The interaction of oxytocin and vasopressin systems in reducing relapse to methamphetamine abuse

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**Winner Best Student Poster Presentation*

Statement of Originality and Ethical Approval

I, Nicholas Adams Everett, declare that this submission is my own work and does not represent the work or view of others, except where acknowledged in the text. No part of this thesis has been submitted for a higher degree to any other university or institution.

Ethics approval was obtained from the Macquarie University Animal Ethics Committee.

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Baracz, S. J.

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Abstract

Methamphetamine (METH) is a highly addictive psychostimulant. Currently there are no approved pharmacotherapies for METH dependence, although animal models of drug use have highlighted the therapeutic potential of the neuropeptide oxytocin (OXY) in treating METH dependence. The nucleus accumbens (NAc) core, an important brain region for addiction and relapse, has been identified as an important site for OXY-METH interactions. However, the inhibitory effect of OXY administration on relapse to METH-seeking behaviour does not appear to be mediated by the oxytocin receptor (OTR) within the NAc core, and further it has not yet been shown if the effect of systemic administration of OXY to reduce METH-seeking behaviour is mediated by the OTR. Due to the increasing evidence of OXY interactions with the vasopressin V1a receptor, the present thesis investigated a role for this receptor in mediating the attenuating effect of exogenous OXY on METH-related behaviours. The intravenous drug self-administration model of reinstatement was utilised to explore this interaction following systemic administration, and locally within the NAc core. This study is the first to demonstrate that the V1a receptor, but not the OTR, plays an important role in mediating the effects of systemic administration of OXY to attenuate METH-primed reinstatement of drug-seeking and acute METH-hyperactivity. The involvement of the V1a receptor in these inhibitory effects of OXY within the NAc core on METH-seeking behaviours remains undetermined. Results are discussed in light of other neural substrates potentially involved in OXY-METH interactions, and the advancement of OXY-based pharmacotherapies for METH-dependence.

Chapter One: Introduction

Drug addiction is a corrosive disorder which pervades most countries and societies, and takes a massive toll on the approximately 5% of the global population that abuse psychoactive drugs, as well as their families and communities (United Nations Office on Drug and Crime, 2012). Drug addiction is characterised by the compulsion to seek and administer drugs, loss of control in limiting this intake, symptoms of withdrawal when drug access is restricted, and chronic relapse to drug taking following periods of abstinence (Koob & Le Moal, 1997). Importantly, not all individuals who engage in drug use meet DSM-V criteria for a diagnosis of drug addiction (American Psychiatric Association [APA], 2013). Indeed, there is epidemiological evidence to suggest that escalation to this dependent stage of drug use only occurs in up to 15-17% of the population (Anthony, Warner & Kessler, 1994). This stage is further characterised by the valuation of the drug over previously important aspects of the user's life, and continued use despite substantial adverse consequences to the individual and others (Hyman, Malenka, & Nestler, 2006; Koob, 2008). Even though this pattern of drug abuse is associated with a multitude of problems for the user, the vast majority of drug-dependent individuals do not seek formal treatment (Abelson & Miller, 1985; Rawson, Gonzales, & Brethen, 2002), and for those that do, the rate of relapse is as high as 90% even after prolonged periods of abstinence (Hunt, Barnett, & Branch, 1971; Dejong, 1994).

Methamphetamine (METH) is amongst the most harmful of all illicit drugs abused around the world in terms of overall harm to the user, closely trailing crack cocaine and heroin, and leads in categories such as loss of relationships, impairment of mental functioning, drug related-mortality, and dependence (Nutt, King, & Phillips, 2010). Globally, amphetamines have become the second most commonly used illicit substance, trailing only cannabis (United Nations Office on Drug and Crime, 2012), and Australia now has one of the highest usage rates of METH, with over 2% of the general population reporting recent use (Australian Institute of Health and Welfare [AIHW], 2013). Furthermore, 66% of regular

drug users have reported METH use, with 20% now preferring METH as their drug of choice (Australian Crime Commission, 2013).

The prevalence of METH use has remained stable over the past 3 years, however, the type and frequency of METH use has changed substantially (AIHW, 2013). Among active METH users, consumption of the more potent crystal form 'ice' increased dramatically from 22% in 2010 to 50% of overall METH use in 2013. The frequency of METH consumption also rose in this time, with weekly use increasing from 9.3% to 15.5% in all METH users, and from 12.4% to 25% in ice users (AIHW, 2013). Taken together, this suggests a clear shift towards ice as the main form of METH consumption.

The long-term abuse of METH by individuals takes a substantial toll on the public health system. It is estimated that each year, METH abuse costs the Australian economy $8 billion through drug seizures, cleaning up of clandestine laboratories, arrests, custodial care of dependent children, hospitalisations, and treatment of health conditions associated with chronic METH use (AIHW, 2013). Whilst moderate improvements in METH-dependent individuals have been shown following intensive psychological therapy, there are currently no approved pharmacological therapies which are effective in treating METH-dependence (Ciketic et al., 2012). Given the corrosive effects METH abuse has on the individual and society, there is a clear need for further development of pharmacological treatments which reduce METH use, and facilitate long term abstinence.

1.1 Methamphetamine (METH)

1.1.1 Pharmacology and routes of administration

Methamphetamine (N-methly-O-phenylisopropylamine) is a synthetic psychostimulant, initially derived from amphetamine in 1919, and now more commonly reduced from ephedrine (Freye, 2009; Zorick, Rad, Rim, Tsuang, 2008). As the more

lipophilic of the two molecules, METH has superior penetration of the central nervous system (CNS), resulting in higher concentrations in the brain, greater potency, and increased addictive potential, compared to amphetamine (Freye, 2009; Shoblock, 2003; Zorick et al., 2008; Rose & Grant, 2008). METH also has a relatively long half-life of 12 hours, with METH and its metabolites still detectable in urine and blood 2 to 3 days post administration (Cruickshank & Dyer, 2009). Furthermore, when manufactured as a salt, METH is highly soluble in water or ethanol, increasing the ease of administration (Freye, 2009).

Methamphetamine can be readily administered via several routes, and depending on the method used, bioavailability and therefore the profile of effects vary greatly. Following oral ingestion, serum METH levels begin to rise after 30 minutes, taking up to 3 hours to peak (Cook, 1991). This results in a less intense euphoric 'rush', and typically manifests in less of a desire to take METH again. In stark contrast to this, when METH is injected or smoked, serum concentrations of METH peak within minutes of consumption, resulting in more profound acute effects, increasing the likelihood of compulsive patterns of use, and a greater range and severity of adverse effects (Freye, 2009). This rapid onset of hedonic and rewarding effects following smoking or injection elevates interest in environmental stimuli, potentially enhancing the development of drug-associated cues, and thus, contributing to the addictive potential of METH use (Freye, 2009; Meredith, Jaffe, Ang-Lee, & Saxon, 2005; Gawin & Ellinwood, 1988). Problematically, the more potent routes of administration account for up to 87% of total METH use (AIHW, 2013).

1.1.2 Mechanism of action

The administration of METH produces euphoric and addictive effects through the potent modulation of monoamine neurotransmission in the brain. Following acute METH administration, numerous events occur to result in the rapid release and enduring synaptic

concentrations of dopamine, noradrenaline and serotonin (Rothman et al., 2001; Zorick et al., 2008). Methamphetamine differentially inhibits and reverses the monoamine transporters, with greatest effect on dopamine, over noradrenalin and serotonin transport (Sulzer, Sonders, Poulsen, & Galli, 2005; Giros et al., 1996). Additionally, the metabolism of monoamines in the synaptic cleft is delayed by METH administration transiently inhibiting function of the enzyme monoamine oxidase (Gorelick & Cornish, 2009; Zorick et al., 2008). Furthermore, it has been suggested that at high concentrations, METH can diffuse into synaptic terminals through vesicular transporters (VMAT), liberating dopamine from intraneuronal vesicular stores, and causing further availability of dopamine for release into the synapse (Fleckstein, Volz, Riddle, Gibb, & Hanson, 2007). Consequently, acute METH exposure has a profound and lasting effect on synaptic monoamine concentrations.

1.1.3 Acute effects of METH use

The potent mechanisms of METH to increase synaptic monoamine concentrations are associated with a variety of subjective, emotional, cognitive and physiological effects. Following acute METH administration, users typically report experiencing a desirable, intense and euphoric 'rush' which is characterised by heightened energy, attention, curiosity, sexual libido and confidence, and decreased anxiety, fatigue and appetite (Meredith et al., 2005; Zorick et al., 2008). This 'high' can last up to 8-12 hours following ingestion, although as previous discussed, this depends on the route of administration, chosen form (e.g. 'ice'), and quantity consumed (Freye, 2009). Furthermore, METH consumption has been reported to be used for cognitive enhancement, such as improved reaction time and sustained attention (Meredith et al., 2005; Cruickshank & Dyer, 2009). Additionally, METH use has diverse and largely undesirable effects on sympathetic autonomic functioning, with self-reported and measurable physiological symptoms including hyperthermia, tachypnea, tachycardia, hypertension, and vasoconstriction (Scott et al., 2007). Other undesirable effects of METH

use may be experienced including anxiety, irritability, hallucinations, paranoia and insomnia, especially in first-time users (Elkashef et al., 2008; Darke, Kaye, McKetin, & Duflou, 2008).

The acute effects of METH administration are relatively short lived and are generally only experienced while the drug is still active, however, ongoing consumption can lead to enduring and sometimes devastating physical, cognitive and psychological complications even in the absence of drug-taking.

1.1.4 Problems associated with repeated METH consumption

Repeated abuse of METH tends to occur in a pattern of binging and crashing, whereby METH is self-administered successively, and in increasing dosage to maintain the positive, euphoric effects, and to abate the negative withdrawal symptoms following abstinence (Meredith et al., 2005; McKetin, Kaye, Clemens, & Hermens, 2013). This increase in dosage is necessary to counteract the rapid development of tolerance to METH which buffers the effects of the initial 'rush' (Darke et al., 2008). Subsequently, the concentration of METH in the bloodstream and in the brain increase dramatically as dosage and frequency of use increases to counteract the developing tolerance (McKetin et al., 2013).

The chronic ingestion of METH, particularly at high doses, can greatly affect the monoamine system, causing neurotoxicity to dopaminergic, and to a lesser extent, serotonergic systems (Meredith et al., 2005). During abstinence, following a binge pattern of METH use, rodents show persistent reductions in dopamine and serotonin levels, as well as their transporters and metabolites (Ricaurte, Schuster, & Seiden, 1980; Wagner et al., 1980). Dopaminergic cell bodies in the striatum also show substantial degeneration following chronic METH use (Ricaurte et al., 1980). Acute exposure to METH can partially restore many of these neurotoxic effects, although this effect is transient (Cass & Manning, 1999).

This dysregulation of monoamine systems is associated with substantial physical and mental health consequences.

Numerous physical complications may arise from long term METH abuse. Hospital admissions records often report that chronic METH users may suffer from cardiovascular problems (Turnipseed, Richards, Kirk, Diercks, & Amsterdam, 2003), stroke (Westover, McBride, & Haley, 2007), difficulties in pregnancy (Behnke & Smith, 2013), dental deterioration ("meth mouth"), respiratory problems, malnourishment, and increased risk of blood-borne virus transmission including HIV and sexually transmitted diseases (Hamamoto & Rhodus, 2009). METH-dependent individuals may also participate in the production of METH in clandestine labs, exposing them to toxic fumes which can cause renal failure, and harmful chemicals which can cause substantial burns (Meredith et al., 2005).

Chronic METH users are also more likely to experience emotional disturbances, neurocognitive deficits and psychiatric symptoms compared to the general population (Newton, Kalechstein, Duran, Vansluis, & Ling, 2004). This includes increased rates of psychosis (McKetin et al., 2006), hyperactivity, impulsivity, and attention deficit hyperactivity disorder which persists into adulthood (American Psychiatric Association, 2013). Severe anxiety and depressive-like symptoms are also common particularly during periods of abstinence (Scott et al., 2007). Whilst these symptoms do tend to improve within three weeks, a return to baseline typically requires at least a year of continued abstinence from all drugs (Meredith et al., 2005). Indeed, a large study of psychiatric symptoms in METH-dependent individuals found that 27% of their sample reported having attempted suicide at least one time in their life (Zweben et al., 2004). Furthermore, the severity of depression and suicidal ideation was highest amongst injecting users (Domier, Simon, Rawson, Huber, & Ling, 2000). This is especially worrying given that symptoms of anxiety and depression may contribute to the propensity to relapse during abstinence.

