to alter dopamine transporter binding or tyrosine hydroxylase activity in rats (J. P. Hansen et al., 2002; Stone, Merchant, et al., 1987). Nonetheless, MDMA administration increases extracellular dopamine levels, as determined by *in vivo* microdialysis (for review, see Schenk (2011)). This increase is more modest than the MDMA-produced increase in extracellular 5-HT levels. For example, there was a 300% increase in extracellular dopamine concentrations in the nucleus accumbens following 3 mg/kg MDMA, but an 1800% increase in extracellular 5-HT concentrations (Baumann, Clark, & Rothman, 2008).

A focus on 5-HT

A question remains as to which of these effects of MDMA might be related to its self-administration. A wealth of data indicate that the reinforcing efficacy of a drug is directly related to its ability to increase synaptic levels of dopamine. For example, dopamine agonists reduced self-administration in a manner consistent with a leftward shift in the dose-response curve (Gardner, 2000; Yokel & Wise, 1978), suggesting enhanced reinforcement. On the other hand, dopamine antagonists produced responding consistent with a rightward shift in the dose-response curve (de Wit & Wise, 1977; Ettenberg, Pettit, Bloom, & Koob, 1982; Gardner, 2000; Yokel & Wise, 1975), suggesting a decrease in reinforcement. Similarly, neurotoxic, 6-OH-DA, lesions also reduced the reinforcing efficacy of drugs of abuse (Gardner, 2000; Lyness, Friedle, & Moore, 1979; Roberts, Corcoran, & Fibiger, 1977; Roberts & Koob, 1982).

As is true with other drugs of abuse, the reinforcing efficacy of MDMA, and thus the self-administration of MDMA, results from dopamine release. As indicated above, however, MDMA preferentially increases 5-HT, an effect that is incompatible with self-administration. For example, stimulation of 5-HT release inhibited (Rothman et al., 2005), while neurotoxic 5,7-DHT lesions enhanced (Bradbury et al., 2014; Loh & Roberts, 1990) self-administration. Self-administration of amphetamine-type drugs was inversely related to affinity for the 5-HT transporter (Ritz & Kuhar, 1989), or potency to stimulate 5-HT release (Wee et al., 2005). With specific reference to MDMA, the (+) isomer that selectively releases dopamine was more readily self-administered than the (-) isomer that selectively releases 5-HT (Z. Wang & Woolverton, 2007). That is, higher levels of 5-HT release are inhibitory to self-

administration in general, and to MDMA self-administration in particular. Thus, MDMA-produced 5-HT release would be expected to inhibit MDMA self-administration, yet, as outlined above, some rats will eventually self-administer MDMA reliably. It is possible that some rats are less responsive to these 5-HTergic effects and so self-administer MDMA more readily.

This hypothesis was recently directly tested in our laboratory. Firstly, the 5-HTergic response to an initial dose of MDMA was determined by *in vivo* microdialysis before MDMA self-administration began. 5-HT release produced by this initial exposure to MDMA was lower in the rats that did acquire MDMA self-administration than in those that did not, while dopamine release was similar for both groups. Secondly, the effect of a neurotoxic 5,7-DHT lesion on acquisition of MDMA self-administration was determined. The lesion reduced 5-HT tissue levels by up to 67%. Of interest, 100% of the lesion group acquired MDMA self-administration, compared to approximately 50% of controls, and the latency to acquisition was greatly reduced in the lesion group (Bradbury et al., 2014). Thus, lower 5-HT release produced by MDMA, either endogenous or exogenously produced by a lesion, was associated with enhanced self-administration. These findings support the hypothesis that MDMA-produced 5-HT release is inhibitory to the acquisition of MDMA self-administration, but a question remains as to the mechanism for this inhibitory effect.

It has been suggested that the development of MDMA as an efficacious reinforcer in the self-administration paradigm is due to neuroadaptations that occur in response to regular MDMA exposure, and that the same neuroadaptations could underlie the development of ecstasy SUDs (Schenk, 2011; Schenk & Aronsen, 2015). Microdialysis studies have shown that the 5-HTergic response to MDMA is attenuated after repeated exposure (Baumann, Clark, Franken, Rutter, & Rothman, 2008; Reveron, Maier, & Duvauchelle, 2010; Shankaran & Gudelsky, 1999), an effect that would be expected to facilitate MDMA self-administration. It has been hypothesised that this reduced 5-HTergic response to MDMA disinhibits the dopaminergic response, enhancing the reinforcing efficacy of MDMA and making it comparable to other drugs of abuse (Schenk, 2011). Furthermore, neuroadaptations in 5-HT receptors, as a result of MDMA exposure, have been suggested to enhance problematic behaviours, like impulsivity, that are associated with SUDs (Schenk & Aronsen, 2015).

Thus, repeated exposure to MDMA reduces the 5-HTergic response to MDMA, enhancing its reinforcing effects and producing behaviours that may contribute to

problematic drug taking. If the reinforcing effects of MDMA rely on 5-HTergic deficits, the variability in acquisition of MDMA self-administration might be due to increased vulnerability to MDMA-produced 5-HTergic neuroadaptations in some rats. Because 5-HTergic deficits enhance MDMA self-administration via a disinhibition of dopamine, there are likely specific 5-HT receptors that modulate the dopaminergic response to, and thus the self-administration of, MDMA.

There are 14 different 5-HT receptor subtypes, arranged into 7 receptor families, and spread widely throughout the brain (Hoyer et al., 1994). The 5-HT_{1A} and 5-HT_{1B} receptor subtypes have a role in the regulation of dopamine and the dopaminergic response to drugs of abuse, and as such changes in the activation of these receptor subtypes might be expected to alter the reinforcing effects of MDMA.

5-HT_{1A} and 5-HT_{1B} receptors

The 5-HT_{1A} receptor is a seven transmembrane receptor that couples to G_i/G_o to inhibit adenylyl cyclase and produce hyperpolarisation (Hamon et al., 1990; Innis, Nestler, & Aghajanian, 1988; Schoeffter & Hoyer, 1988). In the brain the 5-HT_{1A} receptor is located both pre- and post-synaptically. Pre-synaptically, the 5-HT_{1A} receptor is an autoreceptor on 5-HT neurons in the dorsal and median raphe nuclei, where activation inhibits 5-HT synthesis, and release of 5-HT in terminal regions (Hamon et al., 1988; Riad et al., 2000; Yoshimoto & McBride, 1992). 5-HT_{1A} receptors have also been localised to the hippocampus, amygdala, prefrontal cortex (PFC), and the ventral tegmental area (VTA) where they act as heteroreceptors on dopamine, glutamate, and GABA cells (Doherty & Pickel, 2001; Hajós, Gartside, Varga, & Sharp, 2003; Hume et al., 2001; Maeda et al., 2001; Palchaudhuri & Flügge, 2005; Pompeiano, Palacios, & Mengod, 1992; Puig, Artigas, & Celada, 2005; Puig, Watakabe, Ushimaru, Yamamori, & Kawaguchi, 2010).

The 5-HT_{1B} receptor is also a seven transmembrane protein that couples to G_i/G_o to inhibit adenylyl cyclase and produce hyperpolarisation (Hartig, Branchek, & Weinshank, 1992; Hoyer & Middlemiss, 1989; Sari, 2004; Seuwen, Magnaldo, & Pouysségur, 1988; C. Wang et al., 2013). In the brain the 5-HT_{1B} receptor is located pre-synaptically on the terminals of 5-HTergic or non-5-HTergic cells, as auto- or heteroreceptors, respectively (Boulenguez et al., 1996; Offord, Ordway, & Frazer, 1988; Sari et al., 1999; Vergé et al., 1986). 5-HT_{1B} receptor binding was high in globus pallidus, substantia nigra, nucleus accumbens, frontal cortex, striatum, and

hippocampus (Bonaventure, Schotte, Cras, & Leysen, 1997; Lindhe et al., 2011). 5-HT_{1B} mRNA was also abundant in the hypothalamus, thalamus, and amygdala (Bonaventure et al., 1998). As well as being present on 5-HTergic neurons, 5-HT_{1B} receptors have been localised to dopaminergic (Sarhan & Fillion, 1999), GABAergic (Darrow, Strahlendorf, & Strahlendorf, 1990), and glutamatergic (Raiteri, Maura, Bonanno, & Pittaluga, 1986) terminals.

Changes in 5-HT_{1A} and 5-HT_{1B} receptor protein levels, mRNA levels, or binding to G proteins, have been shown in response to general interventions such as exercise (Chennaoui et al., 2001; Fuss et al., 2013), stress (Iyo et al., 2009; S. Wang, Zhang, Guo, Teng, & Chen, 2009), and steroid administration (Ambar & Chiavegatto, 2009; Kindlundh, Lindblom, Bergström, & Nyberg, 2003). More importantly, changes have also been shown after repeated exposure to 5-HTergic ligands. 5-HT_{1A} autoreceptors were down-regulated by chronic exposure to selective 5-HT reuptake inhibitors (SSRIs) (Castro, Diaz, del Olmo, & Pazos, 2003; Le Poul et al., 2000), although no changes in 5-HT_{1A} autoreceptors were detected after repeated exposure to MDMA (Schenk, Abraham, Aronsen, Colussi-Mas, & Do, 2013). Chronic SSRI treatment also increased post-synaptic 5-HT_{1A} receptor agonist-stimulated binding of [^{35}S]GTPγS to G proteins (Castro et al., 2003; Moulin-Sallanon et al., 2009). Similarly, up-regulation of post-synaptic 5-HT_{1B} receptors has been suggested as a result of repeated SSRI treatment (Le Poul et al., 2000). These findings suggest that the large increases in synaptic 5-HT produced by MDMA could also produce changes in these receptor subtypes.

The role of 5-HT_{1A} and 5-HT_{1B} receptors in dopamine modulation

The most commonly used 5-HT_{1A} receptor agonist, 8-hydroxy-2-dipropylaminotetralin (8-OH-DPAT), has high affinity for 5-HT_{1A} receptors (Peroutka, 1986). Low doses of 8-OH-DPAT preferentially activated 5-HT_{1A} autoreceptors, while higher doses also activated heteroreceptors (Alex & Pehek, 2007; Hjorth & Magnusson, 1988). Low doses of 8-OH-DPAT simulated dopamine cell activity in the VTA (Gronier, 2008) and increased extracellular dopamine concentrations in the PFC (Arborelius, Nomikos, Hacksell, & Svensson, 1993) and VTA (Chen & Reith, 1995). Thus, activation of 5-HT_{1A} autoreceptors enhances dopamine cell activity and extracellular dopamine concentrations.

Higher doses of 8-OH-DPAT inhibited dopamine cell firing in the VTA (Arborelius, Chergui, et al., 1993) and decreased extracellular dopamine levels in the

nucleus accumbens (NAc) (Ichikawa & Meltzer, 2000) and striatum (Rasmusson, Goldstein, Deutch, Bunney, & Roth, 1994), as measured by *in vivo* microdialysis. The relatively new 5-HT_{1A} receptor agonists, F13640, and F15599, both have >1000 fold selectivity for the 5-HT_{1A} receptor (Colpaert et al., 2002; Newman-Tancredi et al., 2009), while the slightly older BAY × 3702 has approximately 30 fold selectivity for the 5-HT_{1A} receptor (De Vry et al., 1998). Each of these agonists, when administered locally in the PFC, dose-dependently increased extracellular dopamine levels in the PFC (Díaz-Mataix, Artigas, & Celada, 2006; Díaz-Mataix et al., 2005; Lladó-Pelfort, Assié, Newman-Tancredi, Artigas, & Celada, 2012; Lladó-Pelfort, Assié, Newman-Tancredi, Artigas, & Celada, 2010). It was suggested that this effect was due to inhibition of PFC GABA and glutamate cells, since endogenous 5-HT release inhibited electrophysiological recordings from PFC glutamate and GABA cells, and this effect was attenuated by a 5-HT_{1A} receptor antagonist (Hajós et al., 2003; Puig et al., 2005; Puig et al., 2010; Sakaue et al., 2000). Thus, the effect of 5-HT_{1A} receptor activation on dopamine release is region specific – activation of autoreceptors, or heteroreceptors in the PFC, increased extracellular dopamine concentrations, while global activation of heteroreceptors decreased extracellular dopamine concentrations in the NAc and striatum.

There is evidence that activation of 5-HT_{1A} receptors is inhibitory to the dopaminergic response to drugs of abuse. The 5-HT_{1A} receptor agonist, 8-OH-DPAT, inhibited amphetamine-induced dopamine release in the PFC (Kuroki, Ichikawa, Dai, & Meltzer, 1996), striatum and NAc (Ichikawa, Kuroki, Kitchen, & Meltzer, 1995) as determined by microdialysis. 5-HT_{1A} receptor agonists generally inhibited the hyperactive response to amphetamine, methamphetamine, and MDMA (Müller, Carey, Huston, & Silva, 2007), a response that has been associated with enhanced dopamine neurotransmission (Wise & Bozarth, 1987). Furthermore, the expression and development of cocaine or amphetamine sensitisation in mice was inhibited by 5-HT_{1A} receptor agonist administration (Ago et al., 2006; Przegaliński, Siwanowicz, Baran, & Filip, 2000). Thus, increased activation of 5-HT_{1A} receptors during MDMA self-administration might be expected to inhibit the dopaminergic response to MDMA.

In vitro studies showed that activation of 5-HT_{1B} receptors inhibited the release of dopamine (Sarhan & Fillion, 1999), GABA (Johnson, Mercuri, & North, 1992; Yan & Yan, 2001b), and glutamate (Muramatsu, Lapiz, Tanaka, & Grenhoff, 1998), but these studies do not consider interactions between neurotransmitter systems. One of

the most widely used 5-HT_{1B} receptor agonists, RU 24969 (5-Methoxy-3-(1,2,5,6-tetrahydro-4-pyridinyl)-1H-indole), has high affinity for 5-HT_{1B} receptors (Ki = 0.38 nM), but also displays appreciable affinity for the 5-HT_{1A} receptor (Ki = 2.5 nM) (Peroutka, 1986; Wolf & Kuhn, 1991). Systemic administration of RU 24969 decreased extracellular GABA concentrations in the VTA *in vivo*, but had no effect on extracellular dopamine concentrations (Parsons, Koob, & Weiss, 1999). However, local administration of the 5-HT_{1B} receptor agonist, CP 93129, which has 150 fold selectivity for 5-HT_{1B} over other 5-HT receptors (Macor et al., 1990), increased extracellular dopamine concentrations in the PFC (Iyer & Bradberry, 1996), striatum (Galloway, Suchowski, Keegan, & Hjorth, 1993), and NAc (Hållbus, Magnusson, & Magnusson, 1997; Yan & Yan, 2001a). Similarly, administration of CP 93129 in the VTA increased extracellular dopamine levels in the NAc (O'Dell & Parsons, 2004; Yan & Yan, 2001a; Yan, Zheng, & Yan, 2004) and decreased extracellular GABA concentrations in the VTA (O'Dell & Parsons, 2004; Yan et al., 2004), without altering extracellular glutamate concentrations in the VTA (O'Dell & Parsons, 2004). Together these findings suggest that activation of 5-HT_{1B} receptors enhances dopamine release, possibly via an inhibition of GABA neurotransmission.

There is evidence that activation of 5-HT_{1B} receptors enhances the dopaminergic response to drugs of abuse. Cocaine produced significantly greater increases in extracellular dopamine, and significantly greater reductions in extracellular GABA, in the NAc after systemic administration of the 5-$HT_{1B/1A}$ receptor agonist, RU 24969 (Parsons et al., 1999). A similar response to cocaine was found after infusion of the 5-HT_{1B} receptor agonist, CP 93129, in the VTA (O'Dell & Parsons, 2004). Systemic administration of the 5-HT_{1B} receptor agonist, CP 94253, which has approximately 45 fold selectivity for 5-HT_{1B} over other 5-HT receptors (Koe, Nielsen, Macor, & Heym, 1992), significantly prolonged the increase in extracellular dopamine in the NAc produced by systemic administration of ethanol (Yan, Zheng, Feng, & Yan, 2005). 5-HT_{1B} receptor agonists produced a leftward shift in the cocaine self-administration dose response curve, increased the break points achieved in cocaine progressive ratio tasks (Parsons, Weiss, & Koob, 1998; Pentkowski, Acosta, Browning, Hamilton, & Neisewander, 2009; Przegaliński, Gołda, Frankowska, Zaniewska, & Filip, 2007), and produced a leftward shift in the self-administration dose response curve for the dopamine uptake inhibitor, GBR 12909 (Parsons, Weiss, & Koob, 1996). Therefore, activation of 5-HT_{1B} receptors during

MDMA self-administration might be expected to enhance the dopaminergic response to MDMA.

Thus, 5-HT_{1A} and 5-HT_{1B} receptors might be expected to impact the initial reinforcing effects of MDMA, via dopaminergic modulation. Specifically, activation of 5-HT_{1A} receptors would be expected to decrease the dopaminergic response to MDMA, while activation of 5-HT_{1B} receptors would be expected to enhance the dopaminergic response to MDMA. Furthermore, alterations in the activation of these receptors might explain the enhancement in the reinforcing efficacy of MDMA after repeated exposure. As outlined below, individual variability in these receptor populations, or MDMA-produced changes in these receptor populations, might also impact the reinforcing effects of MDMA and explain behavioural deficits seen in regular ecstasy users.

5-HT_{1A} and 5-HT_{1B} receptors and impulsivity

Impulsivity has been broadly defined as action without foresight, referring to behaviours that are poorly thought out, prematurely executed, or risky (Winstanley, Eagle, & Robbins, 2006). In drug users impulsivity is a risk factor for initiating drug taking, escalating drug use, and for developing SUDs (De Wit, 2009; Perry & Carroll, 2008). For example, impulsive traits in youth and young-adulthood positively predicted future drug use, an earlier onset of drug taking, and the likelihood of developing an SUD (De Wit, 2009; Kirisci, Tarter, Mezzich, & Vanyukov, 2007; Sher, Bartholow, & Wood, 2000; Tarter, Kirisci, Feske, & Vanyukov, 2007).

A role of impulsivity in different aspects of drug self-administration in animals has been determined. Some studies have looked at the acquisition and maintenance of self-administration, based on the idea that highly impulsive subjects, as is the case with humans, might be more prone to take drugs (Perry & Carroll, 2008). Typically, impulsivity is measured by a model of behavioural inhibition, such as the 5 choice serial reaction time task (5CSRTT), or a model of choice preference for a delayed reward, such as the delay discounting paradigm. These measures show good validity as they are variants of those used to assess aspects of impulsive behaviour in humans (Evenden, 1999b; Robbins, 2002). Delay discounting and reaction time tasks can be used to determine impulsivity scores across a group of animal subjects, which can then be divided into 'low impulsivity' (LI) groups and 'high impulsivity' (HI) groups. HI subjects are usually defined as those in the upper quartile of impulsivity scores, with LI subjects being those with impulsivity scores in the bottom quartile. These two

groups can then be compared to determine the relationship between impulsivity and drug self-administration.

When impulsivity was determined using a delay discounting task HI rats consumed more ethanol (Poulos, Le, & Parker, 1995), or cocaine (Koffarnus & Woods, 2013; Perry, Larson, German, Madden, & Carroll, 2005; Perry, Nelson, & Carroll, 2008), and cocaine self-administration was acquired more quickly and in a higher percentage of HI rats (Perry et al., 2005; Zlebnik & Carroll, 2015). Similarly, HI rats, as measured by 5CSRTT performance, acquired nicotine self-administration more readily (Diergaarde et al., 2008), and a strain of mice with high impulsivity showed enhanced ethanol self-administration (Loos, Staal, Smit, De Vries, & Spijker, 2013). Following acquisition, HI rats, as determined by the 5CSRTT, self-administered more cocaine per hour than LI rats, and exhibited an upward shift in the cocaine dose response curve (Dalley et al., 2007). Furthermore, impulsivity as determined by the 5CSRTT predicted the magnitude of the drug-seeking response for MDMA in the reinstatement paradigm (Bird & Schenk, 2013). Thus, higher levels of impulsivity would be expected to facilitate self-administration.

Systemic administration of the 5-HT_{1A} receptor agonist, 8-OH-DPAT, increased premature responding on the 5CSRTT (Carli & Samanin, 2000) while the 5-HT_{1A} receptor antagonist, WAY 100635 (N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl)cyclohexanecarboxamide), which has >100-fold selectivity for the 5-HT_{1A} receptor over other receptors (A. Fletcher et al., 1995), supressed impulsive action in a 3CSRTT (Ohmura et al., 2013). The effects of 5-HT_{1A} ligands on impulsivity appear to be due to autoreceptor activation, because neither local administration of 8-OH-DPAT in the PFC, nor systemic administration of the 5-HT_{1A} post-synaptic preferring receptor agonist F15599, affected premature responding on the 5CSRTT (Carli, Baviera, Invernizzi, & Balducci, 2006; Lladó-Pelfort et al., 2010; Winstanley et al., 2003).

In humans, 5-HT_{1B} receptor gene polymorphisms are associated with impulsive aggression (Zouk et al., 2007). Mice that lack the 5-HT_{1B} receptor gene from birth show increased impulsivity in a behavioural model of response inhibition (Nautiyal et al., 2015; Pattij et al., 2003). Interestingly, knockdown of 5-HT_{1B} autoreceptors did not affect impulsivity, suggesting the effect of 5-HT_{1B} receptor activation on impulsivity is due to heteroreceptor action (Nautiyal et al., 2015). Studies of the effects of 5-HT_{1B} ligands on impulsivity have been limited due to the fact that agonists have a range of

behavioural effects that disrupt operant responding (Evenden, 1999a; van den Bergh, Bloemarts, Groenink, Olivier, & Oosting, 2006). However, the limited available data suggest that activation of 5-HT_{1B} receptors reduces impulsive behaviour (Evenden, 1999a). Therefore, activation of 5-HT_{1B} receptors during MDMA self-administration would be expected to reduce impulsive behaviour, and thus inhibit self-administration.

5-HT_{1A} and 5-HT_{1B} receptors and learning

Before reliable self-administration behaviour can be demonstrated, the subject must learn the association between performance of the operant response and the infusion of drug. Enhanced or inhibited ability to learn this association would clearly also enhance or inhibit the acquisition of self-administration. There is also another learned association that has been shown to be incredibly important in the self-administration paradigm - the Pavlovian association between the drug effect and the contextual stimuli (e.g. the light). These unconditioned contextual stimuli develop conditioned reinforcement properties over repeated pairings with a drug (Ahrens, Singer, Fitzpatrick, Morrow, & Robinson, 2016; W. M. Davis & Smith, 1976; P. J. Fletcher & Korth, 1999b) and these conditioned reinforcers are a powerful driver of self-administration behaviour. For example, one experiment assessed the acquisition of nicotine self-administration in two groups of rats – one in which the nicotine infusion was paired with the illumination of a light, and another in which the infusion was paired with no specific cues. Rats in the nicotine + cue group took less time to show a preference for the active self-administration lever and consumed significantly more nicotine than the nicotine only group, suggesting the Pavlovian association between drug effect and contextual cues facilitated acquisition of self-administration (Caggiula et al., 2002). Therefore, enhanced or inhibited learning of either operant or Pavlovian associations would be expected to enhance or inhibit self-administration, respectively.