1.1.5 METH in reward and addiction

As previously discussed, METH administration causes potent and enduring increases in synaptic dopamine concentrations. The highly addictive properties of METH are attributable to this excessive dopamine release within regions of the mesocorticolimbic system (Kelley & Berridge, 2002; Koob, 1992; Shimosato & Ohkuma, 2000). The ventral tegmental area (VTA) is densely populated with dopaminergic cell bodies which project to a variety of regions that comprise this pathway, including the hippocampus, prefrontal cortex (PFC), amygdala and nucleus accumbens (NAc; Koob, 1992; Hyman & Malenka, 2001; Due, Huettel, Hall, & Rubin, 2002). Due to their innervation by dopamine terminals, all of these regions are potently stimulated by METH administration, an effect much more profound than natural rewards such as food (Wise, 1996) and social rewards such as monogamous pair bonding or sex (Young & Wang, 2004). Furthermore, the potent activation of the mesocorticolimbic dopamine pathway by repeated METH exposure has been associated with substantial dysregulation of the cellular and molecular mechanisms within this circuitry, as well as excessive depletion of dopamine systems (Koob, 2009; Nestler, Hope & Widnell, 1993; Robinson & Berridge, 2004).

With repeated METH use, the mesocorticolimbic system becomes activated by cues associated with the reward, important drivers for the escalation of METH use, development of drug cravings, and relapse to drug-taking (Due et al., 2002; Volkow et al., 2009). Furthermore, the incentive sensitisation theory of addiction suggests that dysregulation of the mesocorticolimbic system causes drug-related cues to become more salient to the user, whilst reducing the motivation to seek social and natural reward, further isolating the user from non-drug cues and interactions (Kelley & Berridge, 2002; Robinson & Berridge, 2004). These neuroadaptations render this circuitry hypersensitive to repeated drug administration, impair executive control, and increase the motivational ‘breaking point’ of the drug (Robinson &

Berridge, 2008). In summary, the potent activation and dysregulation of the dopaminergic reward circuitry by METH administration is critical for the transition to addiction, a devastating disorder that is notoriously challenging to treat (Koob, 2009).

1.2 A review of potential pharmacotherapies for METH dependence

There is promising evidence for the effectiveness of psychosocial treatments such as cognitive behavioural therapy, cue-exposure therapy, contingency management and group counselling for the management of addiction to METH (see reviews: Lee & Rawson, 2008; Ciketic et al., 2012; Baker & Lee, 2003). However, given the considerable cognitive impairments, psychosis, emotional instability and behavioural disorders which often characterise long term METH use, these therapies may not be suitable for all individuals (Aharonovich, Nunes, & Hasin, 2003). As such, the ongoing development of novel pharmacological interventions is necessary to assist the substantial proportion of addicts for whom no effective treatments currently exist.

The efficacy of a range of pharmacotherapies for METH dependence has been examined in both human clinical trials and pre-clinical animal models. Broadly, there is considerable literature investigating the use of antidepressants, antipsychotics, stimulants, opioid antagonists, and novel vaccines to ameliorate withdrawal symptoms, reduce METH reward, and attenuate relapse.

1.2.1 Antidepressants

There is evidence to suggest that alterations in serotonergic systems are involved in drug cravings and the expression of depressive symptomatology (Ciccocioppo, 1999; Albert & Benkelfat, 2013). Due to this, antidepressants specific for serotonergic modulation such as selective serotonin reuptake inhibitors (SSRI), have been explored for their effectiveness in reducing withdrawal symptoms and METH consumption. The initial suggestion of an

antidepressant therapy for METH dependence emerged following the demonstration that daily administration of the tricyclic antidepressant imipramine could triple the retention rates of treatment (Galloway et al., 1996). However, no reductions in METH use, depression scores or self-reported cravings were demonstrated. More promising results were obtained following SSRI treatment with fluoxetine administration producing a significant amelioration in METH-cravings, but not METH use itself (Bakti et al., 1999). The SSRI paroxetine was shown to reduce METH use after five weeks of treatment, albeit with a small effect and sample size (n=3; Piasecki, Steinagel, Thienhaus, & Kohlenberg, 2002). Indeed, the authors concede that attrition may be enhanced by the side effects of SSRI medications, with sedation, weight gain and sexual dysfunction highly polarised to the effects which METH users typically desire (Piasecki et al., 2002). In addition, not all SSRIs show promise for treating METH dependence as daily treatment with sertraline showed an increase in METH use when compared to placebo controls (Shoptaw et al., 2006).

The tetracyclic antidepressant mirtazapine has also been evaluated for its efficacy in treating METH dependence. The first clinical study demonstrated that 14 days of treatment with mirtazapine improved self-reported withdrawal symptoms, as well as anxiety and hyper arousal scores, but not depression ratings (Kongsakon, Papadopoulos, & Saguansiritham, 2005). Similarly to the previously discussed SSRI studies, mirtazapine was also associated with adverse side effects. A more recent trial of mirtazapine combined with counselling suggested its effectiveness in reducing METH use (Colfax et al., 2011). However, lack of controls for mirtazapine-only and counselling-only treatments, in conjunction with low medication adherence limits the interpretation of drug efficacy for METH dependence.

Antidepressant treatments show some ability to reduce anxiety, hyperactivity, and general drug withdrawal symptoms, yet the research shows a consistent inability for these treatments to stem METH use, and to alleviate depression-like symptoms which contribute to

METH relapse. It has been suggested that the mechanisms producing depression during METH abstinence are distinct from other depressive disorders (Newton et al., 2004) limiting the efficacy of current antidepressant therapies for METH dependent individuals. Taken together, this literature suggests that antidepressant pharmacotherapies are ineffective, and some may even be detrimental to the treatment of METH dependence.

1.2.2 Antipsychotics

Antipsychotic medications have also been explored in an attempt to buffer the positive effects of METH on the dopamine system. One such study examined the effects of the dopamine D2 receptor antagonist haloperidol on the subjective and physiological effects of acute METH administration (Wachtel, Ortengren, and de Wit, 2002). Whilst haloperidol treatment caused a sedative-like effect, there was no attenuation of the METH-induced alertness and euphoria. Pre-clinical investigations in rats demonstrated similar null effects of haloperidol treatment on METH-induced cognitive deficits (Nagai et al., 2007). The anti-psychotic chlorpromazine has been somewhat effective in attenuating acute METH effects *in vitro* and in rodents, however this has not yet translated to clinical trials (Buening & Gibb, 1974; Gibb & Kogan, 1979). Furthermore, there are substantial side effects associated with this entire class of medications, with weight gain, restlessness, depression and motor disturbances commonly reported. Thus, there is minimal evidence for the use of antipsychotic medications in preventing the acute stimulant and rewarding effects of METH, and no indication that they may reduce the propensity to relapse to METH use, although they may be effective in treating METH-induced psychoses (Curran, Byrappa & McBride, 2004).

1.2.3 Stimulants

Medications which partially mimic the dopaminergic activity and thus the reinforcing effects of METH have the potential to assist in abstinence, reduce withdrawal symptoms, and

ultimately prevent relapse (Brackins, Brahm, & Kissack, 2011). The most widely studied of these medications is bupropion, an antidepressant which inhibits dopamine and noradrenalin reuptake to have stimulant like effects. Bupropion has been shown to reduce the acute subjective effects of METH use and METH cravings (Newton et al., 2006) and to improve abstinence in low level METH users (Elkashef et al., 2008; Heinzerling et al., 2014). *In vitro* models have also shown the effectiveness of bupropion at inhibiting METH-induced dopamine release (Simmler, Wandeler, and Liechti, 2013). However, other studies have not supported these findings, with bupropion administration ineffective at reducing the reinforcing and physiological effects of METH (Stoops, Pike, Hays, Glaser and Rush, 2015), as well as having no effect on end-of-trial METH abstinence or METH self-administration compared to placebo (Anderson et al., 2015; Shoptaw et al., 2008). Overall, these mixed results suggest that bupropion may have limited use in treating METH dependent individuals.

More recent trials have examined the therapeutic potential of modafinil, a stimulant-like medication which slowly inhibits dopamine reuptake, thereby increasing extracellular dopamine concentrations over a longer duration than occurs following acute METH administration (Murillo-Rodriguez et al., 2007). Although there is some concern that this may in itself be rewarding, postmarketing surveillance suggests that the abuse potential of modafinil is limited (Stoops et al., 2005; Myrick, Malcolm, Taylor, & LaRowe, 2004). Modafinil treatment significantly reduced the severity of withdrawal symptoms during abstinence from METH (McGregor et al., 2008), reversed METH-induced impairments in working memory, cognitive processing and inhibitory control (Gonzalez et al., 2014; Kalchstein, De La Garza and Newton, 2010; Dean et al., 2011), and when combined with cognitive behavioural therapy, substantially reduced METH consumption (McElhiney, Rabkin, Rabkin, & Nunes, 2009). Furthermore, relatively high doses of modafinil have been shown to be safe for use in METH-dependent individuals (Galloway, Garrison & Mendelson,

2014). Overall, modafinil has shown promise as a potential treatment for METH dependence, although there are currently no active clinical trials registered in Australia or the USA investigating its use.

1.2.4 Opioid receptor antagonists

The opioid system regulates hedonic responses to rewarding stimuli, and its receptors are broadly distributed throughout brain regions associated with reinforcement and addiction, including the VTA, NAc, hippocampus and PFC (Merrer, Becker, Befort & Kieffer, 2009). As such, pharmacological manipulation of the activity of these receptors has been proposed as a strategy for modulating reward circuitry (Merrer et al., 2009). Naltrexone is a potent antagonist of the μ-opioid receptor, activation of which causes the characteristic euphoria, sedation and analgesia of drugs like heroin and morphine (Crabtree, 1984). A single pre-treatment with naltrexone in amphetamine-dependent individuals reduced subjective effects produced by the administration of amphetamine (Jayaram-Lindstrom et al., 2008a). Following this, a twelve week trial in amphetamine-dependent users reported that treatment with naltrexone increased negative urine samples and reduced self-reported craving and consumption of amphetamines (Jayaram-Lindstrom et al., 2008b). More recently, a promising trial in non-treatment seeking METH-dependent individuals found that naltrexone modestly attenuated the physiological effects and cravings induced by METH associated cues, and decreased many of the hedonic effects following METH administration (Ray et al., 2015). Conflicting with this, neither naltrexone, bupropion nor a combination of both medications reduced intranasal self-administration or the subjective effects of METH, although this may be reflective of a small sample size (n=7; Stoops et al., 2015). Overall, there is mixed yet promising evidence for the use of naltrexone as a pharmacotherapy for METH-dependence.

1.2.5 Immunotherapy

A novel approach to treating drug-dependence has been the development of immunotherapies which stimulate the enduring production of antibodies against the drug, restricting access to the brain, thereby reducing the rewarding and hedonic effects of drug administration (Miller et al., 2013). There is substantial pre-clinical evidence in animal models for the efficacy of a vaccine for cocaine (Kinsey, Kosten, & Orson, 2010), nicotine (Hartmann-Boyce et al., 2012), morphine and heroin (Anton et al., 2009; Baral et al., 2007). However, only limited evidence in rodents exists for an effective vaccine against METH. One potential vaccine, MH6 was shown to attenuate METH-induced temperature changes and locomotor activity in rats (Miller et al., 2013). Importantly, METH administration resulted in higher circulating METH levels and lower brain concentrations of METH in vaccinated rats, suggesting that MH6 restricted CNS penetration of METH. Furthermore, another candidate vaccine SMA-KLH has been shown to reduce acute METH-hyperactivity, as well as attenuating the formation of a conditioned place preference by METH in mice (Shen et al., 2013). Impressively, this behavioural effect was observed 10 weeks after the final booster administration of the vaccine, and serum antibodies remained elevated for another 5 weeks following this. Whilst there is early evidence for the efficacy of various METH vaccines in animals, these therapies are far less developed and understood compared to other vaccines which have reached clinical trials, such as for cocaine dependence (Martell et al., 2009). Overall, the potential for infrequent vaccinations to inoculate against METH-dependence is a novel and exciting prospect, although no suitable candidates have reached the stage of clinical trials.

This brief review of the evidence for the efficacy of pharmacological interventions for METH dependence suggests that some medications show promise, such as naltrexone, modafinil, bupropion and novel METH vaccines. However, there is currently no widely

accepted pharmacotherapy that effectively alleviates withdrawal symptoms, or reduces METH consumption above and beyond placebo. Importantly, no pharmacotherapy for METH-dependence has been approved by any major regulatory body. Clearly then, further investigations are warranted to discover novel treatments that may facilitate abstinence, and would be suitable for use as an adjunct to psychosocial therapies.

1.3 Oxytocin

Oxytocin (OXY) is a nine-amino acid mammalian peptide which was initially discovered in 1906, and was first synthesised in 1953 (Pow & Morris, 1989; Du Vigneaud et al., 1953). Since then, the OXY system has primarily been investigated for its role in facilitating uterine contractions and lactation in child birth (Soloff, Alexandrova, & Fernstrom, 1979). More recently, OXY has been investigated for its role in an array of psychological symptoms and disorders including autism, anxiety, depression and drug addiction. This incredibly varied role of OXY is likely attributable to its unique neurochemical actions within the brain.