The strengthening of stimulus/reward associations is markedly impacted by pharmacological manipulation of 5-HT_{1A} receptors. Systemic 5-HT_{1A} receptor agonist administration impaired performance on an appetitive Pavlovian conditioned responding task (Blair, Bonardi, & Hall, 2004), increased errors in a repeated acquisition of response sequence task (Winsauer, Rodriguez, Cha, & Moerschbaecher, 1999) and delayed acquisition of operant responding maintained by a food reinforcer (Frick, Bernardez-Vidal, Hocht, Zanutto, & Rapanelli, 2015). Furthermore, the 5-HT_{1A} receptor agonist, 8-OH-DPAT, administered after an initial training session, impaired further operant responding for food (Meneses, 2007). The lack of comprehensive dose-

response functions in these studies limits the degree to which the relative roles of 5-HT_{1A} auto- and heteroreceptors can be disentangled. Importantly, the above results were noted over a range of 8-OH-DPAT doses that would be expected to activate pre- and post-synaptic 5-HT_{1A} receptors (up to 1.0 mg/kg). When low doses of 8-OH-DPAT were used, operant learning was enhanced (Meneses & Hong, 1994b), and this effect was reversed by the tryptophan hydroxylase inhibitor, pCPA (Meneses & Hong, 1994a). Together, these results suggest that activation of 5-HT_{1A} autoreceptors enhances, while activation of 5-HT_{1A} heteroreceptors inhibits, learning of stimulus/reward associations.

The non-selective 5-HT receptor agonist, mCPP, inhibited operant stimulus/response learning, and this effect was reversed by the non-selective 5-HT_{1B} receptor antagonist, propranolol (Meneses & Hong, 1997). Moreover, the 5-HT_{1B} receptor agonist, CGS 12066 impaired (Meneses, 2007), while the 5-$HT_{1B/1D}$ receptor antagonist, GR 127935, improved (Meneses, Terrón, & Hong, 1997) performance on the same task. Similarly, the 5-HT reuptake facilitator, tianeptine, enhanced operant stimulus/response learning, and this effect was reversed by the 5-HT_{1B} receptor inverse agonist, SB 224289 (Meneses, 2002). These findings suggest that activation of 5-HT_{1B} receptors inhibits the consolidation of operant learning, and so activation of 5-HT_{1B} receptors during MDMA self-administration might be expected to inhibit the development of self-administration.

5-HT_{1A} and 5-HT_{1B} receptors and anxiety

Anxiety disorders are frequently comorbid with SUDs (Ipser, Wilson, Akindipe, Sager, & Stein, 2015; Merikangas et al., 1998). It has been suggested that anxiety may underlie the initiation of drug taking, in order to alleviate a negative emotional state, and negatively reinforce the continuation of drug use to mitigate withdrawal symptoms (Altman et al., 1996; Belin, Belin-Rauscent, Everitt, & Dalley, 2015; Lejuez et al., 2008). In animal models, anxiety is often operationalised in rodents as an aversion to open or brightly lit spaces (Belin et al., 2015). A popular method for measuring anxiety is the elevated plus maze (EPM), in which a preference for the closed (protected) arms of the maze over the open arms is regarded as an 'anxious' response (Pellow, Chopin, File, & Briley, 1985). An alternate measure of rodent anxiety is self-grooming behaviour in response to an environmental change (Homberg et al., 2002). Rats in the upper quartile for time spent grooming in a novel environment reached higher break points in progressive ratio cocaine self-

administration than the lower quartile group (Homberg et al., 2002). This effect was not replicated when high anxiety was determined by performance on the EPM, however in this case high and low anxiety were determined using a median split, thus possibly masking an effect of anxiety (Bush & Vaccarino, 2007). Higher anxiety on the EPM was associated with escalation of cocaine self-administration (Dilleen et al., 2012), and propensity to self-administer alcohol (Spanagel et al., 1995). Thus, higher levels of anxiety would be expected to facilitate self-administration.

Time spent in the open arms of the EPM was increased by systemic administration of low doses of the 5-HT_{1A} receptor agonist, 8-OH-DPAT (Kwieciński & Nowak, 2009; Lalonde & Strazielle, 2010), an effect reversed by the 5-HT_{1A} receptor antagonist, WAY 100635 (Collinson & Dawson, 1997), suggesting 5-HT_{1A} autoreceptor activation had an anxiolytic effect. Higher doses of systemically administered 8-OH-DPAT had an anxiogenic effect in the same task in mice (Miheau & Van Marrewijk, 1999).When injected into the dorsal or median raphe, 8-OH-DPAT increased time spent in the open arms of the EPM (De Almeida, Giovenardi, Charchat, & Lucion, 1998; File & Gonzalez, 1996; File, Gonzalez, & Andrews, 1996), while injections into the hippocampus (Cheeta, Kenny, & File, 2000a; File et al., 1996; File, Kenny, & Cheeta, 2000), PFC (Solati, Salari, & Bakhtiari, 2011), or septum (Cheeta, Kenny, & File, 2000b; De Almeida et al., 1998) increased anxiety-like behaviour in the EPM. Thus, activation of 5-HT_{1A} autoreceptors had anxiolytic effects in the EPM, while activation of post-synaptic 5-HT_{1A} receptors was anxiogenic.

Early investigations of the role of 5-HT_{1B} receptors in anxiety states found that non-selective 5-HT_{1B} receptor agonists decreased time spent in the open arms of an EPM (Benjamin, Lal, & Meyerson, 1990; Critchley & Handley, 1987; Pellow, Johnston, & File, 1987), suggesting that activation of 5-HT_{1B} receptors was anxiogenic. The role of 5-HT_{1B} receptors in modulating anxiety was more recently confirmed; entries into the open arms of the EPM were dose-dependently reduced by the 5-HT_{1B} receptor agonist CP 94253, and this effect was reversed by the 5-$HT_{1B/1D}$ receptor antagonist, GR 127935 (Lin & Parsons, 2002). The relative contribution of 5-HT_{1B} auto- and heteroreceptors to this effect is not clear. 5-HT_{1B} heteroreceptors on GABAergic amygdala neurons have been suggested as a possible neuronal mechanism (Lin & Parsons, 2002; Sari, 2004) because 5-HT_{1B} manipulations of these projections altered behaviour in the EPM (Audi, De Oliveira, & Graeff, 1991). Furthermore, activation of 5-HT_{1B} receptors in the PFC produced anxiogenic effects in the EPM

(Solati et al., 2011) however, a role of 5-HT_{1B} autoreceptors cannot be ruled out (Sari, 2004).

Summary

MDMA is widely used recreationally in the form of the street drug, ecstasy. Although the majority of users consume ecstasy intermittently, there is concern that MDMA produces a range of deficits in regular ecstasy users. Among these deficits, ecstasy users show increased anxiety and impulsivity, and impaired learning and memory. Problematically, these behavioural changes might be expected to facilitate further ecstasy taking.

MDMA is unique among drugs of abuse in that it primarily acts as a 5-HT releasing agent. 5-HT release has been hypothesised to inhibit the self-administration of drugs in general, and of MDMA in particular. Nonetheless, MDMA self-administration is acquired in roughly 50% of animal subjects. It is possible that MDMA-produced 5-HT release inhibits the reinforcing efficacy of MDMA via activation of specific 5-HT receptors, but there is likely variability in the 5-HTergic response to MDMA between individuals. Furthermore, it is possible that neuroadaptations in 5-HT receptors underlie both the facilitated reinforcement produced by MDMA after repeated exposure, and the cognitive and behavioural deficits seen after regular use.

The 5-HT_{1A} and 5-HT_{1B} receptors are good candidates for these effects of MDMA. Both receptors modulate the reinforcing effects of other drugs of abuse by regulating dopamine release. Furthermore, these receptors mediate a number of behaviours associated with self-administration that are impacted by regular ecstasy use, and receptor up- or down-regulation has been documented in response to a number of different interventions.

This thesis will explore two ways in which alterations in 5-HT_{1A} and/or 5-HT_{1B} receptors could influence MDMA self-administration. Firstly, underlying differences in 5-HT_{1A} and/or 5-HT_{1B} receptors could predispose some subjects to self-administer more readily. This may explain the variability in acquisition of MDMA self-administration. If so, it is hypothesised that manipulations that alter 5-HT_{1A} and/or 5-HT_{1B} receptors will reduce the variability in the acquisition profile for MDMA self-administration.

Secondly, MDMA exposure during self-administration might produce changes in these receptor populations that might facilitate further drug taking and produce behavioural deficits. If so, it is hypothesised that these changes will be evident after substantial MDMA self-administration. Theoretically, if these changes are not the result of neurotoxicity, they could be partially reversed by repeated administration of selective agonists or antagonists.

General Methods

Subjects

Male Sprague-Dawley rats were bred in the Victoria University of Wellington vivarium. They were housed in groups of 4 in a temperature- (19-21ºC) and humidity- (55%) controlled environment until they reached weights of 300-350g, after which they were housed individually. The housing colony was maintained on a 12 h light/dark cycle (lights on at 7.00 am) and all tests were conducted during the light portion of the cycle. Food and water were freely available except during testing.

Drugs

RU 24969 hemisuccinate, WAY 100635 maleate, lithium chloride, ±8-OH-DPAT hydrobromide, (Tocris, New Zealand), and d-amphetamine sulfate (BDG, New Zealand) were dissolved in sterilised saline. GR 127935 hydrochloride (Tocris, New Zealand) was dissolved in distilled water. All injections were a volume of 1.0 ml/kg. ±MDMA hydrochloride (BDG, New Zealand) for self-administration was dissolved in sterilised saline containing 3IU heparin per ml.

All doses refer to salt weights.

Apparatus and procedures

Water consumption

Water consumption was measured in the home cage. Water bottles were removed for 24 hours. Drug administration occurred before water bottles were reintroduced, at times specified in each study. Consumption was measured for a 30 minute period. Fluid consumption was determined by weighing water bottles before and after the test.

Locomotor Activity

Locomotor activity testing was conducted in clear Plexiglas chambers (Med Associates Inc., USA; model ENV-515) measuring 42×42×30 cm, set in sound-attenuating boxes. Forward locomotion was measured with two sets of 16 infrared beams and sensors spaced evenly along the sides of the chambers producing squares measuring 25mm × 25mm. The interruption of three adjacent beams (the approximate size of the body of a rat) was recorded as one activity count. A white noise generator was used during experiments to mask any outside noise, and chambers were washed with Virkon ‘S’ disinfectant (Southern Veterinary Supplies, NZ) after testing to control for olfactory confounds. Experiments were run in a dark room, except for a red light

that was used to illuminate the room during drug administrations. Locomotor activity counts were recorded in 5 minute intervals.

Surgery

For rats that underwent self-administration testing, a silastic catheter was implanted into the right jugular vein under deep anesthesia produced by i.p. injection of ketamine (90 mg/kg) and xylazine (9 mg/kg). Areas surrounding skin that was to be cut were shaved and washed with ethanol and iodine, and eye lubricant (Refresh lacri-lube) was administered to avoid drying. The catheter was secured in place using surgical string and a small amount of adhesive (Bostick superglue). The distal end of the catheter was passed subcutaneously to an exposed part of the skull, attached to a 3 cm piece of 22 gauge stainless steel tubing (BD needles), fixed in place with screws and a small amount of adhesive, and embedded in dental acrylic (Ostron 100). The silastic tubing was coated with silicone (Selleys wet area silicone) to protect from the corrosive nature of the adhesive. Following surgery an analgesic (Carporfen ®, 5.0 mg/kg, s.c.) and electrolyte replacement (Hartman's solution, 12 ml, s.c.) were administered. Carprofen was also administered on each of two days following the surgery. Testing began once pre-surgery weight had been attained, generally within 4-6 days.

Self-administration

Every day, before self-administration testing, rats were weighed and administered penicillin dissolved in heparinised saline (0.2 ml, i.v.) to help maintain general health and catheter patency.

Self-administration was conducted in operant chambers (Med Associates ENV-001) equipped with two levers. Depression of the active lever resulted in a 12 second activation of a syringe pump (Razell, Model A, 1 RPM) resulting in a 0.1 ml intravenous infusion, and the simultaneous illumination of the house light located above the active lever. Depressions of the inactive lever were recorded, but had no programmed consequence. Each self-administration session began with an experimenter-delivered infusion to fill the volume of the catheter. These infusions are not recorded and do not contribute to calculations of total self-administration intake.

Chapter 3: Development of behavioural assays

These first studies were designed to develop behavioural assays for 5-HT_{1A} and 5-HT_{1B} receptor activation, so that further testing of the effects of drug exposure on the function of these receptor subtypes could be conducted. One assay that seemed promising was latent inhibition: the impairment of learning that a stimulus predicts an important event when that stimulus has previously been presented with no consequence (Cassaday, Hodges, & Gray, 1993).

When a neutral stimulus, for example a tone, is paired with a negative consequence, for example a footshock, that stimulus will develop conditioned-stimulus properties and produce freezing behaviour in rodents. A group of rats that had never been exposed to the tone (control group) would learn this association relatively quickly. However, if the tone has previously been presented to another group of rats without consequence (pre-exposure group), learning that the tone now predicts a footshock will take longer in this group. Therefore, after a small number of pairings, the tone will produce less freezing behaviour in the pre-exposure group, because the association between the tone and the footshock is less well learned. Latent inhibition can be operationalised as this behavioural difference (reduced freezing behaviour) between groups. Latent inhibition is a robust effect, found across a range of stimulus-consequence combinations in a wide range of species (Fernández, Giurfa, Devaud, & Farina, 2012; Ferrari & Chivers, 2011; Lubow, 1989).

A number of studies have implicated 5-HT in latent inhibition. Electrolytic, or neurotoxic 5,7 DHT, lesions of the median raphe or NAc blocked the latent inhibition effect (Loskutova, 2001; Loskutova, Luk'yanenko, & Il'yuchenok, 1990; Solomon, Nichols, Kiernan, Kamer, & Kaplan, 1980). Rats in the pre-exposure group showed greater 5-HT metabolism in the striatum and amygdala than rats in the control group, suggesting the latent inhibition effect is associated with increased 5-HTergic activity (Molodtsova, 2003). Additionally, rats with a genetic deletion of the 5-HT transporter showed reduced latent inhibition compared to wildtype counterparts (Nonkes et al., 2012).

The role of 5-HT in latent inhibition is reinforced by studies employing selective pharmacological ligands. The 5-$HT_{1B/1A}$ receptor agonist, RU 24969 (0.5 mg/kg), administered before each pre-exposure, inhibited the development of latent inhibition, while the more selective 5-HT_{1A} receptor agonist, 8-OH-DPAT, had no significant effect (Cassaday et al., 1993), demonstrating a role of 5-HT_{1B} receptors in

latent inhibition. On the other hand, the 5-HT_{1A} receptor antagonist, WAY 100635 (0.5 mg/kg), facilitated latent inhibition in the same task (Killcross, Stanhope, Dourish, & Piras, 1997), suggesting 5-HT_{1A} receptors also impact the expression of latent inhibition. Thus, RU 24969 inhibited the development of latent inhibition, but the relative roles of 5-HT_{1A} and 5-HT_{1B} receptor activation in this effect, and the range of doses over which this effect is produced, have not been determined.

The majority of studies have employed a footshock when investigating latent inhibition. Given the well-established role of 5-HT, and particularly 5-HT_{1A} receptors, in the processing of pain (Avila-Rojas et al., 2015; Colpaert, 2006; Colpaert et al., 2002; Panczyk et al., 2015), I wanted an alternative paradigm to test for latent inhibition. Latent inhibition can be readily demonstrated using the conditioned taste aversion paradigm. Conditioned taste aversion refers to the phenomenon whereby an unfamiliar taste (e.g. a new blend of coffee), paired with a negative internal state (e.g. feeling sick after drinking), results in future avoidance of that taste (the new coffee). This association is rapidly learned if the taste stimulus is novel, but this learning is hampered if the taste stimulus has previously not predicted the illness (e.g. your usual coffee blend). Thus, prior exposure to the neutral stimulus, without negative consequence, inhibits the learning of an association, and so latent inhibition can be demonstrated through an attenuated conditioned taste aversion. In rats this is typically achieved by pairing flavoured water with a drug (e.g. lithium chloride) that produces "internal malaise" (Lubow, 1989, p. 5). One pairing of the flavoured water with the drug is sufficient to ensure the animal avoids the flavoured water in the future, but this effect is attenuated if, previously, the flavoured water has been consumed without consequence (Ellenbroek, Knobbout, & Cools, 1997; Mora et al., 1999). This paradigm has been successfully used to investigate the effects of antipsychotic-type drugs on latent inhibition (Geyer & Ellenbroek, 2003; Moser, Hitchcock, Lister, & Moran, 2000).

As a first attempt to assess the roles of 5-HT_{1A} and 5-HT_{1B} receptors in latent inhibition, the effect of the 5-$HT_{1B/1A}$ receptor agonist, RU 24969, on latent inhibition in a conditioned taste aversion paradigm was tested.

Method

Procedure

Firstly, I aimed to establish the conditioned taste aversion effect, based on the methods of Ellenbroek et al. (1997). All testing was conducted in the home cages. Water bottles were removed from the home cages and made available for 30 minutes per day. Rats (see general methods, n=8 per group) were randomly assigned to have either water (water pre-exposure group) or a 5% sucrose solution (sucrose pre-exposure group) available for drinking. Water bottles were weighed before and after each 30 minute drinking period to measure consumption. Once total consumption during this pre-exposure phase reached 40ml (approximately 3 days; Ellenbroek et al. (1997)), rats in both groups received the 5% sucrose solution for 30 minutes. Immediately after this 30 minute drinking period, lithium chloride (75 mg/kg, i.p.; Ellenbroek et al. (1997)) was administered. The next day both water and the sucrose solution were made available for 30 minutes. Taste aversion was measured as the proportion of sucrose consumed on this test day (amount of sucrose solution consumed divided by total fluid consumption), with lower proportions of sucrose consumption indicative of greater taste aversion. Thus, latent inhibition was indicated by a lower taste aversion (i.e. greater proportion of sucrose consumption) in the sucrose pre-exposure group.

Other groups were tested to determine the effect of RU 24969 pretreatment on this latent inhibition effect. The same protocol were used, but 15 minutes prior to water bottles being available during the pre-exposure phase, rats were injected with RU 24969 (0, 0.03, 0.3, 3.0 mg/kg, s.c.). This range of RU 24969 doses has been shown to be behaviourally effective in different paradigms (Kennett, Dourish, & Curzon, 1987; Tricklebank, Middlemiss, & Neill, 1986). The 15 minute RU 24969 pretreatment time is common (Acosta, Boynton, Kirschner, & Neisewander, 2005; P. J. Fletcher & Korth, 1999b), because maximal effects have been shown between 15 minutes and 4 hours after administration (Tricklebank et al., 1986).

Statistical analyses

Fluid consumption was compared as a function of pre-exposure using one-way analysis of variance (ANOVA). A 3 (RU 24969 dose) × 3 (Session) mixed model ANOVA, with session as the within subjects factor, was used to analyse the effect of RU 24969 on fluid consumption as a function of pre-exposure session. Where appropriate, post-hoc analyses were conducted using Tukey's HSD method.

Results

Experiment 1: Pilot study on the Conditioned Taste Aversion effect

Rats in both pre-exposure groups (n=8 per group) met the fluid consumption criterion in 3 daily pre-exposure sessions. There was no difference in total fluid consumption between the sucrose and water groups ($F(1,14)=2.10$, $p=0.17$). Figure 3.1 shows the water pre-exposure group demonstrated conditioned taste aversion, as indicated by the low proportion of sucrose consumed on the test day. The sucrose pre-exposure group showed significantly greater sucrose consumption than the water pre-exposure group ($F(1,14)=4.53$, $p=0.05$, $\eta_p^2= 0.25$). Figure 3.1 shows that the sucrose pre-exposure group consumed similar amounts of water and sucrose on the test day, suggesting the internal malaise produced by lithium chloride was not associated with the sucrose solution, thus illustrating latent inhibition.

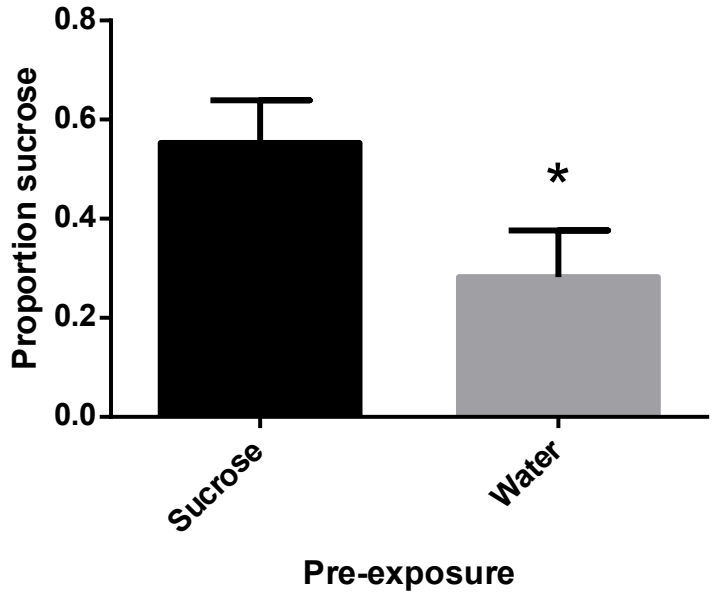


Fig. 3.1 Conditioned taste aversion to sucrose after pairing with lithium chloride in rats either pre-exposed to sucrose or water. The lack of preference for water over sucrose in the sucrose pre-exposure group is indicative of latent inhibition. n = 8 per group, error bars represent SEM. *p=0.05

Experiment 2: The effect of RU 24969 on Conditioned Taste Aversion

Only 11 subjects were available at the beginning of this experiment, so they were divided into groups that received different doses of RU 24969 (0.0, 0.03, 0.3, 3.0 mg/kg, s.c.) and different pre-exposures (water, sucrose; n=2-3 per group) with the intention of adding more subjects to each group as they became available. However,

the initial groups treated with RU 24969 appeared to consume less fluid in the daily 30 minute sessions. Figure 3.2 shows the fluid consumption over the first 3 sessions (collapsed across pre-exposure group) as a function of RU 24969 dose. It is clear that higher RU 24969 dose groups initially consumed less fluid than the lower dose groups. ANOVA confirmed a significant effect of RU 24969 dose ($F(3,54)=25.8$, $p<0.001$, $\eta_p^2=0.59$), and a post-hoc Tukey test showed that the 3.0 and 0.3 mg/kg RU 24969 dose groups both consumed less fluid than the 0.0 and 0.03 mg/kg groups across the first 3 sessions. This decrease in fluid consumption provided a confound that would compromise interpretation of a conditioned taste aversion experiment. Therefore, no further testing was conducted.