1.3.1 Neurochemistry

There is some evidence that OXY may be produced from cells outside of the brain such as in the ovaries and testes (Gimpl & Fahrenholz, 2001), however, it is primarily synthesised by the magnocellular neurosecretory cells of the supraoptic nucleus (SON), paraventricular nucleus (PVN), and accessory nuclei of the hypothalamus (Swanson & Sawchenko, 1983). These neurosecretory cells have characteristically long axons which project to and terminate in the posterior lobe of the pituitary gland (Ludwig & Gareth, 2006). Oxytocin is stored within specialised glial cells and Herring bodies of the posterior pituitary, which assist in its release in to the blood stream for peripheral effects as a hormone (Landgraf & Neumann, 2004). The central effects of OXY, however, are largely attributed to these same

neurosecretory cells projecting centrally to act on receptors widely distributed throughout the brain (Ludwig & Leng, 2006). The magnocellular dendrites are also capable of synthesising proteins, and an abundance of OXY containing vesicles have been found within these dendrites (Ma & Morris, 2002), suggesting dendritic synthesis and vesicular release of OXY occurs at magnocellular dendrites, in addition to terminal release (Landgraf & Neumann, 2004). It has also been shown that dendritic and terminal release of OXY from the same magnocellular cell can differ, depending upon the stimulus (Ludwig & Leng 2006).

Autonomic regions of the medulla, pons and brain stem receive OXYergic projections from the PVN, and there is accumulating evidence for the role of OXY in autonomic cardiac control (Quintana et al., 2013; Lee et al., 2009). However, PVN projections primarily terminate in limbic, forebrain and midbrain regions which are likely responsible for the many higher-order behavioural effects of OXY (Fuxe et al., 2012). The advent of viral neuronal tracing techniques has recently enabled the identification of OXYergic projections from the PVN to the NAc core (Dolen et al., 2013), and from magnocellular SON neurons to a variety of forebrain structures including the NAc and central amygdala (Knobloch et al., 2012).

As a neuropeptide, OXY acts much like a classical neurotransmitter within the brain, however there are some important differences in how it functions. Synaptically released OXY may diffuse within extracellular fluid, binding to distant receptor sites of neurons with no direct connection to the pre-synaptic neuron (Zoli & Agnati, 1996). This synaptic spill-over is further enhanced by OXY temporarily escaping enzymatic degradation, enabling binding at receptor sites for up to 20 minutes (Landgraf & Neumann, 2004). Additionally, depending upon the specific combination of G-proteins coupled to the OXY receptor (OTR), a variety of intracellular signalling pathways can be activated to have diverse extracellular and behavioural effects (Gimpl & Fahrenholz, 2001; Viero et al., 2010).

Distributed widely throughout the rat brain, the OTR is abundantly expressed in various hind, mid and forebrain systems, including the thalamus, hypothalamus, limbic system, brain stem and basal ganglia (Adan et al., 1995; Gimpl & Fahrenholz, 2001). Beyond its own receptor, OXY also binds with varying affinity to the arginine vasopressin receptor subtypes (Chini & Manning, 2007; Tribollet et al., 1988). Overall, OXY has a diverse effect on the brain, owing to the dissociated synaptic and dendritic release, its rapid point-to-point communication between neurons, its slower and enduring volume diffusion, its extended viability in extracellular fluid, and promiscuous binding to varied receptor sites. Consequently, OXY has been implicated in an equally diverse range of behaviours

1.3.2 Effects on social behaviour, memory, anxiety and depression

Oxytocin is classically known for its role in milk let down and uterine contractions, however, it is becoming more widely recognised for its potent and diverse modulation of social, emotional, sexual and memory function in humans (Nishimori et al., 1996). Numerous experiments in humans have explored the involvement of OXY in various behaviours. For example in humans, sexual arousal elevated serum OXY levels in the bloodstream (Blaicher et al., 1999), and exogenous administration has been shown to enhance behaviours associated with social cognition and interpersonal communication, including eye gaze (Guastella, Mitchell & Dadds, 2008), emotion recognition (Domes et al., 2007; Hollander et al., 2007) and trust (Kosfeld et al., 2005). The perception and reciprocation of trust has also been associated with increased endogenous release of OXY (Zak, Kurzban & Matzner, 2005).

There is also evidence for the involvement of OXY in memory processes. Findings from human experiments have suggested OXY impairs recall of non-social stimuli whilst having no effect on facial recognition (Ferrier, Kennett & Devlin, 1980; Kennett, Devlin & Ferrier, 1982; Rimmele, Hediger, Heinrichs & Klaver, 2009), although this has not

consistently been shown (Fehm-Wolfsdorf et al., 1988). More recently, intranasal OXY administration was shown to improve recognition of neutral and angry, but not happy faces, suggesting a specific role of OXY dependent upon the psychobiological relevance of the stimuli (Savaskan et al., 2008). In animals, the amnesic effects following OXY administration have been consistently shown (Kovacs et al., 1979; de Wied et al., 1997; Wu and Yu, 2004), albeit with OXY attenuating or facilitating social cognition in a dose dependent manner (Bielsky et al., 2004; Popik & Vetulani, 1991). Furthermore, OTR knockout studies have demonstrated the necessity of endogenous OXY function for the normal development of social memory, but not non-social memory (Ferguson et al., 2000; 2001). Taken together, the OXY system appears to an important mediator of social but not non-social memory, although the pre-clinical and clinical findings are somewhat inconsistent.

An extensive body of rodent studies has also described the anxiolytic effects of exogenous OXY, with dose-dependent increases in time spent in open fields (Uvnas-Moberg et al., 1994), increased exploration of the open arm on the elevated plus maze (McCarthy et al., 1996), and attenuated stress-induced corticosterone release (Windle et al., 1997). Most convincingly, Ring et al. (2006) demonstrated peripherally and centrally administered OXY had a dose dependent anxiolytic-like effect in three different mouse models of anxiety, which was prevented by OTR antagonism, and of comparable efficacy to the anxiolytic benzodiazepine Alprazolam. Furthermore, mating behaviours which naturally produce sedation and calmness in rats have been shown to be driven by the endogenous OXY system (Waldherr & Neumann, 2007). Lastly, a recent human trial demonstrated that a single intranasal dose of OXY effectively reduced self-reported anxiety and objective skin conductance prior to a public speaking task (de Oliveira et al., 2012). This literature suggests that OXY has great potential as a therapy for symptoms of anxiety.

The administration of OXY has also demonstrated effective antidepressant properties. In animals, OXY treatment decreased depressive symptoms in a manner comparable to a tricyclic antidepressant (Arletti & Bertolini, 1987; Yan et al., 2014). In depressed humans, there is some evidence that the endogenous OXY system has become dysregulated, although plasma and cerebrospinal fluid measurements of OXY have been inconsistent across studies (Scantamburlo et al., 2007; Cryanowski et al., 2008; Slattery & Neumann, 2010; Parker et al., 2010). Due to these promising findings, there are numerous registered clinical trials in the USA investigating the use of OXY as an adjunct therapy for patients with major depressive disorder, dysthymia disorder, post-partum depression, and treatment resistant depression.

1.3.3 Interactions of OXY with drug reward and addiction

Limited human research has investigated the efficacy of OXY as a treatment for drug dependence. A clinical trial of intranasal OXY administration in alcohol-dependent inpatients found that after three days of treatment, OXY was effective at reducing subjective withdrawal symptoms, cravings and anxiety (Pedersen et al., 2013). Additionally, a pilot study in marijuana-dependent individuals found that OXY administration reduced the objective stress response, as well as subjective cravings and anxiety experienced during abstinence (McRae-Clark et al., 2013). Surprisingly, in cocaine dependent individuals in a court-ordered treatment program, OXY modestly increased the salience of cocaine and monetary reward, whilst impairing social cognitive performance (Lee et al., 2014). However, OXY was shown to disrupt the relationship between reactivity to cocaine cues and state anger, a trait known to play a role in drug taking and relapse (Lee et al., 2014; Fox et al., 2008). Other conflicting evidence has suggested that OXY may not be suitable for treating opioid addiction (Moaddab, Hyland & Brown, 2015). As for the endogenous OXY system, circulating OXY levels are positively associated with resilience to addiction, and negatively associated with novelty seeking in heroin users (for review see: Buisman-Piljman et al., 2014; Lin et al.,

2015). Overall, these findings suggest a complex interaction between the endogenous OXY system and addiction, with a promising role of exogenous OXY in reducing withdrawal symptoms during abstinence from alcohol, marijuana and cocaine. Currently, no clinical trials have assessed the efficacy of OXY in reducing drug consumption. Furthermore, there are no completed or registered trials in the USA or Australia exploring the effectiveness of OXY in METH-dependent individuals.

There is however a large and growing body of pre-clinical research using animal models to explore the therapeutic potential of OXY for addiction to METH and other drugs, as well the neural mechanisms by which OXY has its effects. Numerous rodent studies have demonstrated that peripheral or central administration of OXY can decrease tolerance to, and self-administration of cocaine (Sarnyai & Kovacs, 1994), heroin (Kovacs, Borthaiser, & Telegdy, 1985a), morphine (Kovacs et al., 1985b) and alcohol (McGregor & Bowen, 2012). Administration of lithium, a chemical known to cause endogenous OXY release, has also been shown to prevent the cannabinoid withdrawal syndrome in rats (Cui et al., 2001). Although not completely understood, OXY may be having these effects by inhibiting dopamine function within the NAc and other mesocorticolimbic regions, and may be reducing the incentive motivational properties of drugs (Kovacs et al., 1990; Sarnyai et al., 1990; McGregor et al., 2008; Qi et al., 2008; Baracz et al., 2013).

With respect to METH, OXY administration has been shown to interfere with many of the acute and chronic effects of METH exposure. In rodents, OXY delivered centrally or peripherally was effective at attenuating METH induced hyperactivity (Qi et al., 2008; Carson et al., 2010a). Additionally, OXY administered intracerebroventricularly (ICV) or locally within the NAc core or subthalamic nucleus (STh) abolished the METH-induced formation of a conditioned place preference (Qi et al., 2009; Baracz et al., 2012). Findings by Subiah et al. (2012) are in conflict with these data, demonstrating a modest enhancement of

the METH-induced place preference by subcutaneous (SC) OXY pre-treatment. However, this may have been due to the SC delivery not exerting central effects to the same extent as the intracranial or intraperitoneal (IP) routes found to be effective in the aforementioned studies. Furthermore, IP OXY administration decreased self-administration of METH in rats, as well as producing a partial block of METH-induced relapse following a period of extinction (Carson et al., 2010a). An effect by OXY administration to reduce METH-primed relapse has been shown consistently, with OXY injected IP, or infused into the NAc core or STh significantly reducing METH-induced reinstatement of METH-seeking behaviours (Cox et al., 2013; Hicks et al., 2014; Baracz, Everett & Cornish, 2015; in press-a). Beyond the effects of acute OXY administration on METH-induced behaviours, chronic pre-treatment with OXY has also been explored in adolescent female rats to show a reduction in the motivation to self-administer METH in adulthood (Hicks et al., 2014). Altogether, the literature suggests that both peripheral and central administration of OXY can attenuate acute METH effects, as well as METH-seeking behaviours during different phases of use.

1.3.4 Mediation of OXY by the arginine vasopressin V1a receptor

Despite a clear interaction between OXY and METH-induced behaviours, the OTR may not be the only receptor involved in the modulatory effects of OXY on METH-induced reinstatement of drug-seeking behaviour. Our group has recently demonstrated that that the attenuation of METH-primed reinstatement by local OXY treatment in the NAc core or STh was only partially reversed by selective blockade of the OTR, suggesting mediation by other receptors in these regions (Baracz et al., 2015; in press-a). Whilst an uncharacterised subtype of the OTR has been proposed (Adan et al., 1995; Chan et al., 2000), there is the possibility that OXY is interacting with the arginine vasopressin (AVP) receptor systems.

Although AVP and OXY have classically been thought to operate in opposition to each other in memory processes and social behaviours (Bohus et al., 1978; Kovacs et al., 1978; 1979), OXY has been shown to activate the three G-protein coupled AVP receptors, with 15x and 13x greater selectivity for V1a than V1b and V2 receptors, respectively (Chini & Manning, 2007; Tribollet et al., 1988). The V1a receptor is also the most widely expressed AVP receptor in the rodent brain, and has been located in a variety of hind and midbrain regions including the NAc (Hernando et al., 2001; Stoop, 2012). Both the OTR and V1a receptors have been localised in the same brain regions, however they are generally expressed in separate subregions (Tribollet et al.,1992).

Functionally, the V1a receptor has been implicated in various social and autonomic effects previously thought to be driven by the oxytocin system. In a rodent model of autism, social and cognitive deficits were rescued following OXY administration, which was subsequently reversed by the selective V1a receptor antagonist SR49059 (Sala et al., 2011). Similarly, the OXY-driven rat behaviour of adjacent lying was reduced following pre-treatment with SR49059 (Ramos et al., 2013). The administration of OXY has also been shown to activate the V1a receptor to induce defensive aggregation in rats (Bowen & McGregor, 2014) and social flank forming in Syrian hamsters (Song et al., 2014). Furthermore, the heart rate, body temperature and pressor effects of OXY treatment in rats were blocked by pre-treatment with SR49059 (Hicks et al., 2014; Wsol et al., 2014). Collectively, this suggests that OXY produces many of its behavioural and autonomic effects not only through activation of the OTR, but also by activation of the AVP V1a receptor.

1.4 Involvement of the nucleus accumbens core in oxytocin and methamphetamine interactions

The nucleus accumbens (NAc) is an important component of the neural circuitry which underpins motivation, memory of reward, and reinforcement learning (Russo et al., 2010; Ito, Robbins, & Everitt, 2004). Together with the olfactory tubercle, the NAc forms the ventral striatum, the largest region of the basal ganglia, and is an important aspect of the mesocorticolimbic dopaminergic pathway (Carlezon & Thomas, 2009). Numerous regions project to the NAc to modulate its activity. Primarily, dopaminergic input is received from the VTA and excitatory glutamatergic input is received from the amygdala, hippocampus and the prefrontal cortex (Joffe, Grueter, & Grueter, 2014; Koch, Schmid & Schnitlzer, 2000). However, the output of the NAc depends upon two discrete morphological and functional subdivisions this region: the dorsolateral core, and the medioventral shell. The NAc shell projects back to the VTA, as well as terminating in the lateral hypothalamus, extended amygdala, substantia nigra pars reticulata, and medial ventral pallidum (Joffe et al., 2014; Tripathi et al., 2010). Conversely, the NAc core projects to the dorsal aspect of the ventral pallidum, the medial globus pallidus, STh and substantia nigra pars compacta (Joffe et al., 2014; Tripathi et al., 2010).

As a whole, the NAc is involved in the early stages of drug addiction (Bjorklund et al., 2008). Acute METH administration increases dopamine release within the NAc, leading to the pleasure and behavioural effects associated with acute reward (Broom & Yamamoto, 2005; De Chiara, 2002). Additionally, exposure to drug-associated cues and contexts has been associated with increased dopamine release within the NAc (Ito et al., 2000). However, owing to the dissociated outputs, the NAc core and shell are involved in different aspects of drug-related reward (Di Chiara, 2002). It has been suggested that the NAc shell is primarily

associated with the initial invigorating and euphoric effects of psychostimulants (De Chiara, 2002; Ito et al., 2004; Broom & Yamamoto, 2005), whilst the NAc core is involved in the motivation to engage in behaviours based on reward-associated stimuli and cues (Ito et al., 2004). Indeed, lesions to the NAc core appear to disrupt the conditioned reinforcement between drug and cues, attenuating drug-seeking behaviour (Ito et al., 2004). Furthermore, a role for dopaminergic signalling within the NAc core in this effect was demonstrated by Saunders et al. (2013), whereby local antagonism of dopamine receptors reduced cue-induced cocaine-seeking in rats. Overall, this highlights the importance of dopamine activity in the NAc core to produce behavioural responses to rewarding, or reward-associated stimuli.

Importantly, several studies have demonstrated that drug-seeking behaviours driven by the NAc core can be attenuated by OXY administration. Early findings reported that local OXY infusions into the NAc core could reduce the self-administration of heroin (Ibragimov et al., 1987), and inhibit acute cocaine stereotyped behaviours (Sarnyai et al., 1991). More recently, OXY was shown to substantially reduce METH-induced c-Fos expression in several discrete brain regions, including the NAc core (Carson et al., 2010b). Building upon these findings, our group has demonstrated the ability of OXY administered into the NAc core to attenuate the formation of a METH-induced conditioned place preference (Baracz et al., 2012). Furthermore, and consistent with the functional NAc core-shell divide (De Chiara, 2002), OXY administered into the NAc core dose dependently attenuated METH-induced reinstatement of previous METH-seeking behaviours, although had no effect on METH-hyperactivity (Baracz et al., in press-a). However, selective blockade of the OTR within the NAc core did not significantly reverse the effect of OXY on METH-primed reinstatement, suggesting that OXY may activate other receptors in the core subregion to have this inhibitory effect on relapse to METH-seeking.

As previously discussed, OXY binds with reasonable affinity to the AVP V1a receptor, which has been localised within the NAc core, and has been implicated in a variety of OXY-driven behaviours. Considering this, and to further the clinical utility of OXY, continued delineation of the receptor interactions of OXY in reducing relapse to METH is required, both globally in the brain and locally within the NAc core. To this end, animal models of drug addiction provide a means of elucidating the central mechanisms of OXY administration to reduce drug-seeking behaviours.

1.5 Intravenous drug-self administration paradigm in rats

To understand the neurobiological processes which contribute to the initiation, maintenance, abstinence and relapse of drug-self administration in humans is vital to furthering addiction medicine. Unfortunately, studies of human self-administration are limited by ethical practicalities, self-report biases, lack of control over cognitive and genetic factors, co-morbidities with other psychological disorders, and often, concurrent use of multiple psychoactive drugs. However, animal models equip researchers with the means to investigate addiction to specific drugs with a high degree of experimental control, to delineate the influence of developmental, environmental and genetic factors, as well as the mechanisms and efficacy of pharmacological interventions.

Of the most commonly used animal models, the drug self-administration paradigm corresponds most directly with human addictive behaviours. Based in operant conditioning theory, self-administration typically involves the animal engaging in a behaviour which produces rewarding stimuli, such as delivery of a drug. In this case, the drug acts as a reinforcer of the otherwise inconsequential behaviour, increasing the likelihood of the animal to engage in that behaviour in future (Panlilio & Goldberg, 2007). The development of this

operant conditioning depends on the nature of the relationship between behaviour and response: the schedule of reinforcement.

The schedule of reinforcement during drug self-administration describes when and how the operant behaviour is reinforced with delivery of the drug. These two factors can have a significant impact upon the rate of responding, as well as the strength of associative learning. Most commonly, the operant behaviour is reinforced on a fixed-ratio (FR) schedule whereby a pre-determined number of responses are required to produce drug reinforcement (e.g. FR-1: Baracz et al., 2015; FR-5: Neugebauer et al., 2007). Fixed-ratio schedules are extensively used as they enable the most direct examination of the relationship between the operant behaviour and the drug infusion (Sanchis-Segura & Spanagel, 2006). Furthermore, FR-1 schedules may most closely resemble human drug taking behaviour. Alternatively, variable and progressive-ratio schedules arithmetically or logarithmically vary the amount of responding required for reinforcement of the operant behaviour to occur (Richardson & Roberts, 1996). These schedules are typically utilised to indirectly measure motivation to self-administer, and to index the reinforcing effects of a drug (Stafford et al., 1998) In this way, the breaking point of different rewarding drugs can be characterised, reflecting the maximal effort expended by an animal to receive drug-reward.

Additionally, the route of drug administration can significantly influence the acquisition of self-administration. The fastest route, and therefore the one which produces the strongest reinforcing effects, is typically through infusions via a surgically implanted intravenous (IV) catheter (Panlilo & Goldberg, 2007). Intravenous drug administration results in rapid central effects, enhancing the formation of operant and pavlovian associations (Panlilo & Goldberg, 2007). Whilst inhalation, insufflation and oral administration can also be utilised in animal models, IV infusions provide the most ecologically valid delivery, especially given the increasing pattern of injection METH use in Australia (AIHW, 2013).

Additionally, the drug reinforcement of an operant response has the potential to elicit classical conditioning. For example, stimuli present in the environment during reinforcement may become associated with the drug, signalling its availability and further reinforcing and maintaining the drug-seeking behaviour (Panlilo & Goldberg, 2007). In this way, lights, audible tones and scents can be reliably paired to the operant behaviour and drug reinforcement. Through this association, these cues may also motivate drug seeking behaviours, in a manner akin to cravings. Presentation or removal of these cues can be manipulated during various stages of the paradigm to model the effects of associated cues on acquisition, abstinence and relapse in humans.

This reliable process of acquiring and maintaining drug self-administration in rodents has been extensively utilised to explore the reinforcing properties and neurobiological mechanisms of many drugs, including nicotine (Corrigall et al., 1992; Clemens et al., 2014), alcohol (Mello, 1976; Willcocks & McNally, 2013), heroin (Babor et al., 1976), mephedrone (Motbey et al., 2013), MDMA (Cornish et al., 2003), cocaine (Caine & Koob, 1993; James, Charnley, Flynn, Smith, & Dayas, 2011), cocaine and heroin 'speedball' (Cornish et al., 2005), amphetamine (Yokel & Pickens, 1974; Calipari et al., 2014) and methamphetamine (Munzar et al., 1999; Baracz et al., 2015; in press-a,b). However, numerous modifications to this paradigm can be employed to enable the examination of a variety of factors associated with drug addiction, such as extended access (Ahmed & Koob, 1998), discrimination between different drugs (Goodwin & Baker, 2000), the impact of choice on drug administration (Ahmed, 2010), the disparate effects of active versus passive drug administration (Galici et al., 2000), and the identification of phenotypes most vulnerable to addiction (Deroche-Gamonet, Belin, & Piazza, 2004).

Importantly, self-administration has been widely used to investigate relapse to drug seeking behaviours following a period of abstinence (Epstein et al., 2006). Generally, animals

are trained to self-administer a drug, and following a period of maintained operant responding, drug infusions are discontinued and the operant behaviour is no longer reinforced (Epstein et al., 2006). This extinction period continues until the behaviour is reduced to a specified level of responding (Shaham et al., 2003). At this point, previous drug seeking behaviours can be reinstated by exposing extinguished rats to a non-contingent drug or non-drug trigger. Successful reinstatement is determined by a significantly higher engagement in the behaviour previously associated with drug administration, when compared to control treatments and previous extinction sessions (Panlilo & Goldberg, 2007; Shaham et al., 2003).

Environmental cues, stressors and exposure to drugs have been shown to reliably reinstate previously learnt behaviour (Bossert et al., 2013). For example, lights or audible tones which were present contingently during drug delivery but not extinction, as well as novel cues can induce reinstatement (Sanchis-Segura & Spangel, 2006). Exposure to a variety of stressors such as bright lights, loud noises, foot shocks and the pharmacological stressor yohimbine can also induce reinstatement (Bosster et al., 2013; Shaham et al., 2003). Lastly, a relatively small dose of the self-administered drug, or even another reinforcing drug can reinstate rats to previous drug-seeking behaviours (Sanchis-Segura & Spanagel, 2006). Importantly, numerous experiments in rats have demonstrated that METH-seeking behaviours can be reliably reinstated following a non-contingent injection of METH (Anggadiredja et al., 2004; Carson et al., 2010a; Reichel & See, 2010; Baracz et al., 2015; in press-a; Hicks et al., 2014).

For these reasons the self-administration paradigm is widely considered to be the ‘gold standard’ of preclinical addiction research. However, the conditioned place preference (CPP) procedure is also a sensitive and reliable tool for understanding the rewarding potential of drugs, and investigating the efficacy of pharmacotherapies in reducing acute reward and motivation to drug-seek (Bardo, Rowlett & Harris, 1995). This technique examines the ability

of rewarding drugs to induce a preference for the context in which the drug was non-contingently administered, and typically involves between one and four of the drug-pairing sessions (Baracz et al., 2012; Bardo et al., 2003). Although CPP can also be used to model cue-induced relapse (Mueller & Stewart, 2010), the non-contingent drug delivery and relatively small exposure to the drug are unlikely to result in the substantial neurobiological changes which occur in human addicts. Thus, the self-administration model of reinstatement may be more appropriate for modelling chronic abuse of and relapse to drugs.

Overall, the reinstatement model of self-administration in animals is a highly amenable technique for modelling a range of factors which influence human drug abuse and relapse, and for developing and trialling potential pharmacotherapies for human drug addicts. This paradigm is also highly suited for combining with contemporary neuroscience techniques such as: *in vivo* microdialysis to dynamically measure neurotransmitters in discrete brain regions; pharmacological and optogenetic excitation or inhibition of specific neural substrates; and transgenic modulation of receptor expression. In summary, given its reliability, high degree of face validity, construct validity and adaptability, the drug self-administration procedure provides an excellent means for investigating potential pharmacotherapies for preventing relapse to drug addiction in humans, and for delineating the neural mechanisms by which they effect drug-seeking behaviour.

1.6 Aims and hypotheses

The promising effects of OXY administration on acute METH reward, chronic abuse and relapse motivate further investigation into the mechanisms which mediate this OXY-METH interaction in the brain. Currently, there is limited understanding of the receptors which OXY activates within the NAc core to attenuate reinstatement to METH-seeking behaviours, and a similar gap in the understanding of which receptors mediate its actions when administered peripherally. In light of this, the current thesis will examine the involvement of the OTR and vasopressin V1a receptors in the attenuating effect OXY has on METH-primed reinstatement to METH-seeking behaviours. This will be explored systemically, and locally in the NAc core.