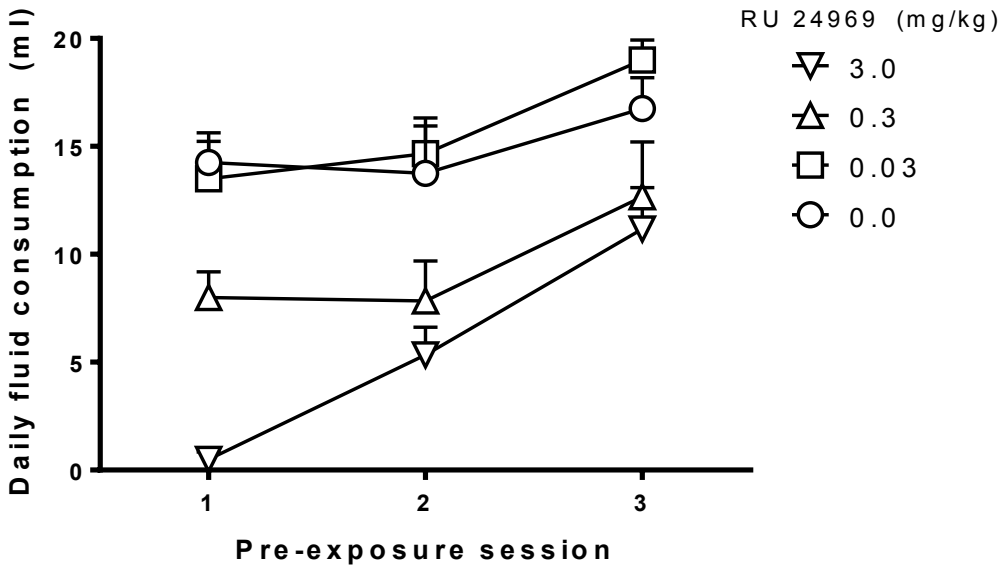


Fig 3.2 The effect of RU 24969 dose on the amount of fluid consumed by fluid-deprived rats during the first 3 drinking sessions. RU 24969 dose-dependently decreased fluid consumption, with the 0.3 and 3.0 mg/kg groups consuming significantly less fluid over the 3 sessions. n=4-6 per group, error bars represent SEM.

Discussion

The latent inhibition effect was successfully produced using the conditioned taste aversion paradigm. However, the impact of RU 24969 on this effect could not be assessed because higher doses of RU 24969 reduced fluid consumption. These results showed that the conditioned taste aversion paradigm was a confounded assay for measuring behavioural responses to RU 24969 under these conditions.

Serendipitously, the results also suggested a more straightforward measure of 5-HT_{1B} activation, that of reduced drinking, or adipsia. This response to RU 24969 had

been alluded to in the literature. For example, RU 24969 non-selectively reduced intake of both water and sweetened ethanol (Silvestre, Palacios, Fernandez, & O'Neill, 1998), responding maintained by water in water-deprived rats (Carli, Invernizzi, Cervo, & Samanin, 1988), and the time spent drinking sweetened condensed milk (Simansky & Vaidya, 1990). To our knowledge there had not been any pharmacological studies to determine whether this decrease in fluid consumption is due to effects at 5-HT_{1A} or 5-HT_{1B} receptors. In many ways the adipsic response to RU 24969 would be a preferable behavioural response to measure, because only one drug exposure is required, and because the effect can be assessed in a relatively short time period. Thus, the next study aimed to determine the parameters of RU 24969-produced adipsia, and the relative contribution of 5-HT_{1A} and 5-HT_{1B} receptor subtypes to this effect.

Chapter 4: Behavioural responses to RU 24969

Parts of this chapter appear in:

Aronsen, Webster, & Schenk. (2014). RU 24969-produced adipsia and hyperlocomotion: Differential role of 5-HT_{1A} and 5-HT_{1B} receptor mechanisms. *Pharmacology, Biochemistry and Behavior, 124*, 1-4. DOI 10.1016/j.pbb.2014.05.008

Aronsen, Bukholt, & Schenk (2016). Repeated administration of the 5-$HT_{1B/1A}$ agonist, RU 24969, facilitates the acquisition of MDMA self-administration: Role of 5-HT_{1A} and 5-HT_{1B} receptor mechanisms. *Psychopharmacology, 233* (8), 1339-1347. DOI 10.1007/s00213-016-4225-x

The previous chapter showed that latent inhibition, assessed using the conditioned taste aversion paradigm, is a confounded behavioural assay due to decreased fluid consumption produced by RU 24969. This decrease in fluid consumption might, however, be a novel response that could be used to characterise RU 24969. This effect had been referred to in the literature, but no study had determined the parameters of this adipsic response to RU 24969, or the contribution of 5-HT_{1A} and 5-HT_{1B} receptors. This was, therefore, one objective of this study.

RU 24969 also produces hyperlocomotion. In contrast to RU 24969-produced adipsia, this behavioural response to RU 24969 has been well studied. RU 24969-produced hyperactivity was not attenuated by depletion of brain 5-HT, suggesting a post synaptic mechanism (Cheetham & Heal, 1993). Studies in mice have generally attributed RU 24969-induced hyperlocomotion to 5-HT_{1B} mechanisms because it was selectively attenuated by pretreatment with 5-HT_{1B}, but not 5-HT_{1A}, receptor antagonists (Cheetham & Heal, 1993; Shanahan et al., 2009). In the rat, however, there is a lack of full parametric analysis of the roles of 5-HT_{1A} or 5-HT_{1B} activation in this behavioural response. For example, the 5-$HT_{1B/1D}$ receptor antagonist, GR 127935, dose-dependently attenuated the hyperactive response to RU 24969 in the Wistar-Kyoto hyperactive rat, but a control strain was not assessed (Chaouloff, Courvoisier, Moisan, & Mormede, 1999). Similarly, GR 127935 blocked the hyperactive response to RU 24969 50-60 minutes after RU 24969 administration (O'Neill & Parameswaran, 1997), while the 5-HT_{1A} receptor antagonists, WAY 100635 and SDX 216-525, but not GR 127935, blocked the hyperactive response to RU 24969 in the first 15 minutes after administration (Kalkman, 1995), before maximal effects of RU 24969 are

evident. Thus, roles of 5-HT_{1A} and 5-HT_{1B} receptors in RU 24969-produced hyperlocomotion have been suggested, but the relative contribution of 5-HT_{1A} and 5-HT_{1B} receptors over the course of RU 24969-produced hyperactivity is not clear.

These studies had 3 aims. Firstly, the adipsic and hyperactive responses to RU 24969 were characterised by administering a range of doses and measuring dose-dependent behavioural responses. Secondly, the relative contributions of 5-HT_{1A} and 5-HT_{1B} receptors to these effects were determined by pretreating rats with a selective 5-HT_{1A} or 5-HT_{1B} receptor antagonist. Lastly, if a behavioural response to RU 24969 was antagonised by a 5-HT_{1A} receptor antagonist the same response was to be tested after administration of the selective 5-HT_{1A} receptor agonist, 8-OH-DPAT. A more selective, well characterised, 5-HT_{1B} receptor agonist was not readily available to us at the time of these experiments, but 8-OH-DPAT has been widely used as a selective 5-HT_{1A} receptor agonist. 8-OH-DPAT has approximately 7000 fold preference for 5-HT_{1A} receptors over 5-HT_{1B} receptors (Hamon, Cossery, Spampinato, & Gozlan, 1986). Therefore, 8-OH-DPAT is a preferable ligand to use when measuring behavioural responses to 5-HT_{1A} receptor activation.

Method

Water consumption

Standard protocol was used (see General Methods). RU 24969 (0.0 – 3.0 mg/kg, s.c.; n = 10 per group) was administered 15 minutes before water bottles were reintroduced. These data provided the dose of RU 24969 that was subsequently used in the antagonist study. Separate groups (n=6-9 per group) were tested in the same manner to assess the contribution of 5-HT_{1A} or 5-HT_{1B} mechanisms. Either the 5-HT_{1A} receptor antagonist, WAY 100635 (0.0, 1.0 mg/kg, s.c.), or the 5-$HT_{1B/1D}$ receptor antagonist, GR 127935 (0.0, 3.0 mg/kg, s.c.) was administered 15 minutes before RU 24969 (1.0 mg/kg, s.c.). These doses were chosen for their documented efficacy in blocking 5-HT_{1A} and 5-HT_{1B} effects, respectively (Acosta et al., 2005; P. J. Fletcher & Korth, 1999b).

Locomotor Activity

Rats were placed in the testing chamber for 30 minutes, followed by an injection of RU 24969 (0.0-3.0 mg/kg, s.c.; n=8 per group), and activity was measured for 45 minutes post-injection. Separate groups (n=6-12 per group) were placed in the activity monitoring chambers and 15 minutes later received either WAY 100635 (0.0,

1.0 mg/kg, s.c.) or GR 127935 (0.0, 3.0 mg/kg, s.c.), followed 15 minutes later by RU 24969 (3.0 mg/kg, s.c.). In order for the data to be directly comparable to the fluid consumption protocol, only data collected from 15-45 minutes following the injection of RU 24969 were analysed.

Separate rats were used to test the hyperactive response to 8-OH-DPAT. Rats were placed in the testing chamber for 30 minutes, followed by an injection of 8-OH-DPAT (0.0, 0.03, 0.1, 0.3, 1.0, 3.0 mg/kg, s.c., n=5-7 per group), and activity was measured for 60 minutes post-injection.

8-OH-DPAT is a selective 5-HT_{1A} receptor agonist but also has appreciable affinity for 5-HT_7 receptors (Bard et al., 1993; Lovenberg et al., 1993). To determine whether 8-OH-DAT-produced hyperactivity was due to 5-HT_{1A} activation we determined the effect of the selective 5-HT_{1A} receptor antagonist, WAY 100635, on 8-OH-DPAT-produced hyperactivity. Rats were placed in the testing chamber and 15 minutes later were injected with WAY 100635 (0, 0.003, 0.3 mg/kg, s.c., n=4-5 per group). Following a further 15 minutes, 8-OH-DPAT (0.3 mg/kg, s.c.) was injected, and activity was measured for an additional 60 minutes.

Data analysis

The effect of RU 24969 on water consumption was assessed using a one-way ANOVA. The effect of RU 24969 on locomotor activity was assessed using a 4 (RU 24969 dose) × 6 (Time after injection) mixed ANOVA with Time as the within subjects factor. The effects of WAY 100635 and GR 127935 on RU 24969-proudced adipsia or hyperlocomotion were assessed using separate 2 (antagonist dose) × 2 (RU 24969 dose) ANOVAs. The effect of 8-OH-DPAT on locomotor activity counts was assessed using a one-way ANOVA. Data for 8-OH-DPAT-produced hyperactivity after administration of WAY 100635 were analysed using a 3 (Dose) × 12 (Time after injection) mixed model ANOVA with Time as the within subjects factor. Post-hoc analyses were conducted using Tukey's HSD method.

Results

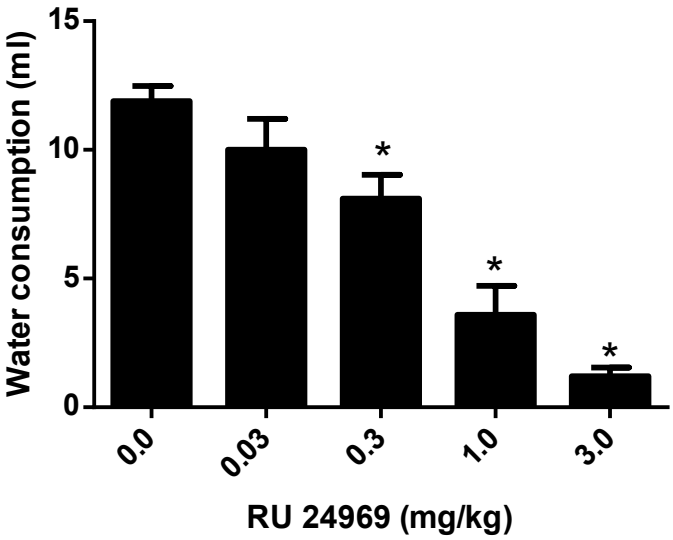


Fig. 4.1 Effect of RU 24969 on water consumption over 30 minutes in water deprived rats. n= 10 per group, error bars represent SEM. *- p<0.05 compared to 0.0 mg/kg dose.

Figure 4.1 shows the effect of RU 24969 on water consumption. ANOVA confirmed an effect of dose (F (4, 45) = 24.56, p<0.001, η_p^2= 0.69), and post hoc Tukey analysis indicated that 0.3, 1.0, and 3.0 mg/kg RU 24969 significantly decreased water consumption (p<0.05). Effects of the antagonists on RU 24969-produced adipsia are presented in Figure 4.2

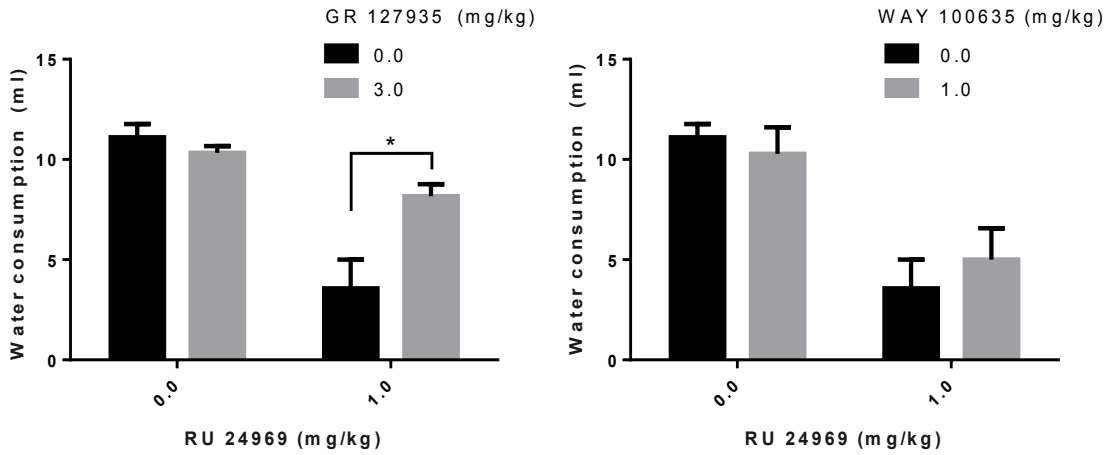


Fig. 4.2 Effect of the 5-$HT_{1B/1D}$ receptor antagonist, GR 127935 (left), or the 5-HT_{1A} receptor antagonist, WAY 100635 (right), on RU 24969-produced adipsia. n=6-9 per group, error bars represent SEM. *- p<0.05.

Analysis of the effect of WAY 100635 (dose RU 24969 × dose WAY 100635) revealed a main effect of RU 24969 (F (1,26) = 26.95, p<.001, η_p^2= 0.51), but no effect of WAY 100635 (F (1,26) = 0.016, *ns*) or an interaction (F (1,26) = 0.83, *ns*). In contrast, analysis of the effect of GR 127935 (dose RU 24969 × dose GR 127935) revealed an effect of GR 127935 (F (1,24) = 4.55, p=0.043, η_p^2= 0.16), an effect of RU 24969 (F (1,24) = 29.44, p<0.001 η_p^2= 0.55) and an interaction (F (1,24) = 9.02, p = 0.006 η_p^2= 0.27). Tukey post hoc comparisons confirmed that GR 127935 significantly reduced RU 24969-produced adipsia (p<0.05).

Figure 4.3 shows that RU 24969 increased locomotor activity (F (3,28) = 8.15, p<0.001 η_p^2= 0.47). There was no effect of Time (F (5,140) = 0.27, *ns*) and no interaction (F (15,140) = 0.45, *ns*). Post hoc Tukey analysis showed the dose of 3.0 mg/kg was the only dose that significantly increased total forward locomotion.

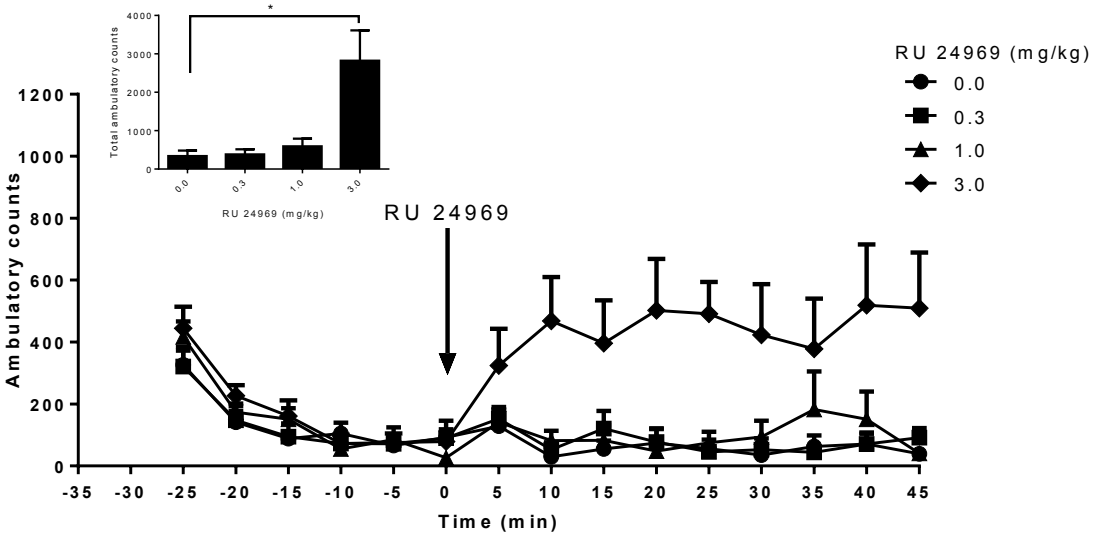


Fig. 4.3 Effect of RU 24969 on locomotor activity. n=8 per group, error bars represent SEM. *- p<0.05.

Effects of the antagonists on RU 24969-produced hyperlocomotion are presented in figure 4.4. GR 127935 failed to alter RU 24969-produced hyperactivity; the effect of GR 127935 (F (1,26) = 0.75, *ns*) and the interaction (F (1,26) = 0.52, *ns*) between the two drugs were not significant. A significant effect of WAY 100635 was found (F (1,36) = 6.73, p = 0.014, η_p^2= 0.16), and an interaction between WAY 100635 and RU 24969 treatment was significant (F (1,36) = 4.44, p = 0.042, η_p^2= 0.11). Tukey post hoc comparisons confirmed that WAY 100635 significantly reduced RU 24969-produced hyperactivity (p<0.05).

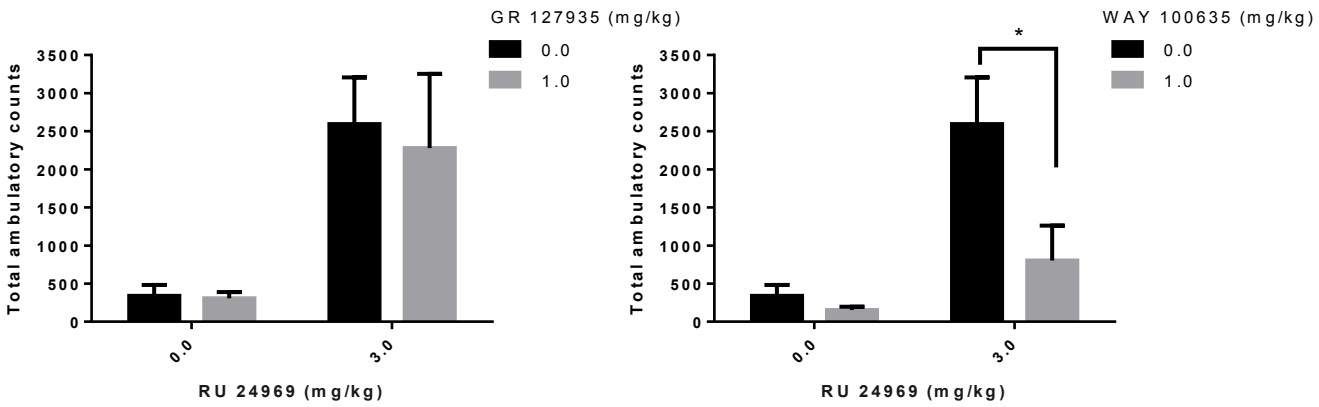


Fig. 4.4 Effect of the 5-$HT_{1B/1D}$ receptor antagonist, GR 127935 (left), or the 5-HT_{1A} receptor antagonist, WAY 100635 (right), on RU 24969-produced hyperactivity. n=6-12 per group, error bars represent SEM. *- $p<0.05$.

8-OH-DPAT dose-dependently increased locomotor activity counts ($F(5,33)=48.63$, $p<0.001$, $\eta_p^2= 0.88$). Post hoc analysis revealed that doses of 0.3, 1.0, and 3.0 mg/kg 8-OH-DPAT significantly increased locomotor activity counts (see fig 4.5).

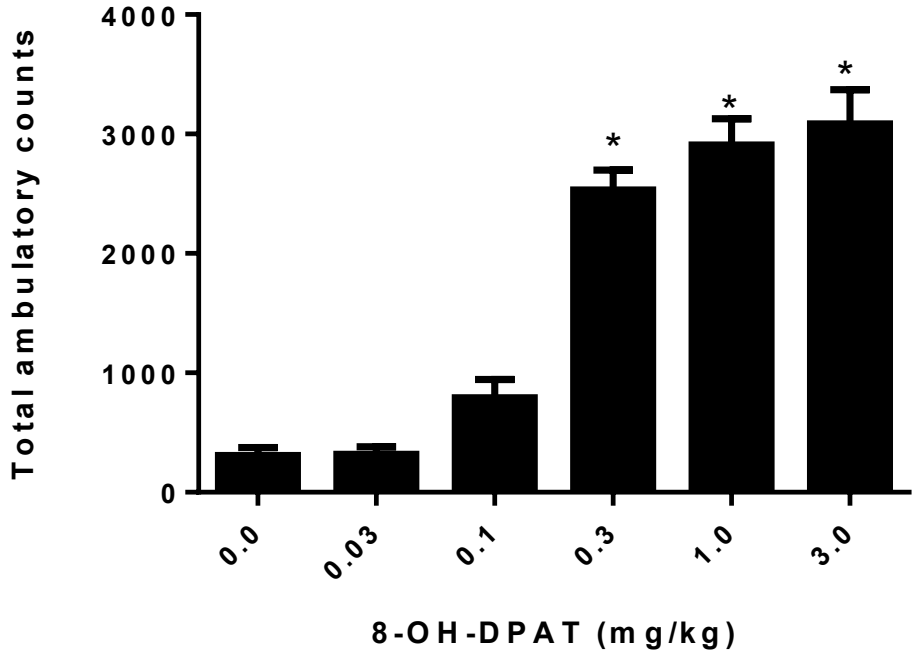


Fig. 4.5 The hyperactive response to 8-OH-DPAT. n=5-7 per group, error bars represent SEM. *- $p<0.05$ compared to 0.0 mg/kg group.

Figure 4.6 (left panel) shows the time course of the effects of WAY 100635 on 8-OH-DPAT-produced hyperactivity. ANOVA showed a significant interaction

between Time after injection and Dose ($F(22,121)=7.66$, $p<0.001$, $\eta_p^2= 0.58$), and significant main effects of Time ($F(11,121)=31.1$, $p<0.001$, $\eta_p^2= 0.74$) and Dose ($F(2,11)=21.5$, $p<0.001$, $\eta_p^2= 0.80$). Post hoc tests revealed a significant decrease in 8-OH-DPAT-produced hyperactivity at Time=5, 10 and 15 minutes following administration of 0.3 mg/kg WAY 100635. The effect of dose is further illustrated in Figure 4.6 (right panel). Post hoc analysis showed a significant decrease in 8-OH-DPAT-produced hyperactivity after the 0.3 mg/kg dose of WAY 100635.

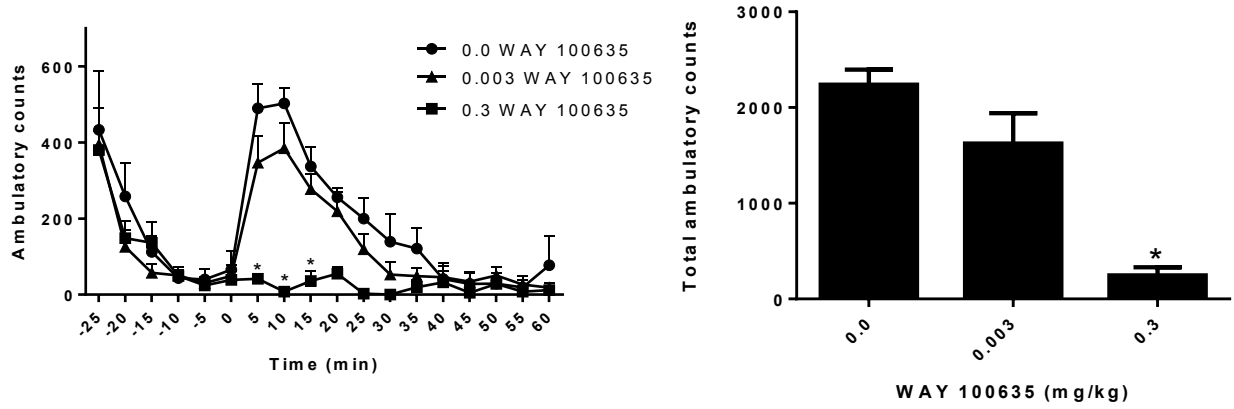


Fig. 4.6 (left panel) Time course of 8-OH-DPAT- (0.3 mg/kg) produced locomotor activity following WAY 100635. (right panel) Effects of WAY 100635 on total locomotor activity following administration of 8-OH-DPAT (0.3 mg/kg). n = 5-6 per group, error bars represent SEM. *- $p<0.05$ compared to WAY 100635 0.0 mg/kg group.

Discussion

The 5-$HT_{1B/1A}$ receptor agonist, RU 24969, dose dependently decreased water consumption and increased locomotor activity. The different potencies of RU 24969 in the two behavioural paradigms were consistent with the differential affinity of RU 24969 for the 5-HT_{1A} and 5-HT_{1B} receptors; RU 24969 has about 6 times greater affinity for the 5-HT_{1B} receptor subtype than the 5-HT_{1A} receptor subtype (Peroutka, 1986). In the behavioural tasks, the lowest dose of RU 24969 that affected fluid consumption was 0.3 mg/kg, while RU 24969-induced hyperlocomotion was only observed after 3.0 mg/kg.

The most convincing evidence of different receptor mechanisms for RU 24969-induced adipsia and hyperlocomotion is that a dose of the 5-$HT_{1B/1D}$ receptor antagonist, GR 127935, which blocked the adipsic effect, failed to alter the

hyperlocomotion effect of RU 24969. Further, a dose of the 5-HT_{1A} receptor antagonist, WAY 100635, which blocked the locomotor activating effects failed to alter the adipsic response to RU 24969. The failure of these doses of WAY 100635 and GR 127935 to alter one behaviour cannot be due to ineffective dosing since the other behavioural effect of RU 24969 was attenuated by the same dose of the antagonist. It might be argued that the decrease in drinking reflects the hyperactive response to RU 24969 that might have interfered with the ability to remain at the drinking spout. This is unlikely since the reduction of fluid consumption was produced by doses of RU 24969 lower than those that increased locomotor activity.

Rather, the data are consistent with the idea that RU 24969-induced adipsia in rats is mediated by 5-HT_{1B}, but not 5-HT_{1A}, mechanisms, and that RU 24969-induced hyperactivity in rats is mediated by 5-HT_{1A}, but not 5-HT_{1B}, mechanisms. Another study (Chaouloff et al., 1999) showed that GR 127935 attenuated RU 24969-induced hyperactivity in Wistar-Kyoto hyperactive rats. This effect might have been non-selective since GR 127935 in that study also decreased basal activity levels. We failed to observe either of these effects in Sprague-Dawley rats, raising the possibility that there are strain differences in the response to the antagonist. Another study (O'Neill & Parameswaran, 1997) also showed that RU 24969-induced hyperactivity was decreased by GR 127935, but this effect was produced 50-60 minutes after RU 24969 administration. This finding raises the possibility that there is an effect of GR 127935 that emerges at time points later than those tested in the present study. In accordance with our conclusion that RU 24969-induced hyperlocomotion is due to this agonist's affinity for the 5-HT_{1A} receptor, 5-HT_{1A} receptor agonists are known to produce hyperlocomotion (Kalkman & Soar, 1990; Tricklebank, Forler, & Fozard, 1984).

GR 127935 has affinity for a number of serotonin receptors (Centurión et al., 2000; Price et al., 1997; Watson, Burton, Price, Jones, & Middlemiss, 1996), but it is noteworthy that the 5-$HT_{1B/1D}$ receptor antagonist is at least 60 times more selective for the 5-HT_{1B} receptor than any of the other receptors that RU 24969 has notable affinity for. Therefore, the most likely explanation for the reversal of RU 24969-induced adipsia by GR 127935 is antagonism of the 5-HT_{1B} receptor.

These results, along with others from the literature, raised the possibility that a 5-HT_{1A} receptor agonist would also produce reliable hyperlocomotion. Given the high selectivity of 8-OH-DPAT for the 5-HT_{1A} receptor, as well as the low affinity for the 5-HT_{1B} receptor (Hamon et al., 1986; Peroutka, 1986), 8-OH-DPAT-produced

hyperactivity would be a preferable behavioural measure of 5-HT_{1A} activation. Thus, the last study in this chapter aimed to determine the parameters under which 8-OH-DPAT produces hyperactivity, and the role of 5-HT_{1A} receptor activation in this effect.

8-OH-DPAT dose dependently increased locomotor activity, with a maximal effect around 1.0 mg/kg. This hyperactive response to the 5-HT_{1A} receptor agonist was reversed by the 5-HT_{1A} receptor antagonist, WAY 100635. It is unlikely that this reversal by WAY 100635 was due to a non-specific decrease in locomotor activity because the higher (1.0 mg/kg) dose of WAY 100635 used in the previous experiment had no significant effect on locomotor activity. Therefore, these results suggest that 8-OH-DPAT-produced hyperactivity is due to 5-HT_{1A} receptor activation.

This result is in accordance with other studies that have investigated the hyperlocomotor response to 8-OH-DPAT. Hyperactivity produced by 8-OH-DPAT was attenuated by the 5-HT_1 receptor antagonist, pindolol (Ahlenius & Salmi, 1995; Hillegaart, Estival, & Ahlenius, 1996), suggesting a 5-HT_{1A} receptor mechanism. 8-OH-DPAT produced hyperlocomotion was not attenuated by depletion of monoamines via reserpine treatment, suggesting this behavioural response to 8-OH-DPAT is not due to alterations in synthesis and/or release of 5-HT via autoreceptor-mediated effects, but instead action on post-synaptic 5-HT_{1A} receptors (Ahlenius & Salmi, 1995; Mignon & Wolf, 2002).

Together, these data show that adipsia and hyperlocomotion provide dissociable behavioural measures of RU 24969 that are produced by 5-HT_{1B} and 5-HT_{1A} activation, respectively. Furthermore, 8-OH-DPAT-produced hyperactivity may be a preferable measure of 5-HT_{1A} activation, because of the selectivity of 8-OH-DPAT for the 5-HT_{1A} receptor. Because RU 24969-produced adipsia and 8-OH-DPAT-produced hyperactivity are selective responses to 5-HT_{1B} and 5-HT_{1A} receptor activation, respectively, these procedures provide straight-forward assays of 5-HT_{1A} and 5-HT_{1B} receptor function, and so will be used in the following chapters.

Chapter 5: Effects of repeated administration of the 5-$HT_{1B/1A}$ receptor agonist, RU 24969, on the acquisition of MDMA self-administration

Parts of this chapter appear in:

Aronsen, Bukholt, & Schenk (2016). Repeated administration of the 5-$HT_{1B/1A}$ agonist, RU 24969, facilitates the acquisition of MDMA self-administration: Role of 5-HT_{1A} and 5-HT_{1B} receptor mechanisms. *Psychopharmacology, 233* (8), 1339-1347. DOI 10.1007/s00213-016-4225-x

As was explained in the General Introduction, self-administration of a range of substances is inhibited by increased synaptic 5-HT (Loh & Roberts, 1990; Ritz & Kuhar, 1989; Rothman et al., 2005; Z. Wang & Woolverton, 2007; Wee et al., 2005). A recent study (Bradbury et al., 2014) tested the idea that MDMA-produced 5-HT release might be inhibitory to MDMA self-administration and attempted to explain both the long latency to acquisition, and the small proportion of rats that meet acquisition criteria. The MDMA-produced increase in synaptic 5-HT was measured by *in vivo* microdialysis before MDMA self-administration began. As has been observed in many studies from the Schenk lab (Colussi-Mas et al., 2010; Schenk et al., 2012; Schenk et al., 2003; Schenk et al., 2007), about 50% of the rats acquired MDMA self-administration. Of interest, MDMA-stimulated 5-HT release was lower for the rats that ultimately met the acquisition criteria, suggesting an inhibitory role of MDMA-produced 5-HT release on the acquisition of MDMA self-administration. This idea was experimentally tested by determining the effect of a neurotoxic, 5,7-DHT, lesion on MDMA self-administration. The lesion reduced 5-HT levels by up to 67%, and greatly facilitated the acquisition of MDMA self-administration; while approximately 50% of control rats met acquisition criteria, 100% of the lesion group acquired. Furthermore, of the control group that acquired, 50% met the criterion within 14 sessions, while only 6 sessions were required for 50% of the lesion group to meet the criterion.

These findings strengthen the idea that variability in the acquisition of MDMA self-administration is due to variability in sensitivity to MDMA-produced 5-HT release. Specifically, 5-HT has an inhibitory impact on MDMA self-administration. A question remains as to the mechanism for this inhibitory effect of 5-HT on the acquisition of MDMA self-administration. One possibility is that high levels of synaptic 5-HT produced by MDMA during initial self-administration sessions led to

neuroadaptive changes in 5-HT receptor mechanisms that modulate responses associated with the acquisition of self-administration.

As outlined in the General Introduction, 5-HT_{1A} and 5-HT_{1B} receptors regulate dopaminergic neurotransmission. Because self-administration is associated with increased dopamine neurotransmission, activation of 5-HT_{1A} and 5-HT_{1B} receptors might be expected to impact self-administration. Of particular interest, activation of 5-HT_{1A} receptors attenuated amphetamine-induced increases in extracellular dopamine levels (Ichikawa et al., 1995; Kuroki et al., 1996) and, as would therefore be expected, a range of 5-HT_{1A} receptor agonists have been shown to inhibit self-administration (Müller et al., 2007). On the other hand, 5-HT_{1B} receptor agonists potentiated the increase in extracellular dopamine produced by cocaine or ethanol (O'Dell & Parsons, 2004; Parsons et al., 1999; Yan et al., 2005), and generally enhanced self-administration, producing leftward shifts in the self-administration dose-response curves for cocaine and GBR 12909 (Parsons et al., 1996, 1998; Pentkowski et al., 2009; Przegaliñski et al., 2007).

A wealth of data indicate a role of 5-HT_{1A} and 5-HT_{1B} receptor subtypes in the maintenance of self-administration (P. J. Fletcher, Azampanah, & Korth, 2002; Neisewander, Cheung, & Pentkowski, 2014; Parsons et al., 1998; Peltier & Schenk, 1993; Przegaliñski et al., 2007) but the role in the acquisition of self-administration has received far less attention. Given that self-administration is driven by increases in dopamine neurotransmission, and that the acquisition of MDMA self-administration was enhanced by a neurotoxic, 5,7-DHT lesion, I wanted to determine whether this facilitation of MDMA self-administration was due to decreased activation of 5-HT_{1A} or 5-HT_{1B} receptors. If so, it should be possible to manipulate receptor mechanisms via repeated agonist or antagonist exposure and to determine the effect on acquisition of MDMA self-administration.

Tolerance to RU 24969-produced hyperactivity was produced following repeated exposure to the 5-$HT_{1B/1A}$ receptor agonist (Oberlander, Demassey, Verdu, Van de Velde, & Bardelay, 1987). As outlined in the previous chapter, we have recently shown that RU 24969-produced hyperactivity in rats is due to activation of 5-HT_{1A}, but not 5-HT_{1B}, receptors (Aronsen, Webster, & Schenk, 2014), suggesting that behavioural tolerance reflects a down-regulation of this receptor subtype. The effect of RU 24969 pretreatment on 5-HT_{1B} receptor mechanisms has not been specifically measured, but RU 24969-produced adipsia provides a means of addressing this

question (Aronsen et al., 2014). Therefore, in the present study we determined the effect of repeated exposure to RU 24969 on the acquisition of MDMA self-administration, and on RU 24969-produced adipsia. In order to assess the effect on 5-HT_{1A} receptor mechanisms we also measured hyperactivity in response to the selective 5-HT_{1A} receptor agonist, 8-OH-DPAT.

Method

Male Sprague-Dawley rats (see General Methods) were used. For rats that underwent self-administration testing, an intravenous catheter was implanted, as outlined in the General Methods.

RU 24969 pretreatment

RU 24969 pretreatment began once pre-surgery weight had been obtained. RU 24969 (3.0 mg/kg, s.c.), or the saline vehicle (1.0 ml/kg), was administered in the home cage daily at 0900hr and 1600hr, for three consecutive days. This protocol was adapted from that used in earlier studies (Callaway & Geyer, 1992; Oberlander et al., 1987) to utilise a dose of RU 24969 that we have previously shown produces both hyperactivity and adipsia (Aronsen et al., 2014).

Acquisition of MDMA self-administration

Self-administration sessions began the day after the last administration of RU 24969. Self-administration was conducted during 2 hour daily sessions, 6 days per week. Each self-administration session began with an experimenter-delivered infusion of drug to clear the line of heparinised saline solution. Thereafter, depression of the active lever produced an infusion of MDMA (1.0 mg/kg/infusion) according to an FR1 schedule. Responses on the active and inactive levers were recorded. Every seventh day catheters were infused with sodium pentobarbital (20.0 mg/kg, i.v.). Failure to demonstrate an immediate loss of the righting reflex suggested a loss of catheter patency and the rat was excluded from the study. Catheter patency was lost in 4 rats (3 RU 24969 pretreated, 1 saline pretreated), and 3 rats in the RU 24969 pretreatment group self-administered lethal doses of MDMA, resulting in final sample sizes of 9 and 8 for the RU 24969 and saline pretreated groups, respectively. Self-administration testing continued for each rat until a total of 90 infusions (90.0 mg/kg) had been self-administered, or for 25 days, whichever came first. This acquisition criterion is the same as has been used previously in our laboratory (Bradbury et al., 2014; Oakly, Brox, Schenk, & Ellenbroek, 2014).

Water consumption and locomotor activity

Separate groups of rats were tested to determine the effects of RU 24969 pretreatment on RU 24969-produced adipsia, or 8-OH-DPAT-produced hyperactivity. The standard water consumption protocol was used (see General Methods), with water bottles removed the day after the last RU 24969 pretreatment administration. RU 24969 (0.0, 1.0, 3.0 mg/kg, s.c., n= 6-8 per group) was administered 15 minutes before water bottles were reintroduced. These doses were chosen based on our previous study (Aronsen et al., 2014) that suggested that adipsia following administration of these doses of RU 24969 was due to 5-HT_{1B} receptor activation.

The effect of the selective 5-HT_{1A} receptor agonist, 8-OH-DPAT, on locomotor activity was assessed 2 days after the last administration of RU 24969, in order to match the delay between pretreatment and the test for RU 24969-induced adipsia. Rats were placed in the testing chamber (see General Methods) for 30 minutes, followed by an injection of 8-OH-DPAT (0.0, 0.1, 0.3 mg/kg, s.c., n=4-7 per group), and activity was measured for 60 minutes post-injection.

To investigate the possibility that RU 24969 pretreatment affected dopaminergic mechanisms, locomotor activity produced by the dopamine releasing agent, d-amphetamine, was assessed 2 days after the last administration of RU 24969. Rats pretreated with either RU 24969, or vehicle, were placed in the testing chamber (see General Methods) for 30 minutes, followed by an injection of d-amphetamine (0.5 mg/kg, i.p., n=10 per group), and activity was measured for 60 minutes post-injection. This dose was chosen because it has previously been used in our laboratory to illustrate dopaminergic sensitisation (Bradbury, Gittings, & Schenk, 2012).

Data analysis

Acquisition of self-administration was compared between pretreatment groups with a survival analysis, using the log-rank test to compare Kaplan-Meier survival estimates (Kaplan & Meier, 1958). Right-censoring was applied to data from rats that did not acquire within the 25 day cut-off period.

RU 24969-produced adipsia was analysed with a 2 (Pretreatment) × 3 (Dose of RU 24969) ANOVA. Effects of each dose of 8-OH-DPAT on locomotor activity were analysed by individual 2 (Pretreatment) × 12 (Time after injection) mixed model ANOVAs with Time as the within subjects factor. Total activity counts as a function of Dose of 8-OH-DPAT and RU 24969 pretreatment were analysed using a 2 (Pretreatment) × 3 (8-OH-DPAT Dose) ANOVA. The locomotor responses to d-

amphetamine was analysed with a 2 (pretreatment) × 12 (Time after injection) mixed model ANOVA with Time as the within subjects factor.

Results

Figure 5.1 shows survival curves for the acquisition of self-administration for saline- or RU 24969-treated groups. RU 24969 pretreatment produced a significant increase in the probability of acquiring MDMA self-administration (χ^2 (1) = 12.21, p<0.01). Of the control group that met the acquisition criterion, 50% met the criterion within 17 sessions, whereas 50% of RU 24969 pretreatment group met the acquisition criterion within 10 sessions. It is noteworthy that three rats in the RU 24969 pretreatment group self-administered lethal doses of MDMA (>20 mg/kg) during the first self-administration session and therefore additional data from these rats could not be obtained. The high intake during the first self-administration session for these 3 rats supports the other data suggesting RU 24969 pretreatment enhanced the initial reinforcing effects of MDMA.

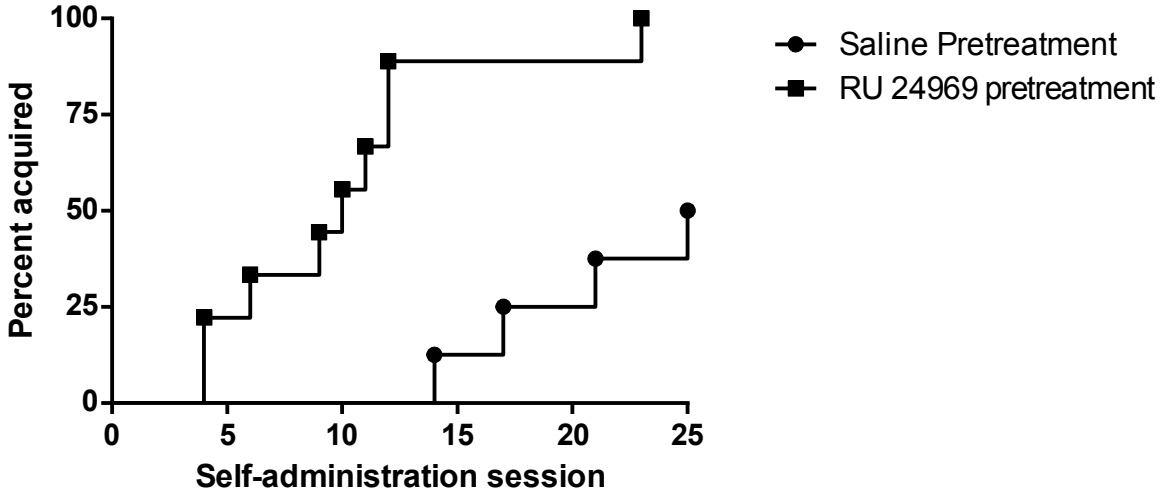


Fig. 5.1 Cumulative percentage of rats that met the criterion for acquisition of MDMA self-administration in the RU 24969 (squares, n=9) and saline (circles, n=8) pretreatment groups.