More specifically, it is hypothesised that in experiment 1:

1) Systemic administration of OXY will significantly reduce METH-primed reinstatement of active lever pressing and locomotor activity;
2) Systemic OTR blockade will have a significant but partial inhibition of this effect; and
3) Systemic antagonism of the V1a receptor will significantly inhibit the effect of OXY to reduce METH-seeking behaviour.

In experiment 2, it is hypothesised that:

1) OXY microinjected into the NAc core will significantly reduce METH-primed reinstatement of active lever pressing, but not locomotor activity;
2) OTR antagonism in the NAc core will partially, but not significantly inhibit these effects; and
3) Pharmacological blockade of the V1a receptors within the NAc core will significantly attenuate the inhibitory effects of OXY on METH-primed reinstatement to active lever pressing

Chapter Two: Methods

2.1 Animals

Thirty-two male Sprague Dawley rats (Animal Resources Centre, Perth, WA, Australia) weighing 250-300g were housed in pairs (cage size: 40x27x16cm) with food and water available *ad libitum* except during experimental procedures. The housing room was lit on a 12-hour light/dark cycle (lights on 06:00), with all experiments conducted in the light cycle. Both the experimental and housing rooms were maintained at 21^{o}C (+/-1). Prior to surgeries, rats were acclimatised to the facility for seven days, and were handled by the primary researcher daily. This extensive habituation precluded handling induced stress in the rats during experimental sessions.

2.2 Drugs

Methamphetamine (99% purity) was purchased from the Australian Government Analytical Laboratories (Pymble, NSW, Australia). METH was dissolved in physiological saline (0.9%) for intraperitoneal (IP) injection (1.0 mg/kg/ml) or filtered through a Millipore syringe filter (0.22 μm) for intravenous (IV) self-administration (0.1 mg/kg/ml).

2.2.1 Experiment 1

Oxytocin (OXY) was synthesised by AusPep Ltd (Parkville, VIC, Australia) and dissolved in saline at a dose of 1 mg/kg. The selective vasopressin V1a receptor antagonist SR49059 (Axon MedChem BV, The Netherlands) was chosen for its high affinity for vasopressin V1a receptors (K_i 2.2nM), its low affinity for V1b (K_i 671nM) and OXY (K_i 100nM) receptors, and recent use in inhibiting OXY-related behaviours (Serradeil-Le Gal et al., 1993; Tribollet et al., 1999; Ramos et al., 2013; Hicks et al., 2015). The selective oxytocin receptor antagonist, Compound 25 was chosen for its high affinity for OXY receptors (K_i 9.5nM) and low affinity for V1a receptors (K_i 1120nM). Compound 25 was

synthesised at The University of Sydney (NSW, Australia) as previously described (Brown et al., 2010), and based on proton, carbon nuclear magnetic resonance spectroscopy and mass spectrometry, was considered to be >95% purity. SR49059 and Compound 25 were dissolved in a vehicle of 15% dimethyl sulfoxide (DMSO), 2% Tween-80, and 83% physiological saline at varying doses (SR49059: 0.3 and 1 mg/kg; C25: 10 mg/kg). All drugs were freshly prepared immediately prior to IP administration at a volume of 1 ml/kg.

2.2.2 Experiment 2

The selective oxytocin receptor antagonist desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT was a gift from Dr. Maurice Manning (Department of Biochemistry and Cancer Biology, The University of Toledo, USA). This antagonist was chosen due to its high affinity for OXY receptors (K_i 0.9nM), relatively low affinity for vasopressin V1a receptors (K_i 100nM; (Manning et al., 2012), and for consistency with our previous research in the NAc core (Baracz et al., 2012; in press-a). OXY, SR49059 and desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT were dissolved in a vehicle of 15% dimethyl sulfoxide (DMSO), 2% Tween-80, and 83% physiological saline at varying doses (OXY: 3 pmol; SR49059: 3 pmol; desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT; 1.5 nmol). All solutions were prepared daily (500 nl delivered to the NAc core, each side).

2.3 Surgeries

Prior to experimentation, all 32 rats were anaesthetised with 3% isoflurane in oxygen (2 L/min), given the non-steroidal anti-inflammatory analgesic Carprofen (5mg/kg/ml, subcutaenous), and underwent surgery to implant a chronic indwelling catheter into the right jugular vein. Catheter construction and implantation was conducted as previously described (Motbey et al., 2013). To ensure patency and prevent infection, catheters were flushed with

0.2ml (60 IU) of the anticoagulant heparin, and 0.2ml of the antibiotic cephazolin sodium (20mg). Immediately following catheterisation, 16 of the 32 the rats were transferred to a heated recovery chamber (27ºC) and monitored for 45 mins before being individually housed for two days.

The remaining 16 rats underwent intracranial surgery to implant bilateral cannulae for the purpose of microinjections into the NAc core. Rats were placed in a standard stereotactic instrument and the skull was exposed. Using NAc core co-ordinates obtained from the rat brain atlas of Paxinos and Watson (2005; with nosebar at -3.3 mm, measured from bregma: anterior/posterior +1.3 mm, medial/lateral +1.5 mm; dorsal/ventral – 6.5 mm), two stainless steel guide cannula (26 gauge, 14 mm) were placed bilaterally through small holes drilled in the skull, and lowered to 1 mm above the NAc core. Four surgical screws were inserted into the skull surrounding the cannulae, and cranioplastic cement was applied to secure the implants in place. Stainless steel stylets were inserted into each cannula to maintain its patency and prevent infection for the duration of the experiment.

All surgical tools and implants were sterilised in an autoclave or disinfected in chlorhexidine with alcohol, and aseptic surgical techniques were used.

2.3.1 Post-operative care

All rats received 7 days of post-operative care prior to the commencement of experimental procedures. For the first two days, rats were treated with Carprofen (5 mg/kg/ml subcutaneous) for pain management. For the entire post-operative period, catheter patency was maintained and infection was prevented with daily IV infusions of the anticoagulant heparin (60 IU in 0.2ml) and the antibiotic cephazolin sodium (20mg in 0.2ml). Weight and behaviour was also recorded daily to monitor general health. Throughout the entire experimental procedure, cannula patency was maintained by replacing missing stylets.

2.4 Self-administration procedure

Operant conditioning chambers (30x24x29 cm; Med Associates, VT, USA) were housed inside of a sound attenuated box (56x56x41 cm). Each chamber had an exhaust fan for providing background noise and ventilation, six locomotor activity detectors, a house light, and two retractable levers. MED-PC IV software (Med-PC, VT, USA) was used to control the chambers and record behavioural activity. One lever ("active") was programmed to activate the infusion pump (MedAssociates, VT, USA) which sat outside of the chamber, whilst the other lever ("inactive") had no consequence when engaged. A syringe was fitted to each infusion pump, which connected to PE50 tubing protected by a metal spring connector (Plastics One, VA, USA). At the beginning of each session, the tubing was flushed with a 70% ethanol solution, and then connected to a syringe filled with freshly prepared METH dissolved in saline (0.1 mg/kg/0.05 ml). Catheters were flushed with 0.1 ml heparinised saline (10 IU/ml) to ensure no blockages were present. Rats were then placed inside of the operant chambers, and attached to the spring connector. The initiation of the session was indicated by extension of the retractable levers, and illumination of the house light.

Figure 1 Animal depressing the active lever to receive an infusion of methamphetamine

During each 2-hour session, rats were trained to self-administer METH on a fixed ratio 1 (FR1; Figure 1). The allocation of the active and inactive levers was counterbalanced across chambers. Depression of the active lever resulted in a 3-second 0.05 ml infusion of METH (0.1 mg/kg). For 20 seconds following an active lever press, the house light was extinguished, and depression of the active lever did not result in an infusion. This time out period ensured the rat did not receive another infusion before experiencing the effects of the first, as well as reducing the risk of overdose. This also enabled the house light to signal the availability of drug, and lever presses were still recorded during this period. Engaging the inactive lever at any time resulted in no programmed consequences. The session ended after either two hours had elapsed, or to prevent risk of overdose, a maximum of 60 infusions had been received.

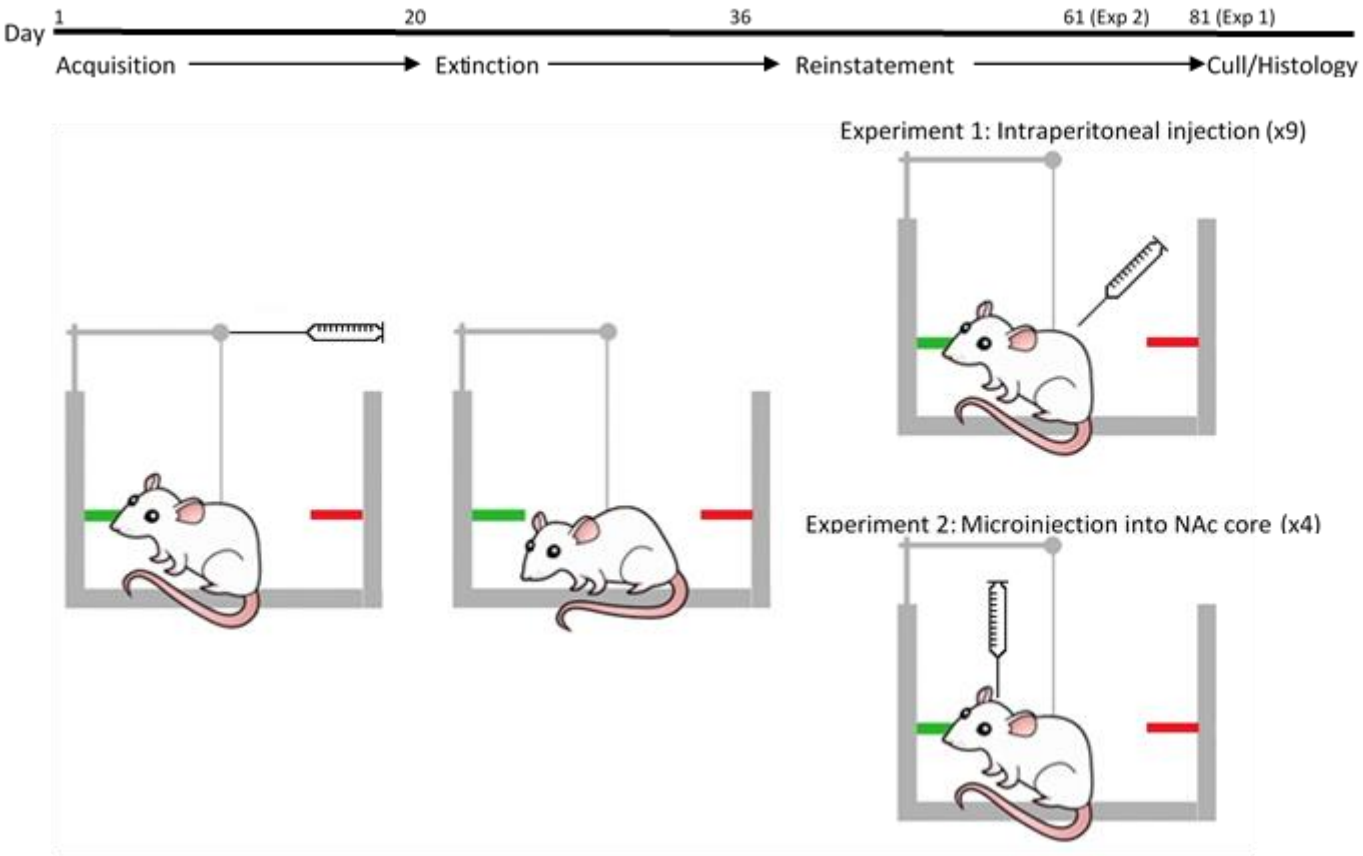

Figure 2 Self-administration and reinstatement timeline for experiment 1 and 2.

Conclusion of the session was indicated by the house light turning off, and the retraction of both levers. After each session, the rats were disconnected from the infusion line, their catheters were flushed with 0.2 ml of cephazolin sodium (100 mg/ml) in heparinised saline solution (300 IU/ml), and they were returned to their home cages. Self-administration sessions were conducted five days per week for four weeks, totalling 20 sessions (Figure 2).

2.5 Behavioural extinction

Following the 20th day of METH self-administration, rats underwent daily 2-hour extinction sessions. During this time, depression of the active lever resulted in a 3-second infusion of saline (0.05 ml). Barring this, extinction sessions were identical to the self-administration days. Each rat continued under these conditions for at least 10 days, and until < 10 active lever presses were made per session for two consecutive days. Furthermore, extinction continued until the entire group no longer demonstrated a statistically significant preference for the active lever for two consecutive days. Immediately following each of the final three extinction sessions, all rats received an IP injection of saline (1 mg/kg). Additionally for experiment 2, all rats received a sham microinjection on the final extinction day. This was done to habituate the rats to the reinstatement treatment procedures.