Figure 5.2 (left panel) shows the effect of RU 24969 pretreatment on RU 24969-produced adipsia. There was a significant interaction between Pretreatment and Dose (F(2,37)=7.85, p=0.01, η_p^2= 0.30) and a significant effect of Dose (F(2,37)=53.55, p<0.01, η_p^2= 0.74). Post hoc tests confirmed a significant difference in

the adipsic response between the RU 24969 and saline pretreatment groups following 0.0 mg/kg, and 3.0 mg/kg RU 24969. Since there was a decrease in basal water consumption produced by repeated RU 24969 treatment, the data were further analysed by expressing drug effects as a percentage of baseline. These data are presented in Figure 5.2 (right panel). A 2×2 (Pretreatment × Dose) ANOVA revealed a significant effect of Pretreatment ($F(1,27)=20.40$, $p<0.01$, $\eta_p^2= 0.43$).

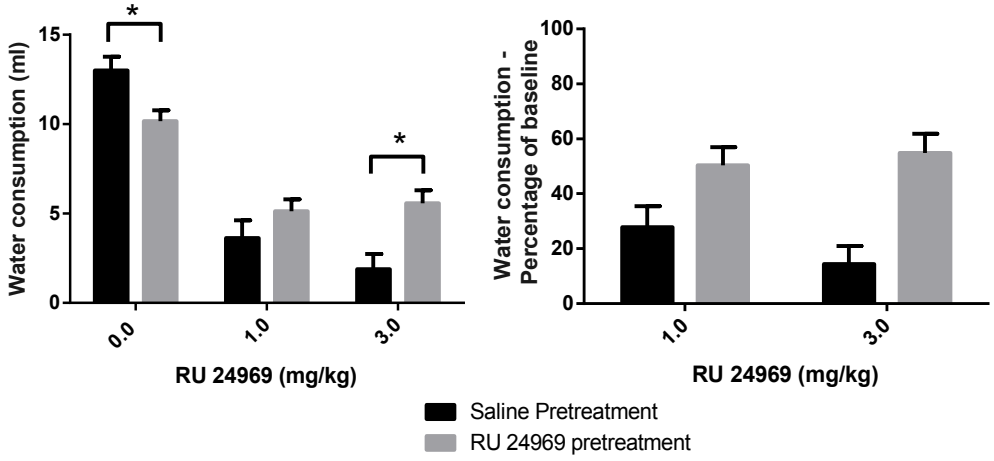


Fig. 5.2 (Left panel) The adipsic response to RU 24969 after repeated exposure to RU 24969 (grey bars) or saline (black bars). (Right panel) Percentage of baseline water intake as a function of RU 24969 dose for RU 24969 and saline pretreated groups. n=6-8 per group. Figures represent the mean + SEM. * - $p<0.05$.

Locomotor activity produced by the various doses of 8-OH-DPAT as a function of RU 24969 pretreatment is shown in Figure 5.3. There were no differences between groups following the 0.0 mg/kg 8-OH-DPAT dose. The data from 0.1 mg/kg 8-OH-DPAT dose produced a Time × Pretreatment interaction ($F(11,110)=4.06$, $p<0.01$, $\eta_p^2= 0.29$) and main effects of Time ($F(11,110)=19.8$, $p<0.01$, $\eta_p^2= 0.66$) and Pretreatment ($F(1,10)=17.6$, $p<0.01$, $\eta_p^2= 0.64$). Post hoc tests revealed significant decreases in activity during Time=10 and 15 minutes following the injection. There was a significant Time × Pretreatment interaction ($F(11,121)=2.77$, $p<0.01$, $\eta_p^2= 0.20$) and main effects of Time ($F(11,121)=62.5$, $p<0.01$, $\eta_p^2= 0.85$) and Pretreatment ($F(1,11)=7.45$, $p<0.05$, $\eta_p^2= 0.40$) for the 0.3 mg/kg 8-OH-DPAT groups. Post-hoc tests revealed a significant decrease in activity at Time=25 minutes. Analysis of total activity counts as a function of Dose and Pretreatment showed a main effect of 8-OH-

DPAT Dose ($F(2,27)=46.0$, $p<0.01$, $\eta_p^2= 0.77$) and a main effect of Pretreatment $F(1,27)=19.5$, $p<0.01$, $\eta_p^2= 0.42$).

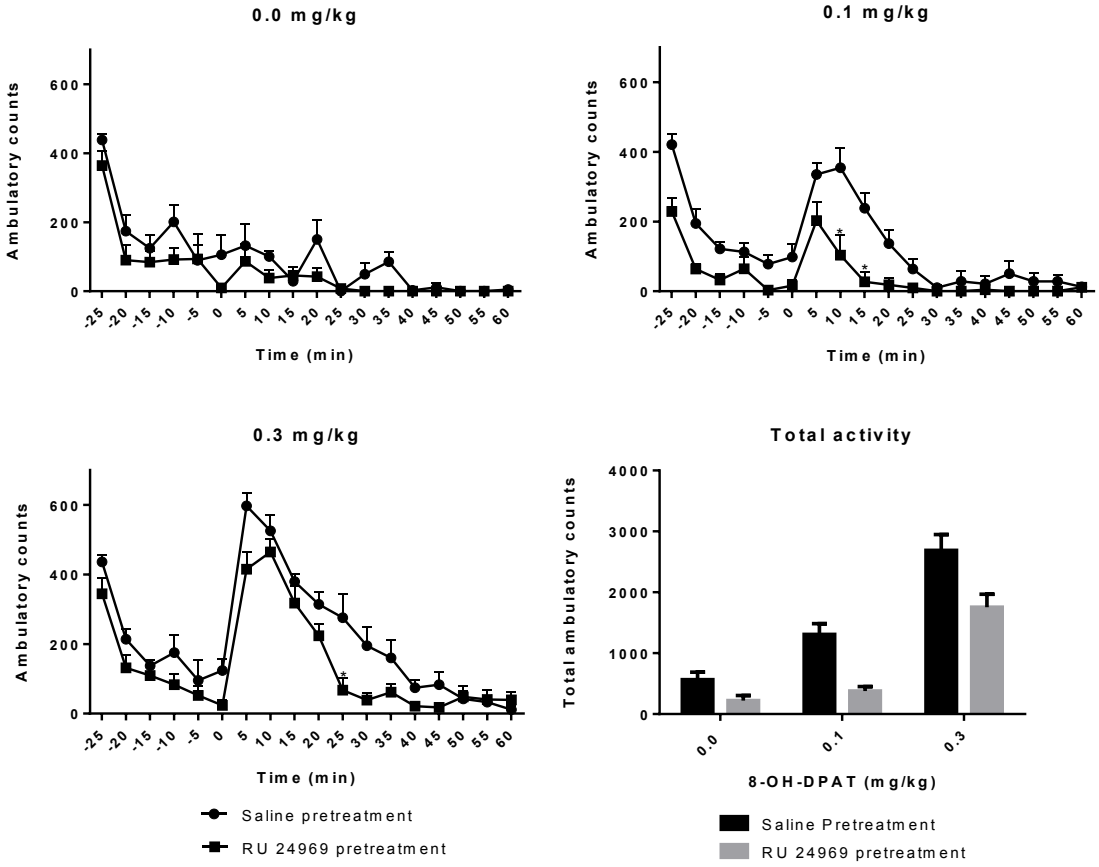


Fig 5.3. Locomotor activating effects of 8-OH-DPAT (Top left – 0 mg/kg, top right – 0.1 mg/kg, bottom left – 0.3 mg/kg, bottom right – totals) as a function of RU 24969 or saline pretreatment. n = 4-7 per group. Symbols represent the mean + SEM. *- $p<0.05$.

During testing of the hyperactive response to d-amphetamine, one rat in the RU 24969 pretreatment group jumped out of the locomotor activity chamber during testing, and so was excluded from analyses. The final sample size for this group was therefore 9. Figure 5.4 (left panel) shows the locomotor response to d-amphetamine for both pretreatment groups over time. ANOVA showed no significant effect of Pretreatment ($F(1,17)=0.19$, $p=0.67$) and no interaction between Pretreatment and Time ($F(11,187)=0.29$, $p=0.99$). Total locomotor activity counts after d-amphetamine injection are shown in figure 5.4 (right panel). As indicated in the previous analysis of variance, there was no effect of pretreatment.

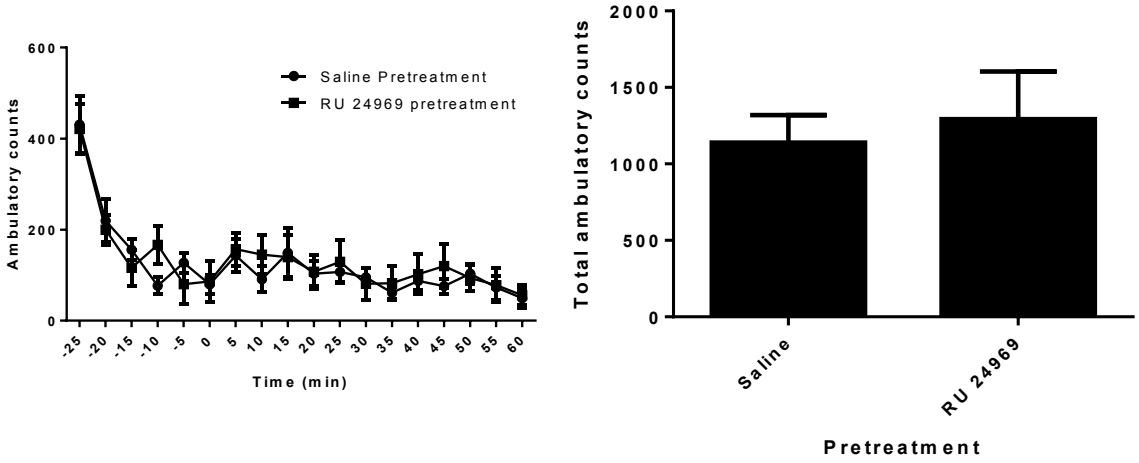


Fig. 5.4 The locomotor response to d-amphetamine (0.5 mg/kg) after pretreatment with either RU 24969 or saline. n=9-10 per group.

Discussion

Pretreatment with RU 24969 decreased the latency to acquisition of MDMA self-administration, and increased the proportion of rats that acquired MDMA self-administration. The leftward shift in the acquisition curve for self-administration might reflect a sensitised reinforcing effect since higher doses of drug have also been shown to decrease the latency to acquisition of self-administration (Carroll & Lac, 1997; Schenk & Partridge, 2000).

A remarkable consequence of pretreatment with RU 24969 was the substantial increase in the proportion of rats that met the criterion for acquisition of MDMA self-administration. As we have previously reported (Bradbury et al., 2014; Schenk et al., 2012), 50% of control rats met the criterion within the 25 day cut-off period. Thus, some rats appear to be inherently more or less sensitive to the reinforcing effects of MDMA. Following RU 24969 pretreatment, however, all of the rats met the criterion for acquisition of MDMA self-administration within the limits of the study (25 test sessions). We have suggested that the initial resistance to self-administration can be overcome by limiting the impact of 5-HT since a similar increase in the percentage of subjects that acquired MDMA self-administration was produced following neurotoxic 5,7-DHT lesions in rats (Bradbury et al., 2014) and in 5-HT transporter knock-out rats (Oakly et al., 2014).

In order to assess the impact of more specific 5-HT mechanisms on the acquisition of MDMA self-administration, the present study repeatedly administered the 5-$HT_{1B/1A}$ receptor agonist, RU 24969, as a pretreatment in an attempt to down-

regulate 5-HT_{1A} and 5-HT_{1B} receptors. We determined effects of the pretreatment by measuring behavioural responses that have been attributed to either 5-HT_{1B} (RU 24969-produced adipsia (Aronsen et al., 2014)) or 5-HT_{1A} (8-OH-DPAT-produced hyperactivity (Hillegaart et al., 1996)) mechanisms.

As previously reported (Aronsen et al., 2014), RU 24969 produced dose-dependent adipsia. The dose-response curve for this response is relatively narrow; minimal effects were produced following administration of 0.3 mg/kg and maximal effects were produced following administration of 3.0 mg/kg (Aronsen et al., 2014). RU 24969 pretreatment decreased basal water consumption and when this was accounted for, RU 24969 pretreatment decreased the subsequent RU 24969-produced adipsic response. These findings are consistent with a rightward shift in the dose-response curve and suggest a down-regulation of 5-HT_{1B} receptors. 5-HT_{1B} receptor down-regulation has previously been evidenced by decreased mRNA levels (Chennaoui et al., 2001; Hiroi & Neumaier, 2009) or decreased binding density (Kindlundh et al., 2003; Suzuki, Han, & Lucas, 2010), both of which could explain the present behavioural data.

RU 24969 pretreatment also shifted the dose-response curve for 8-OH-DPAT-produced hyperactivity to the right; the most pronounced effect of pretreatment was on hyperactivity produced by the lowest does of 8-OH-DPAT tested. This might explain why a similar pretreatment with RU 24969 failed to alter hyperactivity produced by a higher dose of 1.25 mg/kg 8-OH-DPAT (Oberlander et al., 1987).

Although 8-OH-DPAT has appreciable affinity for the 5-HT_7 receptor (Bard et al., 1993; Lovenberg et al., 1993), results from the previous chapter showed that hyperactivity produced by 8-OH-DPAT was attenuated by the selective receptor antagonist, WAY 100635, confirming a 5-HT_{1A} receptor mechanism. Of interest, a similar RU 24969 pretreatment regimen also reduced the locomotor response to RU 24969 (Callaway & Geyer, 1992), a behavioural response that we have attributed to 5-HT_{1A} receptor activation (Aronsen et al., 2014). Therefore, these findings are consistent with a down-regulation of 5-HT_{1A} receptors following RU 24969 pretreatment. 5-HT_{1A} down-regulation has been shown via decreased agonist-stimulated binding of [^{35}S]GTPγS to G proteins (Fuss et al., 2013; Hensler, Vogt, & Gass, 2010), decreased receptor binding densities or immunoreactivity (Fuss et al., 2013; Gui et al., 2011), decreased 5-HT_{1A} mRNA (S. Wang et al., 2009), and

decreased protein levels (Iyo et al., 2009; S. Wang et al., 2009). It would be of great interest to determine which, if any, of these mechanisms can explain the present data.

The available literature is consistent with the idea that MDMA self-administration, like self-administration of other drugs of abuse, progresses as a result of sensitised dopamine and desensitised 5-HT responses. Thus, repeated exposure to MDMA increased dopamine (Colussi-Mas et al., 2010; Kalivas, Duffy, & White, 1998) and decreased 5-HT (Baumann, Clark, Franken, et al., 2008; Reveron et al., 2010; Shankaran & Gudelsky, 1999) synaptic output, as measured by *in vivo* microdialysis, dopamine antagonists reduced MDMA self-administration (Brennan, Carati, Lea, Fitzmaurice, & Schenk, 2009; Daniela, Brennan, Gittings, Hely, & Schenk, 2004), and dopamine, but not 5-HT, agonists potentiated drug-seeking following extinction of MDMA self-administration (Schenk, Gittings, & Colussi-Mas, 2011).

MDMA preferentially releases 5-HT and the ensuing activation of post-synaptic receptors impacts dopamine release, providing potential mechanisms for the enhanced dopamine response. In this study, both 5-HT_{1B} and 5-HT_{1A} receptor mechanisms were down regulated, as measured by behavioural assays. Given the selectivity of RU 24969 for 5-$HT_{1A/1B}$ receptors it is unlikely that alterations in a different receptor mechanism underlies the facilitated acquisition of self-administration found in the present study.

Because activation of 5-HT_{1B} receptors enhanced extracellular dopamine concentrations (Galloway et al., 1993; Hållbus et al., 1997; Iyer & Bradberry, 1996; O'Dell & Parsons, 2004; Yan & Yan, 2001a; Yan et al., 2004) it is possible that repeated administration of RU 24969 sensitised dopamine neurons independently of the effect on 5-HT_{1A} and 5-HT_{1B} receptors. A sensitised dopamine response to MDMA would be expected to facilitate the acquisition of MDMA self-administration. This seems unlikely, however, because RU 24969 pretreatment had no effect on amphetamine-produced hyperactivity. Although the amphetamine dose was chosen based on other sensitisation studies, it is possible that a sensitised dopamine response would have been observed if higher doses of amphetamine had been tested.

Activation of 5-HT_{1B} receptors enhanced basal dopamine neurotransmission (Alex & Pehek, 2007) and the dopaminergic response to drugs of abuse (O'Dell & Parsons, 2004; Parsons et al., 1999; Yan et al., 2005), so the down-regulation of these receptor mechanisms, which would be expected to decrease MDMA-produced

dopamine, cannot easily explain the facilitated self-administration. On the other hand, a wealth of data suggest that activation of 5-HT_{1A} receptors is inhibitory to cocaine self-administration (Müller et al., 2007), possibly via inhibition of dopamine release (Ichikawa & Meltzer, 2000). Therefore, a down-regulation of this receptor subtype might be expected to disinhibit MDMA-produced dopamine, leading to more rapid acquisition of self-administration due to increased reinforcing effects. This might also explain the facilitated acquisition of MDMA self-administration in serotonin transporter knockout rats (Oakly et al., 2014), since this manipulation also desensitised 5-HT_{1A} receptor mechanisms (Homberg et al., 2008).

5-HT_{1A} receptors are widely localised in brain and are well-positioned to modulate activity in a large number of brain systems (Aznar, Qian, Shah, Rahbek, & Knudsen, 2003). Of importance, these receptors are localised on tyrosine hydroxylase immunoreactive cells in the VTA (Doherty & Pickel, 2001) and also in dopamine terminal regions in the NAc (Alex & Pehek, 2007). Systemic administration of 8-OH-DPAT inhibited amphetamine-produced dopamine release in the NAc (Ichikawa et al., 1995). The down-regulation produced by RU 24969 pretreatment would, therefore, be expected to disinhibit stimulated dopamine. Similar studies have not been conducted using MDMA, but this mechanism could explain the facilitated acquisition of self-administration.

The acquisition of self-administration is also influenced by factors in addition to the initial reinforcing effects of the drug and some of these factors are modified by 5-HT_{1A} receptor mechanisms. As explained in the General Introduction, increased impulsivity, anxiety, or learning, could be expected to facilitate the acquisition of MDMA self-administration.

5-HT_{1A} activation increased behavioural measures of impulsivity (Carli & Samanin, 2000). However, individual variability in impulsivity did not predict latency to acquisition of MDMA self-administration (Bird & Schenk, 2013). This might be because the impulsive response to 5-HT_{1A} receptor agonists is due to autoreceptor activation (Carli et al., 2006; Lladó-Pelfort et al., 2010; Winstanley et al., 2003). We have previously shown that repeated exposure to MDMA failed to alter 5-HT_{1A} autoreceptor mechanisms (Schenk et al., 2013). Therefore, alterations in 5-HT_{1A}-mediated impulsivity are unlikely to have impacted the present results.

A down-regulation of 5-HT_{1A} receptors would be expected to reduce anxiety produced by MDMA, because 5-HT_{1A} receptor activation is anxiogenic (Cheeta et al.,

2000a, 2000b; File et al., 1996; File et al., 2000; Solati et al., 2011). However, higher levels of anxiety have been associated with self-administration (Dilleen et al., 2012; Homberg et al., 2002; Spanagel et al., 1995). Therefore an attenuation of 5-HT_{1A} receptor-produced anxiety would not explain the facilitated acquisition of MDMA self-administration.

Reliable self-administration is often facilitated via Pavlovian conditioning processes by pairing delivery of the drug reinforcer with a discrete, discriminative stimulus, like a light, as was done in the present study (Di Ciano & Everitt, 2004). As explained in the General Introduction, strengthening of stimulus/reward associations is markedly inhibited by administration of 5-HT_{1A} receptor agonists (Blair et al., 2004; Frick et al., 2015; Winsauer et al., 1999). These findings raise the possibility that activation of post synaptic 5-HT_{1A} receptors pursuant to MDMA-stimulated 5-HT release limits the acquisition of MDMA self-administration, in some subjects, by interfering with associative learning. If so, our data suggest that this effect is mitigated by exposure to a regimen of RU 24969 pretreatment that down-regulated these receptor mechanisms, thereby facilitating MDMA self-administration as indicated by both a leftward and upward shift in the self-administration acquisition curves. This idea could be tested by administering the same RU 24969 pretreatment as was used in this study and assessing learning in a stimulus/reward association task. If RU 24969 pretreatment facilitated learning in such a task it would strengthen the claim that the facilitation of MDMA self-administration seen in the present study was associated with enhanced learning.

Chapter 6: Predicting the acquisition of MDMA self-administration

In the previous chapter, a manipulation that down-regulated 5-HT_{1A} and 5-HT_{1B} receptors also greatly facilitated the acquisition of MDMA self-administration. As outlined in the discussion, it is possible that a down-regulation of these receptors could explain the facilitated acquisition. However, the correlational nature of that study makes it impossible to ascertain the role of alterations in 5-HT_{1A} and/or 5-HT_{1B} receptor mechanisms in the acquisition of MDMA self-administration.

There is substantial evidence that the magnitude of MDMA-produced 5-HT release predicts the latency to acquire MDMA self-administration. A question remains as to what the mechanism underlying this effect might be. The results from the previous chapter raise the possibility that this mechanism involves individual variability in 5-HT_{1A} and/or 5-HT_{1B} receptor-mediated effects.

Some evidence suggests that activation of these receptor subtypes modulates dopamine neurotransmission, providing a potential mechanism. For example, the 5-HT_{1A} receptor gonist, 8-OH-DPAT, inhibited amphetamine-produced increases in extracellular dopamine (Ichikawa et al., 1995; Kuroki et al., 1996). Thus, activation of 5-HT_{1A} receptors by MDMA-stimulated 5-HT would be expected to inhibit dopamine release. If so, this might explain why MDMA is, at least initially, not a very efficacious reinforcer. Activation of 5-HT_{1A} receptors also impaired learning in a range of operant tasks (Blair et al., 2004; Frick et al., 2015; Meneses, 2007; Winsauer et al., 1999). As a result, 5-HT_{1A} activation during MDMA self-administration could inhibit learning processes associated with the acquisition of self-administration. Therefore, subjects with higher sensitivity to 5-HT_{1A} receptor activation may be less likely to acquire MDMA self-administration, due to inhibition of dopamine release, and/or impaired ability to learn the operant task. The observation that RU 24969 pretreatment enhanced MDMA self-administration and down-regulated 5-HT_{1A} receptors is consistent with this idea.