2.6 METH-primed reinstatement

2.6.1 Experiment 1: *Effects of peripherally administered OXY on METH-primed reinstatement when pre-treated with either SR49059 or Compound 25*

A total of ten reinstatement sessions were conducted, each separated by three extinction days to ensure all drugs had been metabolised, and that any METH-induced relapse to lever pressing had been re-extinguished. Reinstatement sessions were identical to extinction days, except that immediately prior to commencement, rats received an IP prime of

METH (1 mg/kg) or normal saline (1 ml/kg). The time out period on infusions was also disabled to accurately capture the rats' motivation to respond over time. Depending on the treatment schedule rats received either one or two IP treatments prior to the METH prime. All IP injections were separated by 5 min intervals. Rats were then immediately placed in the operant conditioning chamber, connected to the infusion line, and the session commenced.

Table 1. Experiment 1 treatment schedule (peripheral administration)

Session	IP pre-treatment 1		IP pre-treatment 2		IP prime	
1	-		Vehicle		METH	
2	-		Oxytocin (1 mg/kg)		METH	
3	-		SR49059 (1 mg/kg)		METH	
4	-		SR49059 (0.3 mg/kg)		METH	
5	SR49059 (1 mg/kg)	5 minutes	Oxytocin (1 mg/kg)	5 minutes	METH	2-hour session commences
6	Compound 25 (10 mg/kg)		Oxytocin (1 mg/kg)		METH	
7	-		Compound 25 (10 mg/kg)		METH	
8	-		SR49059 (1 mg/kg)		Saline	
9	-		Compound 25 (10 mg/kg)		Saline	
10	Vehicle		Vehicle		METH	

Prior to each of the first four METH-primed reinstatement sessions, rats (n = 16) received an IP injection of vehicle (1 ml/kg), oxytocin or one of two doses of SR49059 (see Table 1). A Latin square design was utilised to prevent the possibility of ordering effects over these four treatments. After ascertaining the independent effects of vehicle, oxytocin and SR49059 on METH-primed reinstatement, five additional sessions were conducted. Session's five and six involved pre-treatment with either SR49059 or Compound 25 respectively, followed by the oxytocin pre-treatment, and then finally the METH prime. For session seven, rats were pre-treated with Compound 25 only before receiving METH. For sessions eight and nine, rats were randomly allocated to receive either Compound 25 or SR49059, followed by saline in lieu of METH. The tenth and final reinstatement session was conducted to ensure

that after extensive testing, rats were still seeking METH to the same extent as the first vehicle+METH reinstatement session. For this, rats were pre-treated with two vehicle injections (1 ml/kg) before receiving the METH prime. This was done to control for the potentially confounding stress induced by a third IP injection in sessions five and six.

2.6.2 Experiment 2: *Effects of OXY administration in the NAc core on METH-primed reinstatement when co-administered with either SR49059 or desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT*

A total of four reinstatement sessions were conducted in a similar manner to experiment 1 (Table 2), except that instead of pre-treating oxytocin with a receptor antagonist antagonists separated by five minutes, they were co-delivered in cocktails. These sessions were conducted on the basis of systemically administered SR49059 significantly and substantially blocking oxytocin's reduction of METH-induced reinstatement to active lever pressing. Prior to these METH-primed sessions, rats received a bilateral microinjection of OXY, vehicle, or OXY co-administered with SR49059 or desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT (1.5 nmol). A Latin-square design was utilised to preclude the ordering effect of drug treatments.

Table 2. Reinstatement schedule of microinjections into the nucleus accumbens core (Experiment 2)

Session	IC pre-treatment		IP prime	
1	Vehicle	5 minutes	METH	2-hour session commences
2	Oxytocin (3 pmol)		METH	
3	Oxytocin (3 pmol) + SR49059 (3 pmol)		METH	
4	Oxytocin (3 pmol) + desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT (1.5 nmol)		METH	

The effects of SR49059 alone were established in Experiment 1, and so were not explored in the NAc core. Similarly, important oxytocin and desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT controls have previously been conducted by our group, and so were not repeated (Baracz & Cornish, 2013; Baracz et al., in press-a). Doses for OXY and desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT were also based on these previous findings. However, SR49059 has only been administered ICV, or intracranially in hindbrain regions, so the dose used in the current study was matched to the OXY dose (Bergen et al., 1997; Kc et al., 2010; Stojičić et al., 2008).

All treatments were bilaterally infused into the NAc core (500 nl/side) over 1 minute. Infusions were delivered through custom made microinjectors (33 gauge; 20 mm) attached by PE50 tubing to a 1 µl Hamilton syringe, and driven by a microinjection pump (Harvard Apparatus, Hooliston, MA, USA).

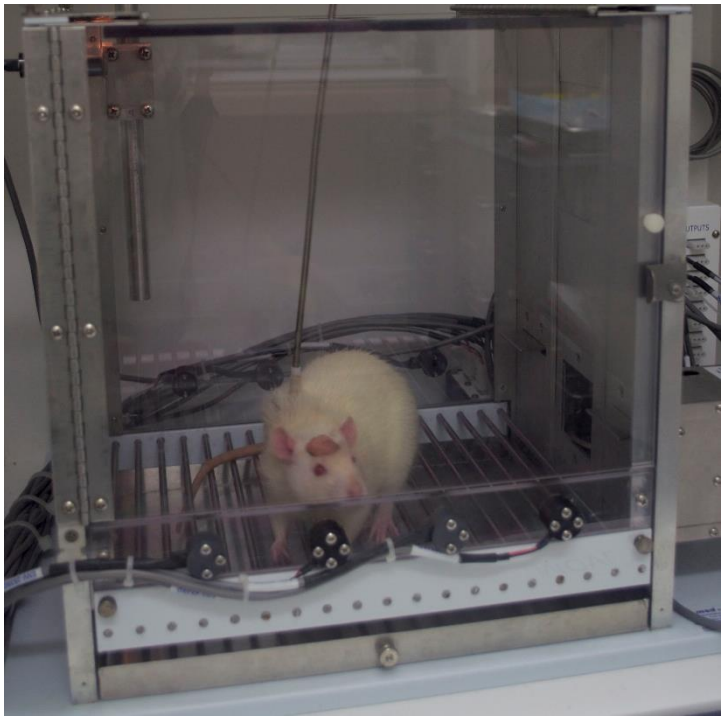

Figure 3 Animal implanted with bilateral intracranial cannulae in operant chamber

2.7 Histology

Upon the completion of testing, rats were sacrificed with an overdose of 1ml sodium pentobarbitone (135 mg/ml, IP) and underwent intracardiac perfusion with saline (0.9%, 40 ml) followed by formalin (10%, 40 ml). Brains were carefully extracted and fixed in a solution of 10% formalin for 7 days. Following this, 60μm thick coronal sections were made using a cryostat, and mounted on to gelatin-coated slides. Under a microscope, and according to the rat brain atlas of Paxinos & Watson (2005), cannulae placements were verified. Only data from rats with bilateral cannulae placements verified to be in the NAc core were included in the following analyses.

2.8 Statistical Analysis

2.8.1 Self-administration

To ensure rats acquired METH self-administration, the number of daily infusions, active lever presses, and inactive lever presses was compared across the 20-day period using repeated measures ANOVAs, with the first and final day of each measure being directly compared. The number of active and inactive lever presses on the final self-administration session was also compared to ensure the cohort could differentiate between the two levers. Locomotor activity was analysed using a repeated measures ANOVA, contrasting the first and the last day. Rats which failed to acquire a preference for the drug-paired lever over the unpaired lever were not included in the final analyses. All contrasts were conducted *a priori*.

2.8.2 Behavioural extinction

Strict criteria were set to ensure rats had extinguished their METH-paired responses, before reinstatement testing could begin. The extinction period extended for a minimum of 10 days, until each rat made < 10 active lever presses for two consecutive sessions. To ensure that the preference for the drug-paired lever had been abolished across the whole group, extinction continued until two consecutive days of a non-significant difference between active and inactive lever presses was achieved, as assessed by paired t-tests. Lastly, the mean active lever pressing and locomotor activity from the last three METH self-administration sessions was compared with the mean active lever pressing and locomotor activity from the last three extinction sessions using a repeated measures ANOVA.

2.8.3 METH-primed reinstatement

Analysis of reinstatement data was conducted on the first hour of each session, due to the time course of the METH-prime. To determine whether rats had reinstated to their previous METH-paired lever responding, active lever pressing across all reinstatement conditions was contrasted to the prior extinction sessions within a repeated measures ANOVA. To ensure that increased lever pressing was not due to METH-hyperactivity, active and inactive lever pressing were compared within each reinstatement condition in a repeated measures ANOVA. Locomotor activity was also compared between reinstatement and extinction sessions, and across reinstatement sessions using a repeated measures ANOVA. Only rats which had reinstated to their previous METH-paired lever responding behaviour (+/- 2 SD) following the initial vehicle+METH session were included in the final analyses.

For both experiments, a series of *a priori* contrasts within a one-way repeated measures ANOVA were conducted to determine the effects of each condition on active lever pressing and locomotor activity. Firstly, the oxytocin conditions were directly contrasted to

the initial vehicle conditions. The magnitude of oxytocin's effects were also assessed using Cohen's D and interpreted using standard cut-offs (Cohen, 1992). Additionally, pre-treatments and co-administrations of SR49059, Compound 25 or desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT with oxytocin were directly compared to the oxytocin+METH conditions in each experiment, to assess whether treatment with any of the receptor antagonists had significantly inhibited oxytocin's effects. To determine whether receptor blockade had partially or completely inhibited oxytocin's effects, these treatments were also compared to the vehicle+METH session.

In experiment 1, the SR49059+METH and Compound 25+METH conditions were contrasted to the initial vehicle+METH session to ascertain their independent effects on METH-induced reinstatement. The initial and final vehicle+METH sessions were also compared to determine whether rats were still reinstating to the same level of lever pressing activity following substantial testing.

Chapter Three: Results

3.1 Experiment 1: *Effects of peripherally administered oxytocin on METH-primed reinstatement when pre-treated with either SR49059 or Compound 25.*

Of the original 16 rats which underwent jugular catheterisation only, 3 were removed from the study due to: non-acquisition of METH IVSA (1), loss of catheter patency (1), and failure to reinstate to METH-seeking behaviours following the METH-prime (1).

3.1.1 METH self-administration

Rats (n = 13) acquired intravenous METH self-administration, as indicated by a significant increase in infusions of METH over the 20-day period (day 1 M = 10.077, SEM = 3.360; day 20 M = 30.846, SEM = 2.917; $F(1, 12) = 23.543$, $P < 0.000$; Figure 4a). Active lever pressing also significantly increased over the self-administration period (day 1 M = 14.923, SEM = 5.509; day 20 M = 40.462, SEM = 4.814; $F(1, 12) = 10.675$, $P = 0.007$; Figure 4b), whilst inactive lever pressing did not (day 1 M = 8.231, SEM = 2.004; day 20 M = 5.231, SEM = 1.991; $F(1, 12) = 0.898$, $P = 0.362$). No significant preference for either lever was observed on day 1 ($t(12) = 1.756$: $P = 0.104$), whilst a significant preference for the active lever was measured on the final day ($t(12) = 7.205$; $P < 0.000$). Locomotor activity also significantly increased across the 20-day period (day 1 M = 2040.077, SEM = 219.276; day 20 M = 4195.000, SEM = 598.919; $F(1, 12) = 15.212$; $P = 0.002$; Figure 4c).