5-HT_{1B} receptor activation, on the other hand, augmented the increases in extracellular dopamine produced by cocaine (O'Dell & Parsons, 2004; Parsons et al., 1999) or ethanol (Yan et al., 2005). Thus, the down-regulation of 5-HT_{1B} receptors produced by RU 24969 pretreatment might be expected to reduce the reinforcing efficacy of MDMA. Furthermore, intra-raphe injections of the neurotoxin, 5,7-DHT, increased 5-HT_{1B} receptor binding in the substantia nigra and NAc (Compan, Segu,

Buhot, & Daszuta, 1998), and intraventricular infusion of 5,7-DHT produced an increase in 5-HT_{1B} binding in the hypothalamus, entorhinal cortex, and substantia nigra (Manrique et al., 1998; Manrique et al., 1994; Manrique, Segu, Hery, Faudon, & François-Bellan, 1993; Weissmann, Mach, Oberlander, Demassey, & Pujol, 1986). This same lesion facilitated MDMA self-administration (Bradbury et al., 2014). In contrast, intraventricular infusion of 5,7-DHT had no impact on 5-HT_{1A} binding in substantia nigra, PFC, hippocampus, hypothalamus, or amygdala (Hensler, Kovachich, & Frazer, 1991; Lawrence, Olverman, Shirakawa, Kelly, & Butcher, 1993; Weissmann et al., 1986). Thus, the role of 5-HT_{1B} receptor populations in MDMA self-administration is not clear. Acquisition was facilitated by separate manipulations that produced both an up- and down-regulation of 5-HT_{1B} receptors, respectively. It is therefore possible that the acquisition of MDMA self-administration is not related to 5-HT_{1B} receptor populations.

The purpose of the following studies was to determine whether individual variability in 5-HT_{1A} and/or 5-HT_{1B} receptors predicted the latency to acquisition of MDMA self-administration. To this end, the behavioural responses to the 5-HT_{1A} receptor agonist, 8-OH-DPAT, or the 5-$HT_{1B/1A}$ receptor agonist, RU 24969, were assessed before MDMA self-administration commenced. Furthermore, to test the idea that RU 24969 pretreatment facilitated acquisition of MDMA self-administration via a down-regulation of 5-HT_{1A} receptors, separate groups of rats were administered the 5-HT_{1A} receptor antagonist, WAY 100635, or vehicle, before each self-administration session. If 5-HT_{1A} receptor activation does inhibit MDMA self-administration, pretreatment with WAY 100635 would be expected to facilitate acquisition.

Method

Subjects and procedures

Male Sprague-Dawley rats underwent catheter surgery for self-administration, as outlined in the General Methods section. Testing began after recovery to pre-surgery weight.

The hyperlocomotor response to 8-OH-DPAT was assessed using the standard locomotor activity methods outlined in the General Methods section. Rats were placed in the activity chambers for 30 minutes, followed by 8-OH-DPAT (0.1, 0.3 mg/kg, s.c., n=24 and 30 respectively) administration, and locomotor activity was measured for another 60 minutes. These doses were chosen because inspection of preliminary data

showed both doses produced hyperactivity with considerable between-subject variability.

Adipsia produced by RU 24969 was assessed using the standard water consumption methods outlined in the General Methods section. RU 24969 (1.0 mg/kg, s.c., n=13) was administered 15 minutes before water bottles were reintroduced. This dose was chosen because in previous studies it produced an adipsic response with considerable between-subject variability (Aronsen et al., 2014).

MDMA self-administration, as outlined in the General Methods, began the day after the behavioural response to 8-OH-DPAT or RU 24969 was measured. Self-administration was conducted during 2 hour daily sessions, 6 days per week. Each self-administration session began with an experimenter-delivered infusion of drug. Thereafter, depression of the active lever produced an infusion of MDMA according to an FR1 schedule. Responses on the active and inactive levers were recorded. Every seventh day catheters were infused with sodium pentobarbital (20.0 mg/kg, i.v.). Failure to demonstrate an immediate loss of the righting reflex suggested a loss of catheter patency and the rat was excluded from the study. Catheter patency was lost in 5 rats (4 after 8-OH-DPAT-produced hyperactivity (3 in the 0.1 mg/kg group, 1 in the 0.3 mg/kg group), 1 after RU 24969-produced adipsia), and one rat self-administered a lethal dose of MDMA on the first day (0.3 mg/kg 8-OH-DPAT group). The same acquisition criterion was used as in the last chapter – a total of 90 infusions (90 mg/kg) self-administered. In order to minimise the number of subjects required, testing continued for 35 sessions in the groups assigned to 0.3 mg/kg 8-OH-DPAT and 1.0 mg/kg RU 24969. Testing in the 0.1 mg/kg 8-OH-DPAT group continued for 25 sessions, whereupon subjects were used for a different study.

Using the same self-administration procedure, separate groups of rats were pretreated with either saline vehicle, or the 5-HT_{1A} receptor antagonist, WAY 100635 (1.0 mg/kg, s.c., n=7 per group) 15 minutes before each daily self-administration session. Of the 14 rats that started self-administration, 4 were removed from the experiment due to loss of catheter patency (3 in the vehicle group, 1 in the WAY 100635 group).

Statistical analyses

Behavioural responses to either 8-OH-DPAT or RU 24969 were correlated with latency to acquisition of MDMA self-administration using a Pearson's product-moment correlation. Data from subjects that did not acquire within the cut off period

were not included in these analyses. Analysis of the effect of WAY 100635 pretreatment on self-administration was not possible due to a high attrition rate, but raw data are presented.

Results

Out of a total of 67 rats that started the locomotor and adipsia studies, 39 met the acquisition criterion within 25 sessions, and a further 9 met the criterion between 26 and 35 sessions.

Figure 6.1 shows the distribution of days to meet the acquisition criterion and locomotor response to 0.1 mg/kg 8-OH-DPAT. There was no significant correlation between these two variables ($r(16)=-0.21$, $p=0.40$).

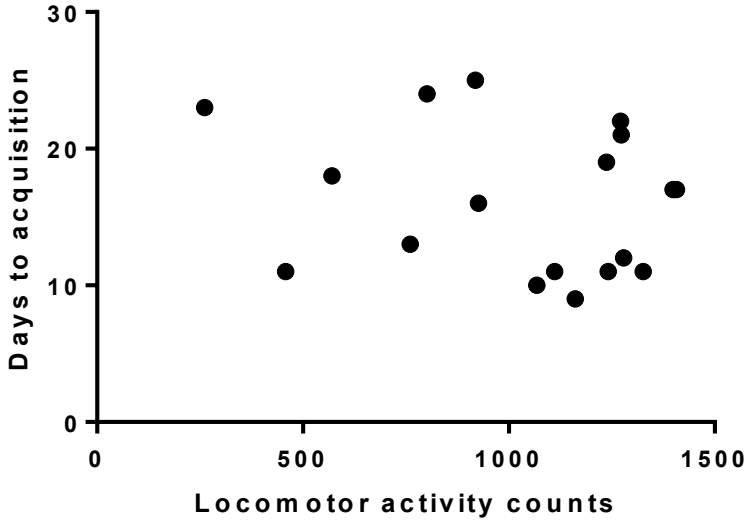


Fig 6.1 Scatterplot of days to acquire MDMA self-administration (y-axis) and locomotor response to 0.1 mg/kg 8-OH-DPAT (x-axis).

Similarly, there was no correlation between days to acquisition and locomotor response to 0.3 mg/kg 8-OH-DPAT ($r(18)=0.004$, $p=0.99$) (see figure 6.2).

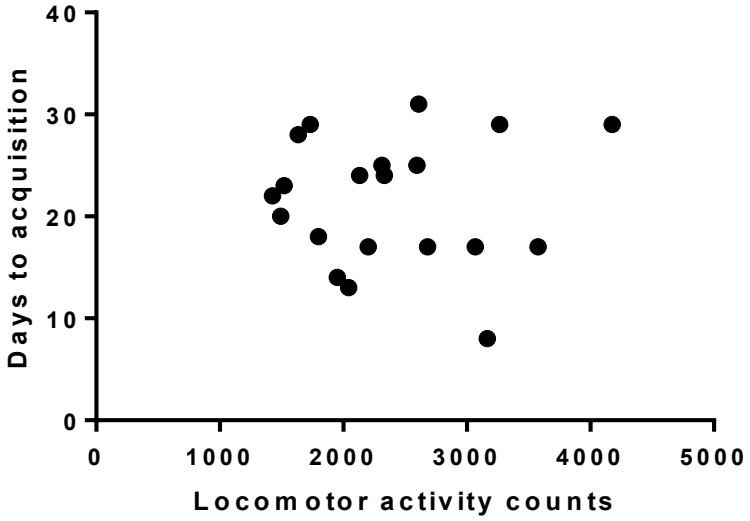


Fig 6.2 Scatterplot of days to acquire MDMA self-administration (y-axis) and locomotor response to 0.3 mg/kg 8-OH-DPAT (x-axis).

The distribution of days to acquire MDMA self-administration and the adipsic response to RU 24969 is shown in figure 6.3. Analysis showed no significant correlation between the two variables ($r(7)=0.26$, $p=0.49$).

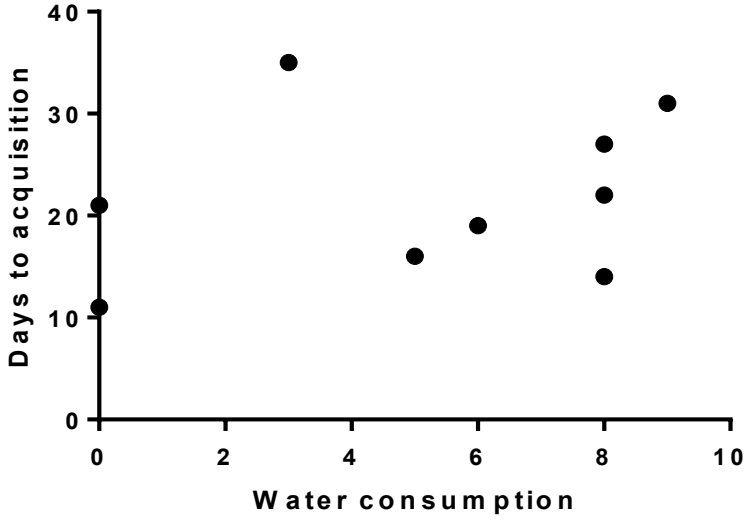


Fig 6.3 Scatterplot of days to acquire MDMA self-administration (y-axis) and adipsic response to 1.0 mg/kg RU 24969 (x-axis).

Data from the WAY 100635 pretreatment groups would have been analysed using a log-rank test to compare Kaplan-Meier survival estimates (Kaplan & Meier, 1958), but given the high attrition rate in the control group this analysis would not be meaningful. Nonetheless the data obtained were interesting, so average self-

administration data over sessions are presented in figure 6.4. It is interesting to point out that, while escalation of intake is evident from around day 6 in the control group, there is no escalation in the WAY 100635 group. This pattern continued beyond day 15, in fact by day 25 the highest total intake in the WAY 100635 pretreatment group was 41 mg/kg. The high variability in the vehicle control group from day 10 is to be expected, because as we have previously shown, intake in some rats increases around this time point (Schenk et al., 2012). Data after day 15 are not presented because, by that stage, only 3 rats remained in the control group (1 reached acquisition criteria, 3 lost catheter patency). Table 6.1 shows the raw data for the number of infusions over different self-administration sessions. These data further illustrate the variability in the vehicle group, due to increased self-administration in some subjects, and is roughly in line with the expectation that approximately 50% of control subjects would acquire MDMA self-administration.

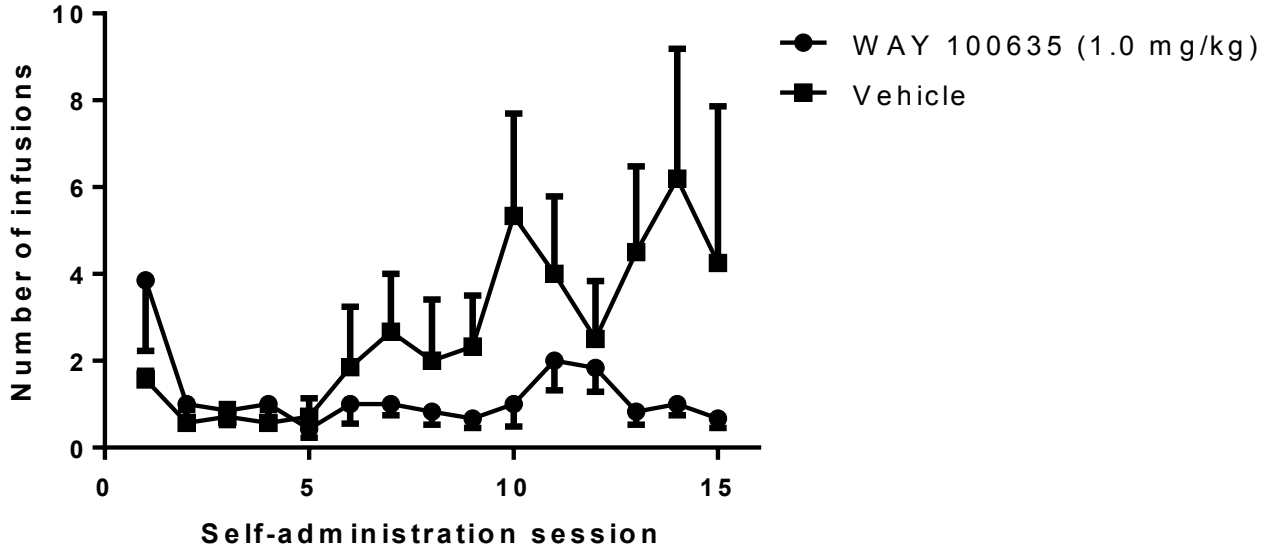


Fig 6.4 Number of MDMA infusions self-administered across sessions in rats treated with either WAY 100635 (1.0 mg/kg) or vehicle, 15 minutes before self-administration commenced. Error bars represent SEM. n=4-7 per data point, see table 6.1 for more detail.

Group	Rat	Session 1	Session 5	Session 10	Session 15
WAY 100635					
	Pūkeko	1	0	*removed*	*removed*
	Rock Wren	4	0	3	1
	Saddleback	2	0	0	1
	Silvereye	12	0	0	1
	Spotless Crake	0	1	0	0
	Spotted Shag	7	1	1	0
	Stichbird	1	1	2	1
Vehicle					
	Oystercatcher	1	0	14	*removed*
	Takahē	2	1	2	2
	Tomtit	2	1	11	15
	Tūī	1	0	4	*removed*
	Weka	1	0	0	0
	White Heron	2	3	*removed*	*removed*
	Yellowhead	2	0	1	0

Table 6.1 The number of MDMA infusions (1.0 mg/kg/infusion) self-administered by subjects treated with either the 5-HT_{1A} receptor antagonist, WAY 100635, or saline vehicle, 15 minutes before each self-administration session.

Discussion

These studies failed to show an association between behavioural response to 5-HT_{1A} or 5-HT_{1B} activation, and latency to acquire MDMA self-administration. These results were surprising given that, in the previous chapter, a treatment that down-regulated both receptor subtypes also facilitated acquisition of MDMA self-administration. Furthermore, lower sensitivity of 5-HT_{1A}, or greater sensitivity of 5-HT_{1B}, receptors would be expected to enhance the dopaminergic response to MDMA, which would be expected to enhance self-administration. Thus, it appears that basal variability in 5-HT_{1A} and 5-HT_{1B} receptors is not associated with the variability in acquisition of MDMA self-administration.

These results might suggest that facilitated self-administration after RU 24969 pretreatment reported in the previous chapter was not due to the effects of the pretreatment on 5-HT_{1A} or 5-HT_{1B} receptors. Indeed, a significant correlation between behavioural response and latency to acquisition in the present studies would have been evidence for a role of 5-HT_{1A} and/or 5-HT_{1B} receptors in the initial reinforcing effects of MDMA. However, caution should be exercised before we conclude that the effect

of RU 24969 pretreatment on MDMA self-administration was independent of the effects on 5-HT_{1A} or 5-HT_{1B} receptors. Firstly, RU 24969 is reasonably selective for these two receptor subtypes, making a non-selective effect less likely. Furthermore, it is possible that the natural variability in the behaviours measured in this study was not substantial enough to show an effect. For example, the mean activity count after 0.1 mg/kg 8-OH-DPAT in the present study was 1003 (*SD*=348), but after RU 24969 pretreatment this mean was 378 (*SD*=164). Thus, it is possible that lower sensitivity to 8-OH-DPAT is indeed predictive of latency to acquire MDMA self-administration, but that significantly lower levels of sensitivity are required.

Further study is required to determine the relative roles of 5-HT_{1A} and 5-HT_{1B} receptors in the facilitation of MDMA self-administration after RU 24969 pretreatment. Pretreatment with RU 24969 and either the 5-HT_{1A} receptor antagonist, WAY 100635, or the 5-HT_{1B} receptor antagonist, GR 127935, would help to clarify the roles of each receptor.

There was an unfortunately high attrition rate in the WAY 100635 pretreatment study. This attrition rate likely reflects a procedural problem during catheter surgery that has since been identified. Measures have now been put in place to avoid such levels of attrition in future. The subsequently small size of the saline pretreatment group precludes meaningful comparisons between the WAY 100635 pretreatment group and its appropriate control. However, our laboratory, and this thesis, have shown that approximately 50% of rats acquire MDMA self-administration (Bradbury et al., 2014; Schenk et al., 2012), and it seems unlikely that saline administration would alter this acquisition rate. Therefore, it becomes interesting that responding for MDMA was so low in the WAY 100635 group. If these findings were replicated in a larger sample, and with an appropriate control, the data would provide evidence for the suggestion that 5-HT_{1A} receptor activation is required for the development of MDMA self-administration.

If so, it would be difficult to reconcile these data with the RU 24969 pretreatment data that showed a down-regulation of 5-HT_{1A} receptors was associated with enhanced MDMA self-administration. Given the limited scope of the present study, it is not possible to rule out a non-specific effect of WAY 100635. Data from chapter 4 suggest that this dose of WAY 100635 does not supress locomotor responding. Furthermore, data from the first self-administration session, and other research from our lab (Schenk et al., Under Review), show that rats are able to perform

an operant response after acute WAY 100635 administration. Thus it seems unlikely that the low levels of responding after WAY 100635 administration were due to motor effects. There were no differences between the weights of subjects in the two groups throughout the experiment (data not shown), suggesting there was no effect of WAY 100635 on eating or drinking.

It might be expected that repeated administration of an antagonist would upregulate receptor populations. Indeed, administration of a high dose (3 mg/kg) of WAY 100635 twice per day for 3 days increased 5-HT_{1A} immunoreactivity in the hippocampus and cortex (Abbas, Nogueira, & Azmitia, 2007). An up-regulation of 5-HT_{1A} receptors might inhibit self-administration via enhanced inhibition of dopamine. Alternatively, WAY 100635 may have protected 5-HT_{1A} receptors from important neuroadaptations in response to high levels of 5-HT. During self-administration session 1 I noticed that the rats in the WAY 100635 group that self-administered a large dose of MDMA did not show the characteristic set of symptoms (hyperthermia, wetness, 'eagle-fear', bleeding nose) typically associated with initial self-administration of high doses. Anecdotally, repeated self-administration of high doses of MDMA produces tolerance to these effects. Thus, WAY 100635 may have been preventing neuroadaptations that produce tolerance to some of the aversive effects of MDMA. Clearly, more research would be required to determine if this is the case.

The results of the present study failed to show an association between basal responses to 5-HT_{1A} or 5-HT_{1B} receptor activation and latency to acquire MDMA self-administration. However, the results of the previous chapter, and inferences drawn from the WAY 100635 study in this chapter, raise the possibility that neuroadaptations in 5-HT_{1A} and/or 5-HT_{1B} receptors are important for the progression of MDMA self-administration. As outlined above, basal variability in these receptor subtypes may not be substantial enough to allow for meaningful analysis. On the other hand, if changes in these receptor subtypes underlie the development of MDMA as an efficacious reinforcer, it might be possible to detect differences in these receptor populations after substantial MDMA self-administration. This possibility will be addressed in the next chapter.

Chapter 7: Response to 5-HT_{1A} and 5-HT_{1B} receptor agonists after self-administration

Parts of this chapter have been adapted from:

Aronsen & Schenk (2016). MDMA self-administration fails to alter the response to 5-HT_{1A} and 5-HT_{1B} agonists. *Psychopharmacology, 233* (7), 1323-1330. DOI 10.1007/s00213-016-4226-9

with permission from the publisher (Appendix A).

Some users regularly consume large quantities of ecstasy (Cottler et al., 2001; Degenhardt, Barker, & Topp, 2004; Topp, Hall, & Hando, 1997), and repeated ecstasy use produces a range of negative consequences, including cognitive and emotional deficits. While these deficits are worrisome in and of themselves, it has been suggested that they could also facilitate further ecstasy taking, and thus contribute to the development of an SUD (Schenk, 2009; Schenk & Aronsen, 2015). The mechanisms underlying these deficits are not, however, well understood.

Ecstasy users showed deficits in learning (Wagner et al., 2013), and in attention and memory (McCann, Mertl, et al., 1999) compared to ecstasy-naïve controls or those with limited ecstasy use. Ecstasy users reported higher levels of depression, impulsiveness, and sleep disturbances than poly-drug users who did not use ecstasy (Taurah et al., 2014). These cognitive and behavioural deficits were persistent, suggesting that regular ecstasy use may cause long-lasting neuroadaptations (Parrott, 2013b).

Animal studies have shown that a number of these adverse effects associated with ecstasy use are modulated by pharmacological manipulation of 5-HT receptors. For example, the 5-HT_{1A} receptor agonist, 8-OH-DPAT, impaired learning and memory in water maze (Carli & Samanin, 1992), passive avoidance (Carli, Tranchina, & Samanin, 1992), and conditioned reinforcement (Meneses, 2007) tasks, while the 5-HT_{1A} receptor antagonist, WAY 101405, improved learning in the Morris water maze (Hirst et al., 2008). 5-HT_{1A} receptor agonists and antagonists also altered performance in the forced swim test and conditioned stress-induced ultrasonic vocalisations (Assié et al., 2010; Lucki, Singh, & Kreiss, 1994) and altered sleep and wakefulness, as measured by EEG and EMG (Monti & Jantos, 1992; Monti et al., 1990). Activation of 5-HT_{1A} receptors increased impulsive responding on the five-choice serial reaction

time task (Carli & Samanin, 2000), while the 5-HT_{1A} receptor antagonist, WAY 100635, supressed impulsive action (Ohmura et al., 2013).

Pharmacological manipulation of the 5-HT_{1B} receptor subtype also affected learning and memory as measured by a conditioned reinforcement task (Meneses, 2001, 2007), altered EEG and EMG recordings of sleep and wakefulness (Bjorvatn & Ursin, 1994; Monti, Monti, Jantos, & Ponzoni, 1995), and affected immobility time in the forced swim test (Dawson et al., 2006; Tatarczynska, Klodzinska, Stachowicz, & Chojnacka-Wojcik, 2004). Therefore, it is possible that some of the cognitive and behavioural deficits that accompany ecstasy use might be due to MDMA-produced neuroadaptations in these receptor mechanisms.

A small number of studies have assessed the effects of repeated exposure to MDMA on 5-HT_{1A} and 5-HT_{1B} receptor mechanisms. Repeated experimenter-administered MDMA reduced 5-HT_{1A} binding in the dorsal raphe, suggesting a down-regulation of 5-HT_{1A} autoreceptors, and increased 5-HT_{1A} binding in the frontal cortex, suggesting an up-regulation of 5-HT_{1A} heteroreceptors (Aguirre, Ballaz, Lasheras, & Del Rio, 1998; Aguirre, Frechilla, García-Osta, Lasheras, & Del RIo, 1997; Aguirre, Galbete, Lasheras, & Del Río, 1995). These effects were only produced following exposure to high doses (2x20-30 mg/kg/day, 4 consecutive days); exposure to lower doses (4x5 mg/kg/day, 2 consecutive days (McGregor et al., 2003)), or intermittent doses (2x10mg/kg/day, every 5th day (Piper, Vu, Safain, Oliver, & Meyer, 2006)) of MDMA failed to alter cortical or subcortical 5-HT_{1A} densities. Repeated administration of racemic MDMA increased 5-HT_{1B} receptor mRNA (Kindlundh-Högberg, Svenningsson, & Schiöth, 2006), and receptor binding densities were increased in some brain regions, but decreased in others, after repeated MDMA administration (McGregor et al., 2003). Repeated administration of (+) MDMA, however, failed to produce persistent changes in 5-HT_{1B} mRNA or 5-HT_{1B} receptor binding (Sexton, McEvoy, & Neumaier, 1999).

Functional evidence for these receptor changes is equivocal. Repeated administration of MDMA attenuated the autoreceptor-mediated decrease in 5-HT release produced by the 5-HT_{1A} receptor agonist, F13640, in mice (Lanteri et al., 2014). Repeated administration of MDMA did not, however, alter 8-OH-DPAT-produced lower lip retraction or hypolocomotion, behaviours associated with 5-HT_{1A} autoreceptor activation (Schenk et al., 2013). On the other hand, 8-OH-DPAT-produced hypothermia was increased after repeated MDMA administration in one

study (Aguirre et al., 1998) but unchanged in others (McNamara, Kelly, & Leonard, 1995; Mechan, O'Shea, Elliott, Colado, & Green, 2001; Piper et al., 2006). MDMA pretreatment also attenuated the 8-OH-DPAT-produced 5-HT syndrome (Piper et al., 2006) and forepaw treading (Granoff & Ashby, 2001), but had no effect on the prosocial response (Thompson, Callaghan, Hunt, & McGregor, 2008), or the hyperactive response (Granoff & Ashby, 2001) to 8-OH-DPAT. Differences might be due to a number of paradigmatic variables including dosing regimen and subject sample.

The hyperactive response to the 5-$HT_{1B/1A}$ receptor agonist, RU 24969, was decreased after repeated administration of racemic MDMA (Callaway & Geyer, 1992), but enhanced after repeated administration of the (+) MDMA isomer (McCreary, Bankson, & Cunningham, 1999). It was suggested that this behavioural response to RU 24969 reflected 5-HT_{1B} receptor activation (Callaway & Geyer, 1992), but some studies have suggested that RU 24969-produced hyperactivity is due to 5-HT_{1A} receptor activation (Aronsen et al., 2014; Kalkman, 1995). Repeated MDMA administration (2x20 mg/kg/day, 4 consecutive days) failed, however, to alter hyperactivity produced by the 5-HT_{1A} receptor agonist, 8-OH-DPAT (Granoff & Ashby, 2001). Therefore, the effect of MDMA exposure on the function of 5-HT_{1B} receptors is equivocal.

Studies on the effects of repeated exposure to MDMA have generally administered a regimen that produces extensive, and persistent, neurotoxic effects. For example, alterations in 5-HT_{1A} binding, decreased tissue levels of 5-HT (Aguirre et al., 1998) and decreased 5-HT transporter binding (Aguirre et al., 1995) were produced by exposure to high doses (2x30mg/kg/day, 4 consecutive days) of MDMA. This high level of exposure is rarely, if ever, experienced by ecstasy users (D. Hansen et al., 2001; Parrott, 2005; Verheyden et al., 2003), which questions the external validity of findings derived from these experiments (Baumann & Rothman, 2009; Cole & Sumnall, 2003; De La Garza et al., 2007; Meyer et al., 2008).

MDMA exposure during self-administration is quite different from most studies that employ experimenter-administered MDMA. In rats, MDMA self-administration is initially limited, but with repeated testing intake gradually increases for some subjects (Schenk et al., 2012). Given the differences in exposure as well as the well documented differences between effects of contingent and non-contingent drug administrations (Dworkin, Mirkis, & Smith, 1995; Miguéns et al., 2008), self-

administered MDMA might be expected to produce different effects than those seen after experimenter-administration. Indeed, self-administered MDMA produced smaller deficits in tissue levels of 5-HT compared to high dose experimenter-administered MDMA (Do & Schenk, 2011; Scanzello et al., 1993; Schenk et al., 2007) even though the total amount self-administered (165-350 mg/kg over 20-30 days of testing) was greater than is generally administered to produce extensive neurotoxicity (20-80 mg/kg in a single day). Additionally, intermittent or low dose exposure to MDMA was neuroprotective against the toxic effects of subsequent high dose administrations (Bhide et al., 2009; Piper et al., 2010).

Because of the limited amount of information concerning effects of self-administered MDMA on brain and/or behaviour and the potential role of specific neuroadaptations in some of the adverse effects of MDMA, this study determined the effect of extensive MDMA self-administration on behavioural responses to 5-HT_{1A} and 5-HT_{1B} receptor agonists.

Method

Subjects and procedures

Male Sprague-Dawley rats underwent catheter surgery as outlined in the General Methods section.

MDMA self-administration

Rats were randomly assigned to self-administer either MDMA, or vehicle, using the standard self-administration equipment outlined in the General Methods section. Self-administration was conducted during 2 hour daily sessions, 6 days per week. Initially, active lever responses were reinforced with MDMA (1.0 mg/kg), or vehicle (0.1 ml) infusions according to an FR1 schedule. The vehicle control group continued on this contingency for the remainder of the experiment. The MDMA self-administration group continued with this contingency until a total of 90 infusions had been self-administered, or 25 test sessions had been completed, whichever came first. Rats that failed to self-administer 90 infusions within this 25 day cut-off period (approximately 50%, as we have previously reported (Schenk et al., 2012)) were not tested further. For those that met this criterion, the dose of MDMA was decreased to 0.5 mg/kg. The reinforcement schedule was then increased to FR2 for a minimum of 5 days and then FR5. Testing continued until a total intake of 350 mg/kg MDMA was self-administered. Between 20 and 58 self-administration sessions were required to

reach a total intake of 350 mg/kg. Where possible, each rat in the vehicle self-administration group was matched to a rat in the MDMA self-administration group to ensure a comparable number of test sessions. A total of 73 rats met the initial criterion of 90 infusions of MDMA (1.0 mg/kg/infusion) within the 25 day cut-off period. Of these, some did not progress further due to loss of catheter patency (n=1), failure to increase responding when the FR schedule was increased (n=12), or MDMA toxicity (n=3). The remaining rats (n=57) completed testing and self-administered 350 mg/kg MDMA. A total of 62 rats initiated vehicle self-administration, but 1 was removed from the study due to an inner ear infection, leaving a total of 61 that self-administered vehicle. Separate groups of rats that completed self-administration testing were then randomly assigned to groups to measure the effects of either 8-OH-DPAT-produced hyperactivity or RU 24969-produced adipsia.

Locomotor activity

Locomotor activity was assessed 2 days after the last self-administration session. Rats were placed in the testing chamber for 30 minutes, followed by an injection of 8-OH-DPAT (0.0, 0.03, 0.1, 0.3, 1.0 mg/kg, s.c., n=5-7 per group). Horizontal activity counts were recorded in 5 minute intervals during the 30 minutes prior to, and 60 minutes following, the 8-OH-DPAT injection.

Water consumption

The day following the last self-administration session, water bottles were removed from the home cages for 24 hours. Fifteen minutes before water bottles were reintroduced, RU 24969 (0, 0.3, 1.0, 3.0 mg/kg, s.c., n= 6-9 per group) was administered, as previously reported (Aronsen et al., 2014). Water bottles were weighed before, and after 30 minutes of access, to measure water consumption.

Data analysis

Effects of 8-OH-DPAT on locomotor activity were analysed by a 2 (self-administration group) × 5 (Dose of 8-OH-DPAT) ANOVA. RU 24969-produced adipsia was analysed with a 2 (self-administration group) × 4 (Dose of RU 24969) ANOVA.

Results

Self-administration

The average amount of MDMA that was self-administered during the last 5 days of testing was 13.2 mg/kg/day (*SEM*=0.55). Figure 7.1 shows the distribution of

the number of rats that self-administered 350 mg/kg of MDMA as a function of test session. Most of the rats met the criterion within 25-44 test sessions. The mean number of test sessions required to complete testing was 35.7 (*SEM*=1.3). The average number of days to complete testing reported in this study is similar to data that we have previously reported. For example, an average of 37 +/- 2.3 days was required to self-administer a slightly lesser total of 315 mg/kg that resulted in decreased tissue levels of 5-HT (Do & Schenk, 2011). The vehicle self-administration group was tested for an average of 36 sessions (*SEM*= 1.4). These rats were matched to the MDMA self-administration rats to minimise any confounds associated with the self-administration procedure.

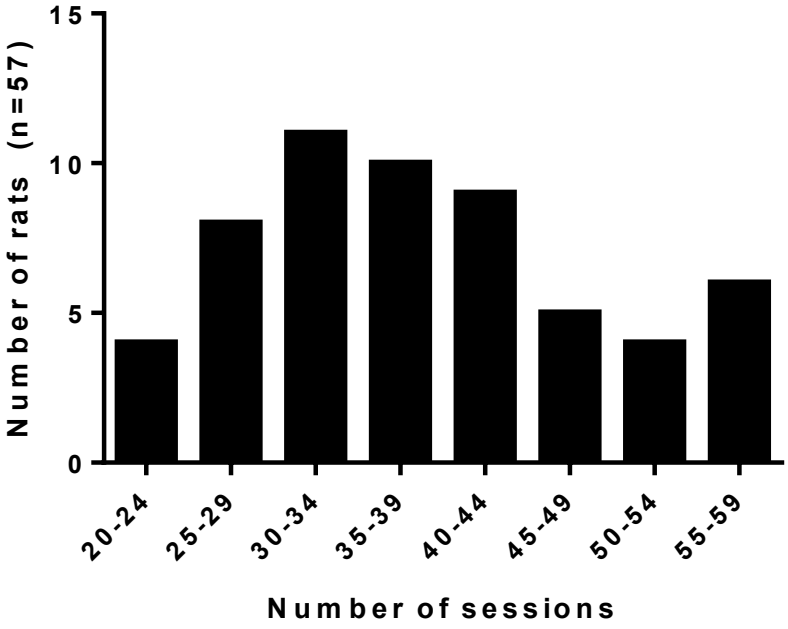


Fig. 7.1 Frequency distribution of the number of rats that self-administered 350 mg/kg MDMA as a function of test session.

8-OH-DPAT-produced hyperactivity

Figure 7.2 shows the hyperactive response to 8-OH-DPAT after self-administration of MDMA or vehicle. ANOVA showed an effect of 8-OH-DPAT dose ($F(4,47) = 27.27$, $p<0.01$, $\eta_p^2 = 0.70$), but no effect of self-administration ($F(1,47) = 0.79$, $p=0.38$), and no interaction ($F(4,47) = 0.50$, $p=0.50$).

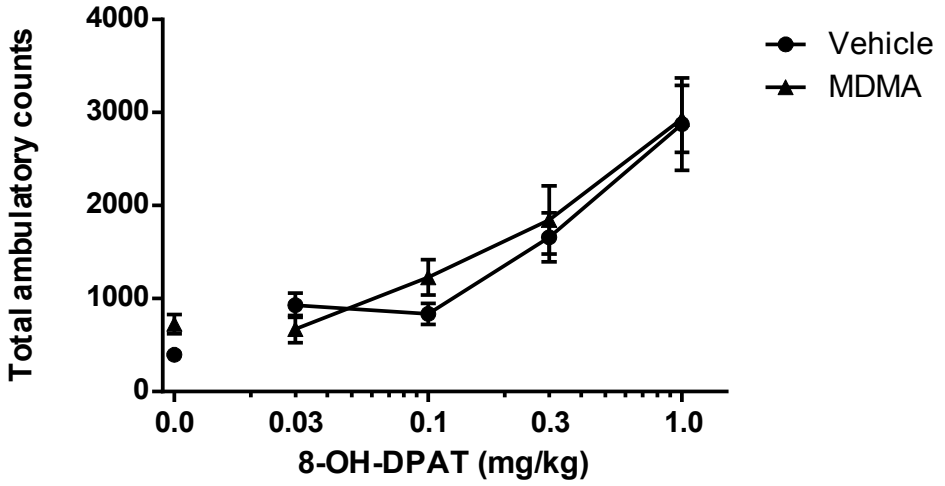


Fig. 7.2 Effect of MDMA self-administration (350 mg/kg total) on 8-OH-DPAT-produced hyperactivity. Rats in these groups met the criterion of 350 mg/kg MDMA after 25-58 test sessions. Symbols represent mean ± SEM. n = 5-7 per group.

RU 24969-produced adipsia

As we have previously shown (Aronsen et al., 2014), RU 24969 produced a dose-dependent adipsic response ($F(3,51) = 65.68$, $p<0.01$, $\eta_p^2 = 0.79$; Fig 7.3). There was no statistically significant effect of self-administration ($F(1,51) = 2.86$, $p=0.10$) and no statistically significant interaction ($F(3,51) = 1.60$, $p=0.20$).

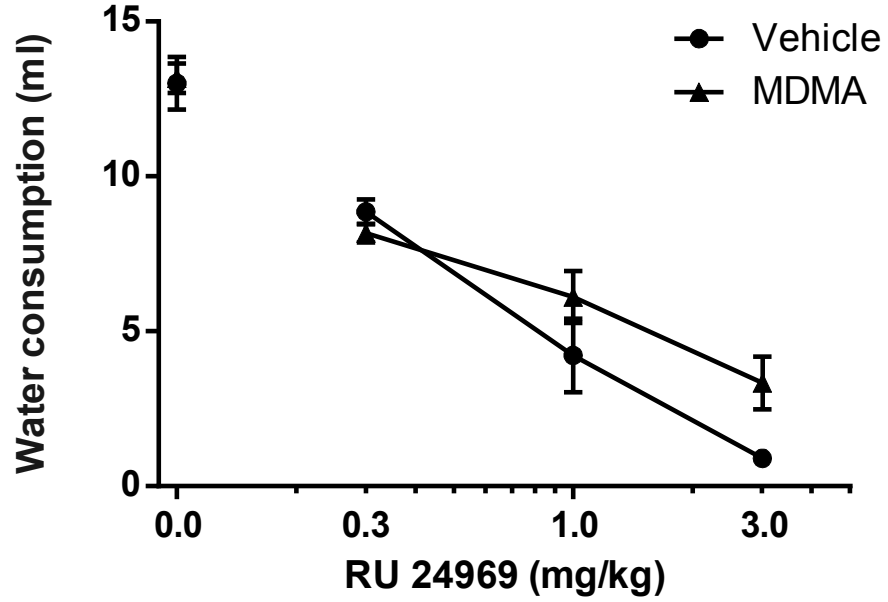


Fig. 7.3 Effect of MDMA self-administration (350 mg/kg total) on RU 24969-produced adipsia. Rats in these groups met the criterion of 350 mg/kg MDMA after 20-58 test sessions. Symbols represent mean ± SEM. n = 6-9 per group.

Discussion

MDMA self-administration failed to alter 8-OH-DPAT-produced hyperactivity, or RU 24969-produced adipsia. It is unlikely that the MDMA exposure was insufficient because similar or lower doses of self-administered MDMA produced decreases in 5-HT transporter binding (Schenk et al., 2007), decreases in tissue levels of 5-HT (Do & Schenk, 2011; Schenk et al., 2011), and behavioural deficits (Do & Schenk, 2011). Instead, the present data suggest that 5-HT_{1A} and 5-HT_{1B} receptor mechanisms are not altered by MDMA self-administration.

These findings were surprising because prolonged activation by MDMA-produced 5-HT release might have been expected to down-regulate these receptor subtypes. Alternatively, the decrease in MDMA-produced 5-HT release that has been reported following MDMA self-administration (Reveron et al., 2010) might have been expected to result in a compensatory up-regulation of these receptors. A neurotoxic 5,7-DHT lesion increased 5-HT_{1B} receptor binding (Compan et al., 1998; Crino, Vogt, Volicer, & Wiley, 1990; Frankfurt, Mendelson, McKittrick, & McEwen, 1993; Manrique et al., 1998; Manrique et al., 1994; Manrique et al., 1993; Offord et al., 1988; Weissmann et al., 1986). Furthermore, repeated agonist treatment decreased 5-HT_{1B} receptor binding (Pranzatelli & Razi, 1994), and behavioural responses to 5-HT_{1A} (De Souza, Goodwin, Green, & Heal, 1986; Hensler, 2003) and 5-HT_{1B} (Frances & Monier, 1991) receptor agonists.

Repeated exposure to other drugs that increase synaptic 5-HT levels altered 5-HT_{1A} and 5-HT_{1B} receptors. For example, chronic treatment with the selective 5-HT reuptake inhibitor (SSRI), fluoxetine, decreased 5-HT_{1B} receptor binding (Duncan, Hester, Hopper, & Franklin, 2010). It is important to note, however, that many of the effects of SSRI treatment reflect alterations that are most likely attributed to autoreceptor, rather than post-synaptic receptor, desensitisation. For example, repeated treatment with fluoxetine (8 mg/kg/day, 2-3 weeks) reduced 5-HT_{1A} mRNA in the raphe nuclei (Le Poul et al., 2000). Higher doses also produced a decrease in 5-HT_{1A} receptor binding (Welner, De Montigny, Desroches, Desjardins, & Suranyi-Cadotte, 1989) and 8-OH-DPAT stimulated [^{35}S]GTPγS binding (Castro et al., 2003) in the dorsal raphe. Repeated exposure to MDMA failed to alter a number of 5-HT_{1A} autoreceptor mediated behavioural or neurochemical responses (Schenk et al., 2013), suggesting differences between effects of these two classes of drugs. Repeated administrations of cocaine increased 5-HT_{1B} receptor binding (Przegaliński, Czepiel,

Nowak, Dlaboga, & Filip, 2003) and 5-HT_{1B} mRNA (Hoplight, Vincow, & Neumaier, 2007). Cocaine self-administration also increased the behavioural and physiological responses to 5-HT_{1A} and 5-HT_{1B} receptor agonists (O'Dell, Manzardo, Polis, Stouffer, & Parsons, 2006).

The present data do not rule out the possibility that repeated ecstasy use leads to cognitive and behavioural deficits via dysregulation of these receptor subtypes, but our results suggest that other 5-HT receptors are more likely to make important contributions. One potential candidate is the 5-HT_{2A} receptor, because it has also been implicated in impulsivity (Cunningham & Anastasio, 2014) , sleep (Sharpley, Elliott, Attenburrow, & Cowen, 1994) and memory (Dhonnchadha & Cunningham, 2008; Howell & Cunningham, 2015), behaviours that are impacted by regular ecstasy use. MDMA exposure increased 5-HT_{2A} receptor binding (Benningfield & Cowan, 2013; Urban et al., 2012) and behavioural responses to the 5-$HT_{2A/2C}$ receptor agonist, DOI (Biezonski, Courtemanche, Hong, Piper, & Meyer, 2009). Additional studies assessing the impact of MDMA self-administration on this receptor mechanism is warranted.

General Discussion

Summary

MDMA is widely used in the form of the street drug, ecstasy. Regular use of ecstasy has been associated with a number of behavioural and neurochemical deficits, and some of these deficits likely contribute to further, problematic drug taking. While most drugs of abuse primarily enhance dopamine neurotransmission, MDMA preferentially releases 5-HT. This 5-HT release has been hypothesised to inhibit the dopaminergic response to MDMA, thus inhibiting the reinforcing efficacy of MDMA. However, with repeated exposure to MDMA, the 5-HTergic response is attenuated, disinhibiting the dopaminergic response and making MDMA similar to other drugs of abuse. The mechanism for this 5-HTergic inhibition of dopamine is not known, but one possibility is that activation of specific 5-HTergic receptors, via MDMA-produced 5-HT release, alters the dopaminergic response to MDMA. Of the 14 different 5-HT receptors, the 5-HT_{1A} and 5-HT_{1B} receptors were investigated because of a documented role in regulating basal and drug-produced dopamine release, as well as behaviours associated with ecstasy use. The purpose of this thesis was to test the role of these receptors in the self-administration of MDMA in rats, and to document any changes in these receptor populations produced by MDMA.

Firstly, appropriate behavioural assays for 5-HT_{1A} and 5-HT_{1B} receptor activation needed to be identified. Latent inhibition, measured using the conditioned taste aversion paradigm, was chosen as a behavioural response to 5-HT_{1B} receptor activation, but this response was confounded by the adipsic response to the 5-$HT_{1B/1A}$ receptor agonist, RU 24969. After further testing, I found that this adipsic response to RU 24969 was dose-dependent, and blocked by a 5-HT_{1B}, but not a 5-HT_{1A}, receptor antagonist. Thus, the adipsic response to RU 24969 was chosen as a behavioural measure of 5-HT_{1B} receptor activation. In contrast, the hyperactive response to RU 24969 was blocked by a 5-HT_{1A}, but not a 5-HT_{1B}, receptor antagonist. A similar result was obtained with the more selective and well characterised 5-HT_{1A} receptor agonist, 8-OH-DPAT, thus the locomotor response to 8-OH-DPAT was chosen as a behavioural response to 5-HT_{1A} receptor activation.