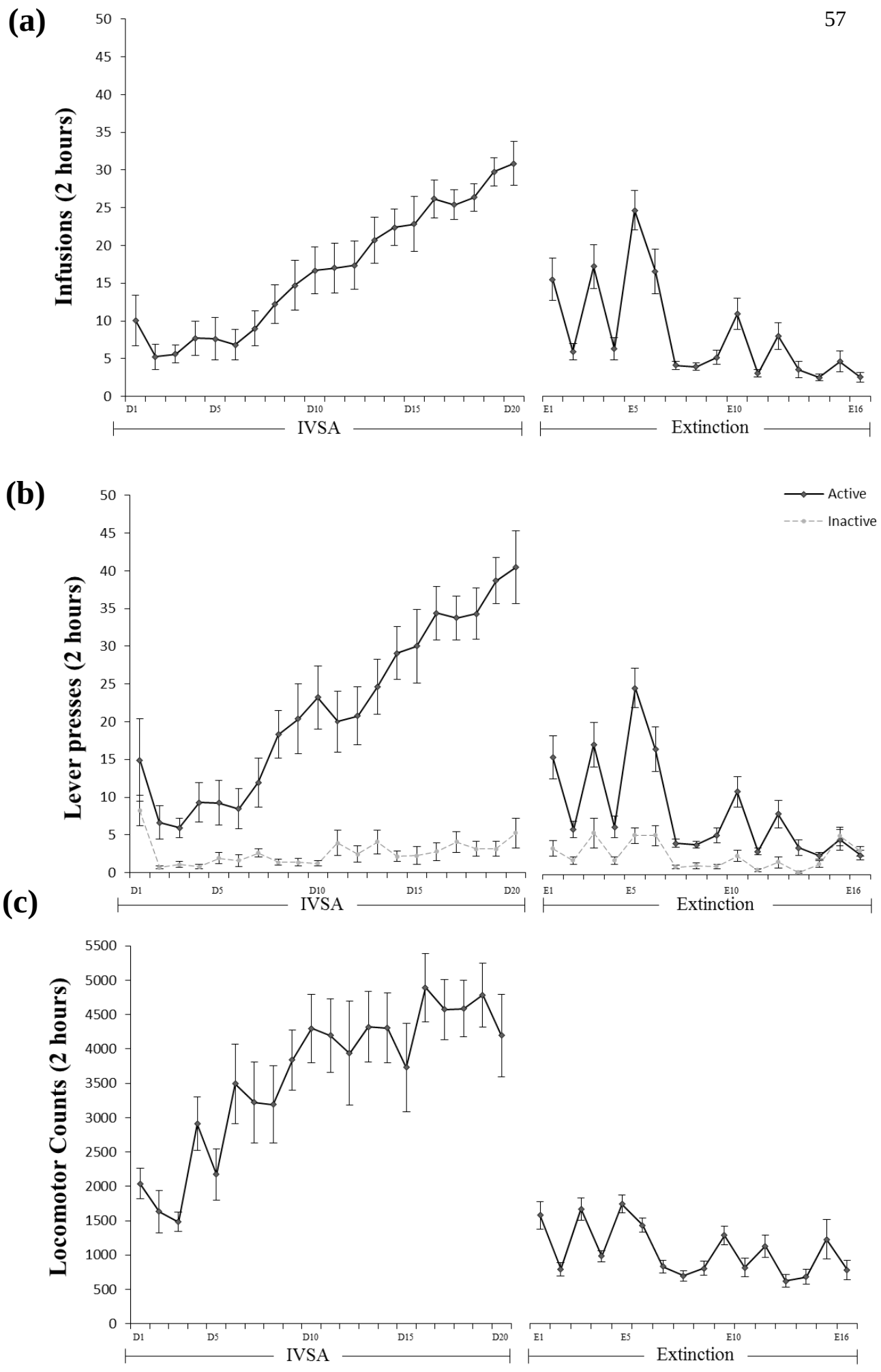

Figure 4 Mean (±SEM) number of (a) infusions, (b) active and inactive lever presses, and (c) mean (±SEM) locomotor activity during METH (0.1 mg/kg, IV) self-administration (D1-20) and extinction (E1-E16).

3.1.2 Behavioural extinction

By the end of the extinction period, active lever pressing had significantly reduced to an average of 4 presses (SEM = 0.827) from an average of 38 presses (SEM = 3.150) over the last three days of METH self-administration ($F(1, 12) = 92.823$; $P < 0.000$; Figure 4b). A significant reduction in locomotor activity was also present across the final three extinction sessions when compared with the final three self-administration sessions ($F(1, 12) = 58.964$; $P < 0.000$; Figure 4c). The end of the extinction period was marked by the observation of a non-significant difference between active and inactive lever pressing for two consecutive days (Day 15: $t(12) = 0.574$; $P = 0.577$; Day 16: $t(12) = 0.762$; $P = 0.461$).

3.1.3 METH-primed reinstatement

METH-primed reinstatement produced active lever presses at a level similar to intravenous METH self-administration, and significantly greater than active lever pressing over the last three days of extinction ($F(1, 12) = 28.320$, $P < 0.000$; Figure 5a). When compared with vehicle, treatment with oxytocin significantly reduced active lever pressing elicited by a METH prime ($F(1, 12) = 29.758$; $P < 0.000$). Cohen's effect size ($d = 2.123$) suggested immense practical significance of the oxytocin pre-treatment. The co-administration of the vasopressin V1a antagonist SR49059 with oxytocin significantly inhibited oxytocin's modulating effects on METH-induced active lever pressing, when compared with oxytocin treatment alone ($F(1, 12) = 16.041$; $P = 0.002$). Furthermore, there was no significant difference in active lever pressing between the SR49059+OXY condition and vehicle, indicating a complete block of oxytocin's effects by administering the V1a receptor antagonist ($F(1, 12) = 0.991$; $P = 0.339$).

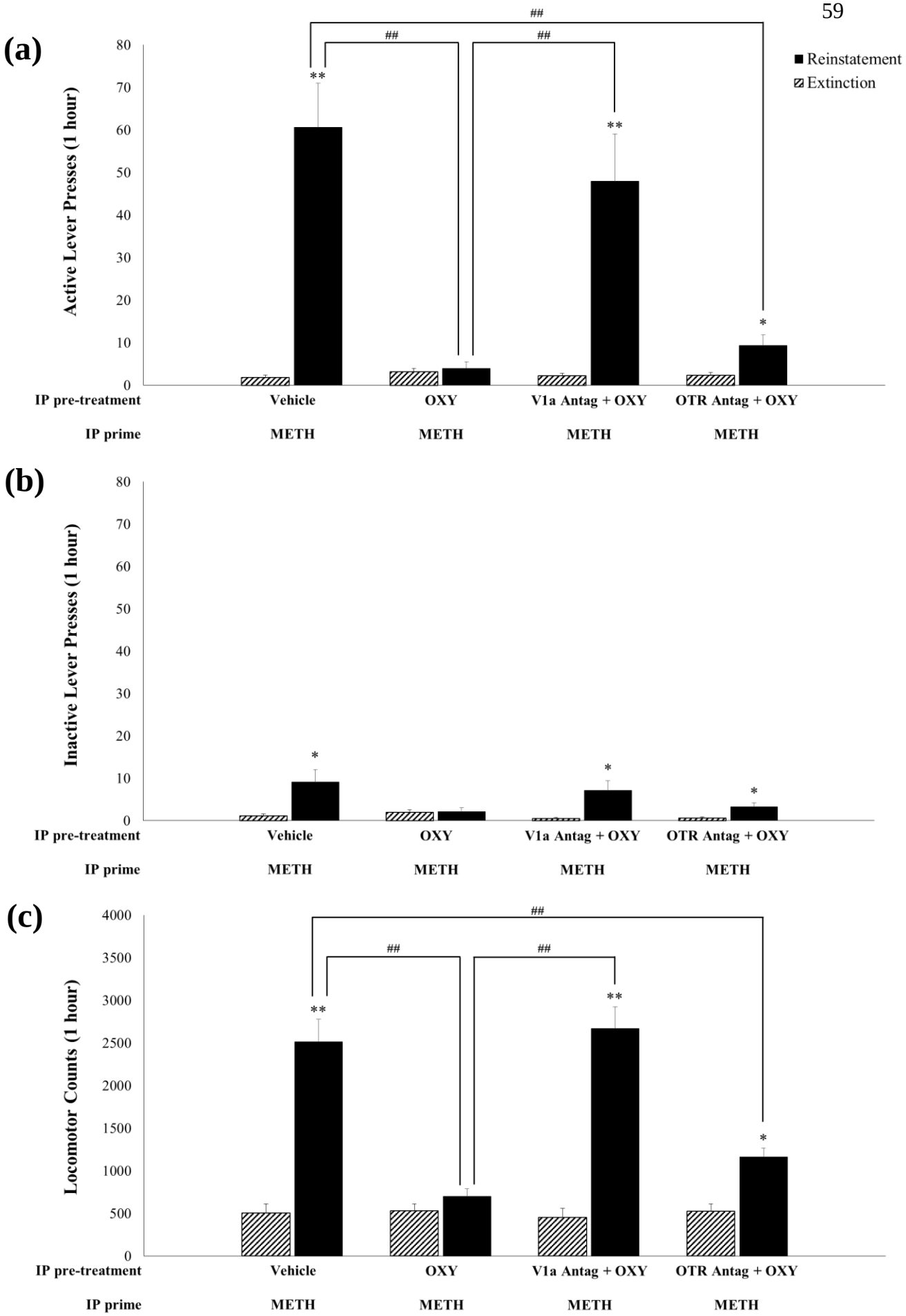

Figure 5 Effects of vehicle, oxytocin, -and co-administration of oxytocin with either the V1a receptor or OTR antagonists on (a) active lever presses, (b) inactive lever presses, and (c) locomotor activity during METH (1 mg/kg, IP) primed reinstatement sessions (n = 13). ##P<0.005 versus reinstatement conditions; *P<0.05, ** P<0.005 versus prior extinction day. Data presented as mean ± SEM.

The active lever pressing produced by Compound 25+OXY treatment was not significantly different than the oxytocin session ($F(1, 12) = 3.033$; $P = 0.107$), and was significantly less than the active lever pressing elicited in the vehicle condition ($F(1, 12) = 22.078$; $P = 0.001$). In addition, when administered alone, neither dose of SR49059 or Compound 25 significantly affected METH-induced active lever pressing compared with vehicle (SR49059: 1 mg/kg: $M = 61.150$, SEM = 8.487; $F(1, 12) = 0.004$; $P = 0.948$; 0.3 mg/kg: $M = 55.77$, SEM = 9.901; $F(1, 12) = 0.219$; $P = 0.648$; Compound 25: $M = 53.62$, SEM = 10.613; $F(1, 12) = 0.609$; $P = 0.450$). The sole administration of SR49059 (1 mg/kg: $M = 1.141$, SEM = 0.404; $t(6) = 0.258$; $P = 0.805$) or Compound 25 ($M = 1.169$, SEM = 0.980; $t(5) = 0.656$; $P = 0.541$) prior to saline instead of METH did not significantly alter active lever pressing when compared with the extinction day prior. Furthermore, reinstatement to active lever pressing did not significantly differ between the initial ($M = 60.615$, SEM = 10.353) and final vehicle sessions ($M = 55.923$, SEM = 9.213; $F(1, 12) = 0.297$; $P = 0.596$), indicating no impairment of the ability of the METH-prime to reinstate rats to active lever pressing after nine reinstatement sessions (data not shown).

Inactive lever pressing was significantly higher during reinstatement sessions than extinction ($F(1, 12) = 17.823$; $P = 0.001$; Figure 5b), except for when pre-treated with oxytocin ($F(1, 12) = 0.176$; $P = 0.682$). However, average active lever pressing ($M = 43.529$, SEM = 6.001) was significantly higher than inactive lever pressing during reinstatement across all conditions ($F(1, 12) = 47.322$; $P < 0.000$) suggesting that rats were differentiating between the levers, and that the METH-prime successfully reinstated previous active lever pressing activity. Inactive lever pressing following the sole administration of SR49059 ($t(6) = 2.291$; $P = 0.062$) or Compound 25 ($t(5) = 0.307$; $P = 0.771$) with a saline prime did not significantly differ significantly from inactive lever pressing on the extinction day prior (data not shown).

Locomotor activity was significantly increased during all METH-primed reinstatement conditions compared to the prior extinction sessions ($F(1, 12) = 148.318$; $P < 0.000$; Figure 5c), except following OXY treatment which had attenuated the METH-induced hyperactivity back to extinction-like levels ($F(1, 12) = 1.653$; $P = 0.223$). However, the SR49059+OXY treatment resulted in significantly higher locomotor activity than oxytocin treatment ($F(1, 12) = 57.490$; $P < 0.000$), up to a level no different than vehicle ($F(1, 12) = 0.531$; $P = 0.480$), suggesting SR49059 was able to entirely prevent the effect of OXY to inhibit METH-induced hyperactivity. Whilst Compound 25+OXY also resulted in a significantly higher locomotor response than the OXY only treatment ($F(1, 12) = 32.147$; $P < 0.000$), it was significantly lower than during the vehicle condition ($F(1, 12) = 19.952$; $P = 0.001$), suggesting Compound 25 only partially prevented the effect of OXY on METH-hyperactivity. When administered alone, neither of the SR49059 doses or Compound 25 elicited significantly different levels of METH-induced locomotor activity to vehicle (SR49059 1 mg/kg: $M = 2803.381$, SEM = 246.132; $F(1, 12) = 4.543$; $P = 0.054$; 0.3 mg/kg: $M = 2715.768$, SEM = 259.761; $F(1, 12) = 1.294$; $P = 0.277$; Compound 25: $M = 2828.853$, SEM = 217.246; $F(1, 12) = 2.067$; $P = 0.176$). Similarly, when administered prior to saline in lieu of METH, there was no effect of SR49059 (1 mg/kg: $t(6) = 0.682$; $P = 0.521$) or Compound 25 ($t(5) = 2.197$; $P = 0.079$) on locomotor activity compared to extinction, suggesting that the increases in locomotor activity elicited by their co-administration with oxytocin were through their interactions with oxytocin (data not shown).

3.2 Experiment 2: *Effects of oxytocin in the nucleus accumbens core on METH-primed reinstatement when co-administered with either SR49059 or desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT*

Of the original 16 rats which underwent both jugular catheterisation and intracranial implantation of bilateral guide cannula, 6 were removed from the study due to: non-acquisition of METH IVSA (2), failure to reinstate to lever pressing behaviour following the METH-prime (2), loss of cannula patency (1), and incorrect cannula placement (1).