To test whether 5-HT_{1A} and/or 5-HT_{1B} receptors regulated MDMA self-administration I attempted to alter the activity of these receptors and measure the

impact on the acquisition of MDMA self-administration. To this end, rats were repeatedly administered a high dose of the 5-$HT_{1B/1A}$ receptor agonist, RU 24969, before commencing MDMA self-administration. The pretreatment down-regulated 5-HT_{1A} and 5-HT_{1B} receptors, and greatly facilitated the acquisition of MDMA self-administration. Because drug produced dopamine release is inhibited by activation of 5-HT_{1A} receptors, but enhanced by 5-HT_{1B} receptors, the impact of RU 24969 pretreatment on acquisition of MDMA self-administration was hypothesised to be associated with the down-regulation of 5-HT_{1A} receptors.

The role of 5-HT_{1A} and 5-HT_{1B} receptors in the acquisition of MDMA self-administration was further tested by investigating the relationship between basal responses to receptor activation and latency to acquire MDMA self-administration. Based on the role of these receptors in regulating the dopaminergic response to other drugs of abuse, and the facilitated acquisition of MDMA self-administration after repeated exposure to RU 24969, it was expected that behavioural responses to activation of these receptors would predict the latency to acquire MDMA self-administration. This hypothesis was not supported in any of the studies. Furthermore, because an inhibitory role of 5-HT_{1A} receptor activation in the acquisition of MDMA self-administration was hypothesised, I investigated the effect of 5-HT_{1A} receptor antagonist treatment during the acquisition phase. Again, results did not support the hypothesis, in fact, the results suggested that 5-HT_{1A} receptor blockade inhibited MDMA self-administration.

Acquisition studies had returned mainly negative results, but there was still reason to believe that 5-HT_{1A} and/or 5-HT_{1B} receptors regulated the self-administration of MDMA. Therefore, behavioural responses to 5-HT_{1A} or 5-HT_{1B} receptor activation were measured after the self-administration of a high dose of MDMA. It was expected that prolonged exposure to MDMA would alter behavioural responses to agonist administration, but again this hypothesis was not supported. Although these studies do not rule out the possibility of 5-HT_{1A} or 5-HT_{1B} receptor neuroadaptations in response to MDMA self-administration, they do suggest that other 5-HT receptors are more likely to make important contributions.

Synthesis of results

Overall, the data presented in this thesis are difficult to reconcile. On the one hand, there is a sound theoretical basis to expect that 5-HT_{1A} and 5-HT_{1B} receptors

would modulate the reinforcing efficacy of MDMA, and that these receptor mechanisms would be altered by prolonged exposure to MDMA. Furthermore, the RU 24969 pretreatment, that down-regulated 5-HT_{1A} and 5-HT_{1B} receptors, also facilitated the acquisition of MDMA self-administration. On the other hand, behavioural responses to 5-HT_{1A} and 5-HT_{1B} receptor activation did not predict the acquisition of MDMA self-administration, acquisition was blocked by the 5-HT_{1A} receptor antagonist, WAY 100635, and there were no changes in dose response curves for 5-HT_{1A} or 5-HT_{1B} mediated responses after the self-administration of a high dose of MDMA.

Together, the most likely explanation for the results of the studies comprising this thesis is that 5-HT_{1A} and 5-HT_{1B} receptors have a limited role in the self-administration of MDMA. If true, this conclusion would suggest that the facilitated self-administration produced by RU 29496 pretreatment was due to some non-specific effect. To test this possibility, it would be important to co-administer a 5-HT_{1A} or 5-HT_{1B} receptor antagonist with RU 24969 during pretreatment and test for latency to acquire MDMA self-administration.

Limitations

It is possible that the conclusions made in this thesis were skewed by the behavioural measures used. Although it was demonstrated that RU 24969-produced adipsia and 8-OH-DPAT-produced locomotor activity are measures of 5-HT_{1B} and 5-HT_{1A} receptor activation, respectively, there is no consensus on what population of 5-HT_{1B} or 5-HT_{1A} receptors produce these effects. Systemic administration of 8-OH-DPAT produced dose-dependent hyperactivity, but this locomotor response is the net result of global 5-HT_{1A} activation. Specific 5-HT_{1A} populations alter locomotor activity in different ways, for example local injections of 8-OH-DPAT in the PFC did not alter locomotor activity (Solati et al., 2011), while administration in the NAc decreased locomotor activity (Hillegaart, Ahlenius, & Larsson, 1991; Plaznik et al., 1994). Similarly, the population of 5-HT_{1B} receptors responsible for the adipsic response to RU 24969 is not known. One study showed that local infusion of RU 24969, or the more selective 5-HT_{1B} receptor agonist, CP 93129, in the NAc reduced responding for water (P. J. Fletcher & Korth, 1999a), but it is not clear what other populations of 5-HT_{1B} receptors might also influence this behavioural response. Thus, care needs to be taken when interpreting these behavioural data. It is possible, for

example, that MDMA self-administration did alter some 5-HT_{1A} and/or 5-HT_{1B} receptor populations, but not those that impact the locomotor response to 8-OH-DPAT or the adipsic response to RU 24969.

Extensive study would be required to address the possibility that the negative results found in this thesis were due to the choice of behavioural responses. Because of the time, rats, and drugs required, it was not possible to investigate further for this thesis, but our lab has started to probe this possibility in further detail. The logical first step is to directly investigate the effect of MDMA self-administration on 5-HT_{1A} and 5-HT_{1B} receptor binding. There is a widely used and well characterised 5-HT_{1A} antibody (Abbas et al., 2007; Kia et al., 1996; Say, Machaalani, & Waters, 2007; Tachibana, Endoh, Fujiwara, & Nawa, 2005), allowing for an immunohistochemistry investigation, but 5-HT_{1B} receptors are best mapped using a radioactively labelled ligand (Domenech, Beleta, & Palacios, 1997; Lindhe et al., 2011). Our lab is currently conducting 5-HT_{1A} immunohistochemistry on tissue from rats that have extensive MDMA self-administration history. Such an approach allows for a detailed, region specific, analysis of the effect of MDMA self-administration. Similar data could be obtained to determine the effect of RU 24969 pretreatment. Receptor populations that are similarly affected by both manipulations might underlie the development of MDMA as an efficacious reinforcer. Thus, local drug administrations in these areas could be used, first to obtain a behavioural response to predict the acquisition of MDMA self-administration, then for pharmacological treatments to reduce MDMA self-administration.

Validity of MDMA doses

The self-administration paradigm was used in this thesis because it likely produces neuroadaptations similar to those produced by ecstasy use in humans. It is important to note that, when compared across species, the MDMA doses self-administered in this thesis were of relevance to human users. The issue of interspecies scaling is based on the fact that, in general, smaller animals have relatively larger organs and a shorter blood circulation time, and so will metabolise drug faster (Mordenti & Chappell, 1989). Therefore, as long as there are no species-specific mechanisms of drug metabolism, smaller animals require larger doses in order for effects to be comparable to those produced in larger animals. Most recreational users consume 1-2 tablets per ecstasy-taking session (Parrott, 2005; Verheyden et al., 2003),

and although the contents of ecstasy tablets procured 'on the street' vary widely, median MDMA content per tablet has been shown to be around 70-80 mg (Vogels et al., 2009). Therefore, a 70kg user is consuming approximately 1-2 mg/kg MDMA in recreational settings. Interspecies scaling can help to determine the doses that should be administered in animal studies to best mimic the effects of such doses in recreational users.

As a starting point for investigating drugs across species the USA Food and Drug Administration (FDA) suggest that drug doses should be scaled across species based on the body weight and surface area of these species (Food and Drug Administration, 2005). The FDA recommendation is that the effects of a 1.0 mg/kg dose in a human are roughly comparable with the effects of a 6.2 mg/kg dose in a rat. The FDA scaling suggestions are not drug-specific and are meant merely as a guideline for determining safe initial doses in clinical trials.

With specific reference to MDMA, some researchers have used the following algorithm to scale doses between species:

$$D_{Human} = D_{Animal} (W_{Human} / W_{Animal})^{k} \qquad \text{(Equation 1)}$$

where D is the drug dose in mg, W is weight in kg, and k is an estimated value that reflects the logarithmic relationship between bodyweight and metabolic rate (that is, the slope of the curve fitted to the log transforms of empirical values for weight and drug clearance times of different species). This process, in which doses are determined by transforming bodyweight to a different physiological variable through a power function, is called 'allometric scaling' (Mordenti & Chappell, 1989). The precise value of the scaling factor, k, has been seriously contended in the literature, with suggestions ranging between 0.67 and 0.77 (Food and Drug Administration, 2005; Mordenti & Chappell, 1989; Travis & White, 1988; Watanabe, Bois, & Zeise, 1992). In studies using MDMA, a k value of 0.7 has been adopted (McCann & Ricaurte, 2001; Ricaurte, Yuan, & McCann, 2000). Based on Equation 1, with k set at 0.7, a 1.0 mg/kg dose in a 70kg human would be equivalent to a 5.0 mg/kg dose in a 330g rat. It should be noted that this suggested dose could vary from 3.4 mg/kg to 5.9 mg/kg if the highest or lowest suggested k value is used, respectively.

Vollenweider, Jones, and Baggott (2001) have suggested that allometric scaling is not relevant to MDMA because there is evidence for species differences in MDMA pharmacokinetics, and because MDMA has active metabolites that may contribute to the drug effect. Instead, they suggest that pharmacokinetic data (e.g. area under the

curve (AUC) of MDMA plasma levels) should be compared between species to determine similar doses. Based on equation 1, McCann and Ricaurte (2001) claim that a 20 mg/kg dose of MDMA in a 220g rat is comparable to a 1.4 mg/kg dose in a human, however AUC of MDMA plasma concentrations in humans after approximately 1.8 mg/kg was 70% lower than the AUC in rats after 20 mg/kg (Vollenweider et al., 2001). Although comparing pharmacokinetic data can account for some potential flaws with allometric scaling, comparisons can only be made with empirical data, so finding similar doses across species becomes a 'trial and error' type task.

Both allometric scaling and comparisons of pharmacokinetic data (AUC) suggest that a human dose of approximately 1 mg/kg is comparable to a rat dose of roughly 5 mg/kg (De La Torre et al., 2000; Fitzgerald, Blanke, & Poklis, 1990). Thus, during initial self-administration sessions, in which rats self-administer less than 5 infusions per session, rats are consuming less MDMA than a human user might be expected to use recreationally. As intake increases, rats will self-administer 5-10 mg/kg per session, which roughly scales to the human recreational dose. The total intake of 350 mg/kg MDMA used in this thesis is comparable to that of a heavy ecstasy user, after roughly 70 recreational doses. Most studies have shown cognitive or behavioural deficits in ecstasy users to be present at levels of total intake around or below 70 doses (Booij et al., 2014; A. K. Davis & Rosenberg, 2014; McCann, Mertl, et al., 1999; Wagner et al., 2013). Therefore, the doses of MDMA self-administered by rats in this thesis are relevant to human ecstasy users, particularly heavy users.

Key findings and future directions

This thesis made a number of novel and important findings. Firstly, characterising the adipsic and hyperactive responses to RU 24969 as 5-HT_{1B} and 5-HT_{1A} receptor mediated, respectively, was important for clarifying previous findings and facilitating further research. Earlier studies had not clearly shown the mechanism by which RU 24969 produced hyperactivity, and some had interpreted the hyperactive response as a behavioural measure of 5-HT_{1B} receptor activation (Callaway & Geyer, 1992). The results from this thesis clearly show a role of 5-HT_{1A}, but not 5-HT_{1B} receptors in RU 24969-produced hyperactivity. Furthermore, this thesis provides a straightforward behavioural assay for 5-HT_{1B} receptor activation, and this behavioural measure might be useful in preclinical tests. As outlined above, further study of the

population of 5-HT_{1B} receptors that produce the the adipsic response to RU 24969 would make this behavioural assay more useful.

As one reviewer pointed out, the facilitation of MDMA self-administration produced by RU 24969 pretreatment is a 'novel and important' finding for the addiction field, although more work needs to be done to understand this effect. I would strongly encourage further investigation of the effects of RU 24969 pretreatment on 5-HTergic systems so that the mechanism by which this pretreatment facilitated MDMA self-administration can be elucidated. Another novel finding made in this thesis was that MDMA self-administration had no effect on behavioural responses to 5-HT_{1A} or 5-HT_{1B} activation. Again, a reviewer commented that these results are interesting and important, even though the results were negative.

The studies in this thesis were based on the theory that a decreased 5-HTergic response to MDMA after repeated exposure could enhance the reinforcing effects of MDMA via altered activation of 5-HT receptors that regulate dopamine neurotransmission. Although this thesis suggests that 5-HT_{1A} and 5-HT_{1B} receptors likely play a limited role in the enhanced reinforcing efficacy of MDMA after repeated exposure, the theoretical basis for these studies is still sound. Thus, it is possible that there are other 5-HT receptors that regulate the reinforcing efficacy of MDMA and that also underlie cognitive and behavioural deficits following repeated exposure. Two 5-HT receptors that have been shown to regulate dopaminergic neurotransmission are the 5-HT_{2A} and 5-HT_{2C} receptors.

5-HT_{2C} receptors are well localised to mediate the dopaminergic responses to drugs of abuse, with high levels of 5-HT_{2C} receptors reported in dopamine terminal areas of the PFC, striatum, and NAcc, and in the VTA (Bubar & Cunningham, 2006; Clemett, Punhani, Duxon, Blackburn, & Fone, 2000; Di Matteo, De Blasi, Di Giulio, & Esposito, 2001; Eberle-Wang, Mikeladze, Uryu, & Chesselet, 1997; Ji et al., 2006). 5-HT_{2C} receptor agonists inhibited, while 5-HT_{2C} receptor antagonists enhanced, the firing rate of VTA dopamine neurons, and extracellular dopamine levels in the nucleus accumbens and PFC (Alex, Yavanian, McFarlane, Pluto, & Pehek, 2005; Di Matteo et al., 2001).

The 5-$HT_{2C/2B}$ receptor antagonist, SB 206553, and the more selective 5-HT_{2C} receptor antagonist, SB 242084, both potentiated the cocaine-produced increase in extracellular dopamine in the nucleus accumbens and striatum (Navailles, De Deurwaerdere, Porras, & Spampinato, 2004).The 5-HT_{2C} receptor agonist, Ro 60-175,

inhibited the self-administration of cocaine, ethanol, and nicotine, an effect that was reversed by the 5-HT_{2C} receptor antagonist, SB 242084 (P. J. Fletcher, Chintoh, Sinyard, & Higgins, 2004; P. J. Fletcher, Rizos, Sinyard, Tampakeras, & Higgins, 2007; Grottick, Corrigall, & Higgins, 2001; Grottick, Fletcher, & Higgins, 2000; Tomkins et al., 2002). Mice that lack the 5-HT_{2C} gene reached higher breakpoints in a progressive ratio paradigm reinforced by cocaine, and also showed enhanced levels of cocaine-induced dopamine release in the NAcc (Rocha et al., 2002).

These findings are consistent with the idea that activation of 5-HT_{2C} receptors is inhibitory to, while blockade of 5-HT_{2C} receptors facilitates, the dopaminergic response to drugs of abuse. As such, it is possible that a down-regulation of 5-HT_{2C} receptors, in response to repeated exposure to MDMA, underlies the development of MDMA as an efficacious reinforcer

Unfortunately, there is no clear evidence for 5-HT_{2C} receptor down-regulation in response to MDMA exposure. On the one hand, male ecstasy users showed blunted neuroendocrine responses to the 5-$HT_{2/1A}$ receptor agonist, m-CPP, compared to MDMA-naïve controls (McCann, Eligulashvili, Mertl, Murphy, & Ricaurte, 1999), and repeated administration of MDMA decreased 5-HT_{2C} receptor protein levels in the hippocampus of young-adult rats (García-Cabrerizo & García-Fuster, 2015), suggesting a possible down-regulation of 5-HT_{2C} receptors after MDMA exposure. On the other hand, repeated exposure to MDMA enhanced the inhibition of MDMA-produced hyperlocomotion by the 5-HT_{2C} receptor agonist, MK 212 (Ramos, Goni-Allo, & Aguirre, 2005) and increased sensitivity to the 5-$HT_{2/1A}$ receptor agonist, m-CPP (Taffe et al., 2002). Furthermore, repeated exposure to MDMA increased 5-HT_{2C} mRNA in cortex and hypothalamus (Kindlundh-Högberg et al., 2006). Further still, some animal studies have failed to show any effect of MDMA exposure on neuroendocrine or behavioural responses to m-CPP (Bull et al., 2003; Jones, Brennan, Colussi-Mas, & Schenk, 2010).

It is entirely possible that repeated exposure to MDMA in the self-administration paradigm would down-regulate 5-HT_{2C} receptors, but so far there is limited evidence to suggest this would be the case. Significantly more research is required to determine the effects of MDMA self-administration on 5-HT_{2C} receptor mechanisms. On the other hand, there is substantial evidence to suggest that neuroadaptations in 5-HT_{2A} receptor mechanisms might underlie the development of MDMA as an efficacious reinforcer in the self-administration paradigm.

5-HT_{2A} receptors are strongly expressed as excitatory 5-HTergic receptors on non-5-HTergic cells in the PFC (Eison & Mullins, 1995), where their activation has been shown to increase dopamine activity in the VTA (Bortolozzi, Díaz-Mataix, Scorza, Celada, & Artigas, 2005). This increased mesocorticolimbic dopamine release is a product of increased glutamatergic activity in projections from the PFC to the VTA (Aghajanian & Marek, 1999; Pehek, Nocjar, Roth, Byrd, & Mabrouk, 2005). The 5-$HT_{2A/2C}$ receptor antagonist, ketanserin, attenuated the dopaminergic response to MDMA in the striatum (Nash, 1990), and a similar effect was produced by local administration of the selective 5-HT_{2A} receptor antagonist, M100907, in the striatum (Schmidt, Sullivan, & Fedayal, 1994). On the other hand the non-selective 5-HT_2 receptor agonists, DOI and 5-MeODMT, both enhanced the dopaminergic response to MDMA in the striatum (Gudelsky, Yamamoto, & Nash, 1994). These data suggest that activation of the 5-HT_{2A} receptor via MDMA-induced 5-HT release would enhance the reinforcing efficacy of MDMA.

Compellingly, repeated MDMA was associated with increased 5-HT_{2A} receptor binding (Benningfield & Cowan, 2013; Urban et al., 2012)(but see McGregor et al. (2003)), suggesting that MDMA self-administration might also up-regulate 5-HT_{2A} receptors. Thus, with repeated exposure to MDMA, enhanced activation of 5-HT_{2A} receptors could underlie the development of MDMA as an efficacious reinforcer. This hypothesis is in agreement with the finding that a neurotoxic 5,7-DHT lesion, which also facilitated the acquisition of MDMA self-administration (Bradbury et al., 2014), produced an increase in 5-HT_{2A} receptor binding density in mice (Heal, Philpot, Molyneux, & Metz, 1985).

An up-regulation of 5-HT_{2A} receptors might also underlie the increased impulsivity produced by repeated exposure to MDMA. The 5-$HT_{2A/2C}$ receptor agonist, DOI, increased premature responding on the 5CSRTT (Koskinen, Haapalinna, & Sirvi, 2003; Koskinen & Sirviö, 2001), and this effect was blocked by the 5-$HT_{2A/2C}$ receptor antagonist, ketanserin (Koskinen, Ruotsalainen, Puumala, et al., 2000; Koskinen, Ruotsalainen, & Sirviö, 2000), while ketanserin (P. J. Fletcher, Tampakeras, Sinyard, & Higgins, 2007; Passetti, Dalley, & Robbins, 2003; Ruotsalainen et al., 1997; Talpos, Wilkinson, & Robbins, 2006) and the more selective 5-HT_{2A} receptor antagonist, M100907 (P. J. Fletcher, Tampakeras, et al., 2007; Winstanley, Theobald, Dalley, Glennon, & Robbins, 2004), decreased premature responses on the 5CSRTT, or the

similar 1CSRTT (Anastasio et al., 2011). Increased impulsivity, due to an up-regulation of 5-HT_{2A} receptors after repeated exposure to MDMA, would be expected to facilitate drug taking, and as such could underlie the development of an MDMA SUD (Schenk & Aronsen, 2015).

The role of the 5-HT_{2A} receptor in MDMA self-administration has not been studied. Some studies have shown no effect of regular, repeated MDMA administration on the head-twitch or locomotor responses to the 5-$HT_{2A/2C}$ receptor agonist, DOI (Granoff & Ashby Jr, 1998), or the behavioural response to the non-selective 5-HT_2 receptor agonist, mCPP (Jones et al., 2010). On the other hand, an intermittent dosing regimen of MDMA increased the head-twitch responses to DOI (Biezonski et al., 2009), suggesting that adaptations in 5-HT_{2A} receptors may be dependent on dosing regimen. Ecstasy use is typically intermittent, and 5-HT_{2A} binding was increased in human ecstasy users, compared to naïve controls, with increased exposure to MDMA associated with increased 5-HT_{2A} binding density (Di Iorio et al., 2012; Urban et al., 2012). Thus, it is possible that an up-regulation of 5-HT_{2A} receptors would be evident after MDMA self-administration.

It would be interesting to selectively up-regulate 5-HT_{2A} receptors and test for latency to acquire MDMA self-administration. An up-regulation may be achieved by repeatedly administering the selective 5-HT_{2A} receptor antagonist, M100907 (Minabe, Hashimoto, Watanabe, & Ashby, 2001). Behavioural assessment is difficult, however, because of the lack of selective 5-HT_{2A} receptor agonists. Ideally, a behavioural response to the selective antagonist, M100907, would be determined. There are some reports that M100907 enhanced the inhibition of a startle response in the pre-pulse inhibition paradigm (Padich, McCloskey, & Kehne, 1996; Zhang, Engel, Jackson, Johansson, & Svensson, 1997), although more parametric work for this behavioural response is required (Geyer, Krebs-Thomson, & Varty, 1999; Varty, Bakshi, & Geyer, 1999)

Conclusion

Repeated exposure to MDMA enhances the reinforcing efficacy of MDMA. It is possible that this increased reinforcement is due to adaptations in 5-HT receptors that regulate dopaminergic responses to MDMA. This thesis showed that 5-HT_{1A} and 5-HT_{1B} receptors likely play a limited role in the self-administration of MDMA, and thus likely do not explain the enhanced reinforcing efficacy of MDMA after repeated

exposure. Future research should consider the role of the 5-HT_{2A} receptor in neuroadaptations that might underlie the self-administration of MDMA.

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Appendix A: Publication details and permissions

Aronsen, Webster, & Schenk (2014). RU 24969-produced adipsia and hyperlocomotion: Differential role of $5HT_{1A}$ and $5HT_{1B}$ receptor mechanisms. *Pharmacology, Biochemistry and Behavior, 124*, 1-4. DOI 10.1016/j.pbb.2014.05.008

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