3.2.1 METH self-administration

Rats (n = 10) acquired intravenous METH self-administration, as indicated by a significant increase in the number of METH infusions over the 20-day period (day 1 M = 16.300, SEM = 3.070; day 20 M = 32.200, SEM = 4.543; $F(1, 9) = 10.913$, $P = 0.009$; Figure 6a). Active lever pressing also increased over the self-administration period (day 1 M = 22.600, SEM = 3.824; day 20 M = 52.200, SEM = 10.837; $F(1, 9) = 6.873$, $P = 0.028$; Figure 6b), whilst inactive lever pressing did not significantly vary, although there was a trending decrease (day 1 M = 25.900, SEM = 10.120; day 20 M = 8.000, SEM = 3.602; $F(1, 9) = 2.704$; $P = 0.135$). No significant preference for either lever was observed on day 1 (Active M = 22.600, SEM = 3.824; Inactive M = 25.90, SEM = 10.119; $t(9) = 0.349$; $P = 0.735$), whilst a significant preference for the active versus inactive lever was evident on the final day (Active M = 52.200, SEM = 34.270; Inactive M = 8.000, SEM = 3.602; $t(9) = 3.670$; $P = 0.005$). Locomotor activity did not significantly vary across the 20-day period (day 1 M = 2902.600; SEM = 262.925; day 20 M = 2516.500, SEM = 510.897; $F(1, 9) = 0.321$, $P = 0.585$; Figure 6c).

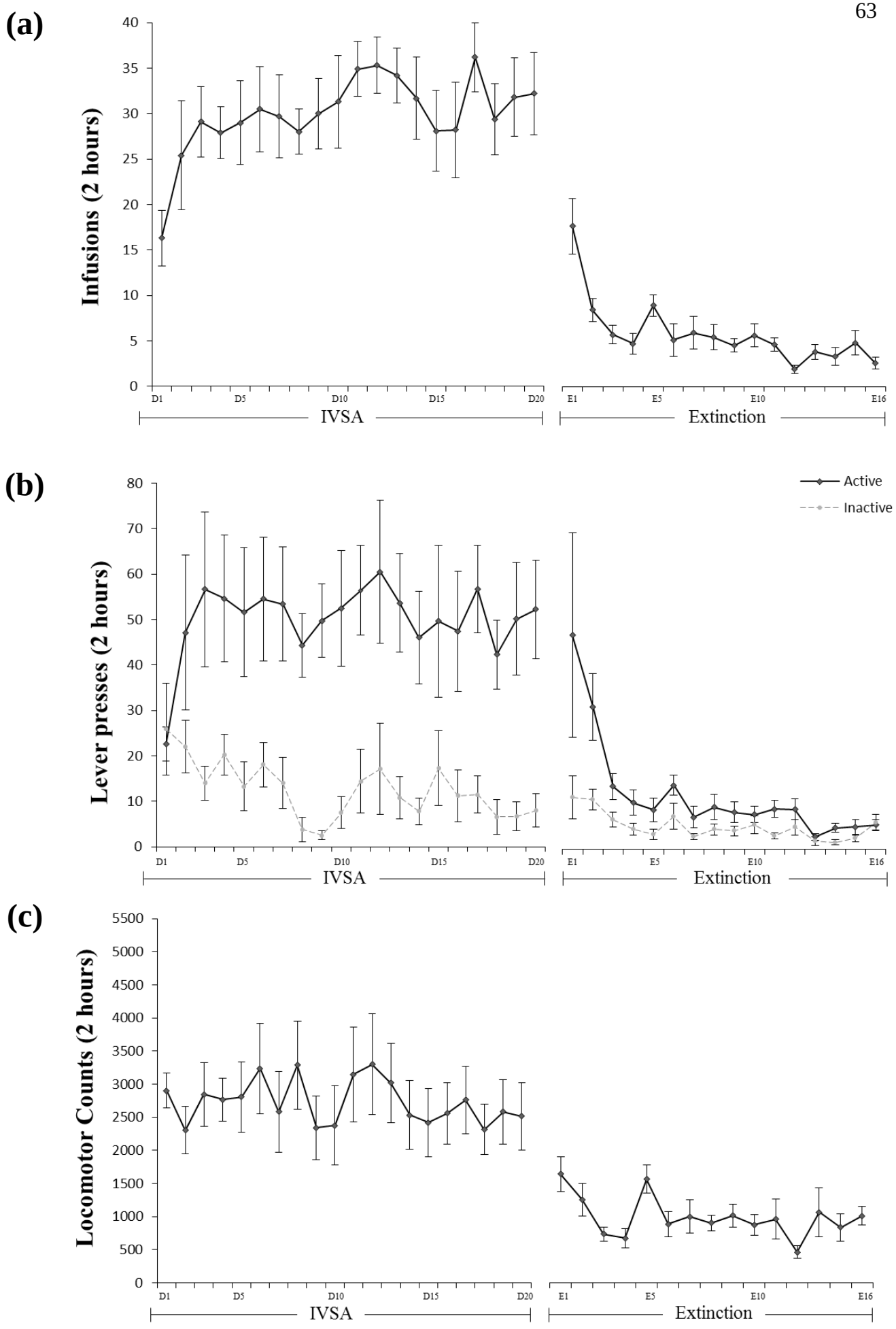

Figure 6 Mean (±SEM) number of (a) infusions, (b) active and inactive lever presses, and (c) mean (±SEM) locomotor activity during METH (0.1 mg/kg, IV) self-administration (D1-20) and extinction (E1-E16).

3.2.2 Behavioural extinction

Over the course of the extinction period, average active lever pressing had reduced to 5 presses (SEM = 0.597) over the final three days; a significant reduction from the 48 presses (SEM = 10.103) averaged over the final three METH self-administration sessions ($F(1, 9) = 20.135$; $P = 0.002$; Figure 6b). A significant reduction in locomotor activity was also observed from the end of self-administration to the end of extinction ($F(1, 9) = 14.491$; $P = 0.004$; Figure 6c). Importantly, there was no significant difference between the number of active and inactive lever presses for two consecutive days (Day 15: $t(9) = 1.861$; $P = 0.096$; Day 16: $t(9) = 0.522$; $P = 0.614$).

3.2.3 METH-primed reinstatement

Overall, METH-primed reinstatement sessions produced active lever pressing to a significantly greater extent than during the extinction sessions prior to testing ($F(1, 9) = 105.456$; $P < 0.000$; Figure 7a), and to a similar level to during intravenous METH self-administration. When compared to vehicle, pre-treatment with oxytocin injected into the NAc core prior to the IP METH-prime significantly reduced active lever pressing ($F(1, 9) = 7.282$; $P = 0.024$); a moderate effect size (d = 0.493). The co-administration of the OTR antagonist desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT with OXY failed to significantly reverse the attenuation of METH-primed reinstatement to active lever pressing, when compared to the OXY-only condition ($F(1, 9) = 0.800$; $P = 0.394$). The co-administration of OXY with the V1a antagonist SR49059 was also unable to significantly attenuate the reduction of METH-primed reinstatement to active lever pressing produced by OXY ($F(1, 9) = 0.001$; $P = 0.981$).

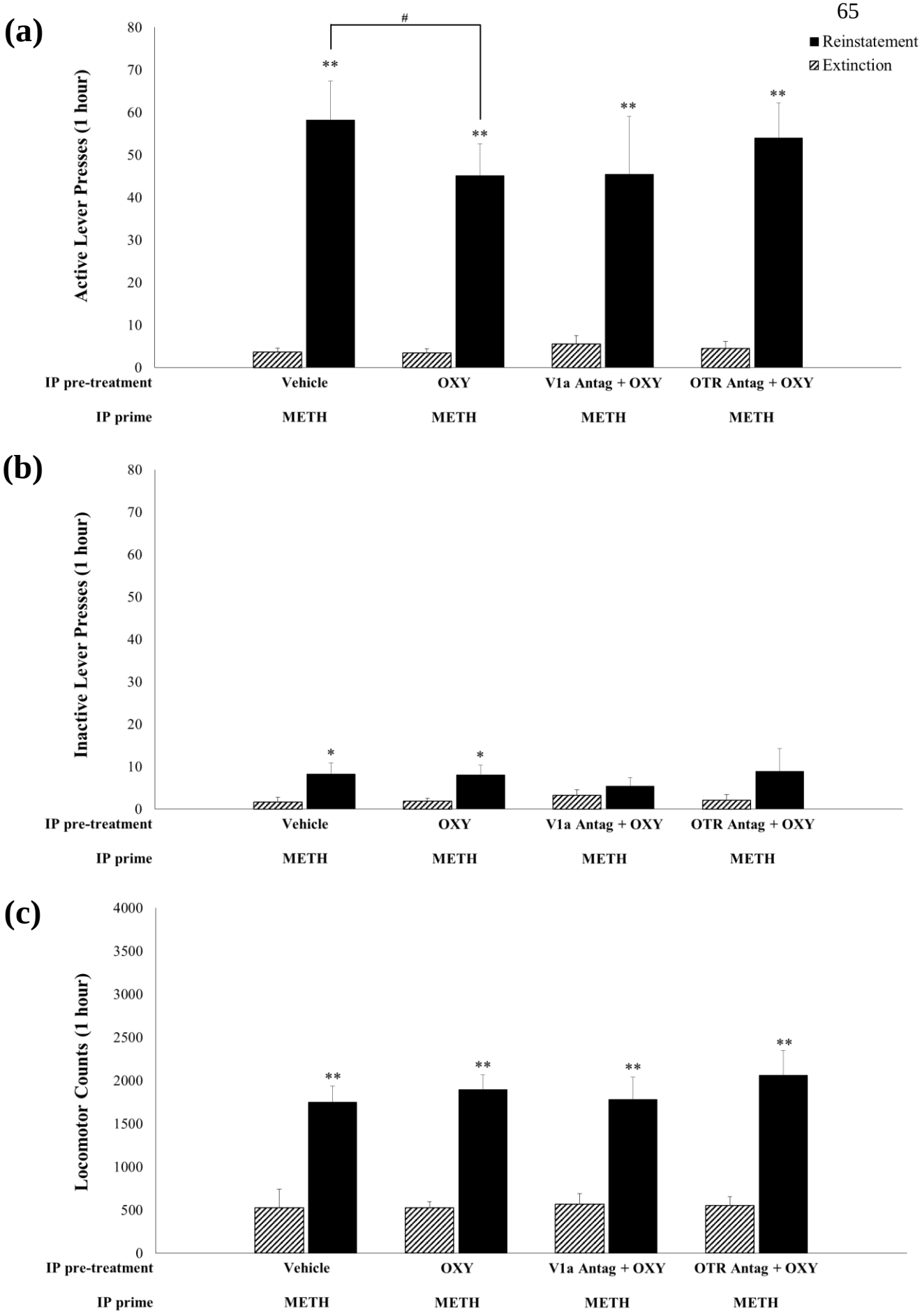

Figure 7 Effects of bilateral NAc core injections (500 nl/side) of vehicle, oxytocin and co-administration of oxytocin with either the V1a receptor or OTR antagonists, on (a) active lever presses, (b) inactive lever presses, and (c) locomotor activity during METH (1 mg/kg, IP) primed reinstatement sessions (n = 10). #P<0.05 versus vehicle+METH condition; *P<0.05, ** P<0.005 versus prior extinction day. Data presented as mean ± SEM.

Overall inactive lever pressing was significantly higher during reinstatement than during extinction ($F(1, 9) = 6.672$; $P = 0.030$; Figure 7b). However, active lever pressing was significantly higher than inactive lever pressing across reinstatement sessions, suggesting that rats continued to differentiate between the two levers ($F(1, 9) = 50.753$; $P < 0.000$). There was also no difference between the inactive lever pressing produced by the oxytocin and vehicle conditions, suggesting the effect of oxytocin in the NAc core on METH were specific to the active lever ($F(1, 9) = 0.004$; $P = 0.950$).

Locomotor activity during METH-primed reinstatement was significantly increased compared to extinction ($F(1, 9) = 50.781$; $P < 0.000$; Figure 7c). Pre-treatment with a microinjection of oxytocin into the NAc core had no effect on METH induced hyperactivity, compared to vehicle ($F(1, 9) = 3.422$; $P = 0.097$). Additionally, neither co-administrations of oxytocin with SR49059 ($F(1, 9) = 0.447$; $P = 0.521$) or desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT ($F(1, 9) = 0.694$; $P = 0.426$) elicited significantly different effects on METH-induced locomotor activity when compared to the oxytocin condition.

3.3 Histological analysis

The histological examination of bilateral cannulae placements resulted in the removal of 1 rat from the sample. Only rats with both guide cannulae correctly implanted in NAc core were included in the final analyses. Figure 8 shows these placements.

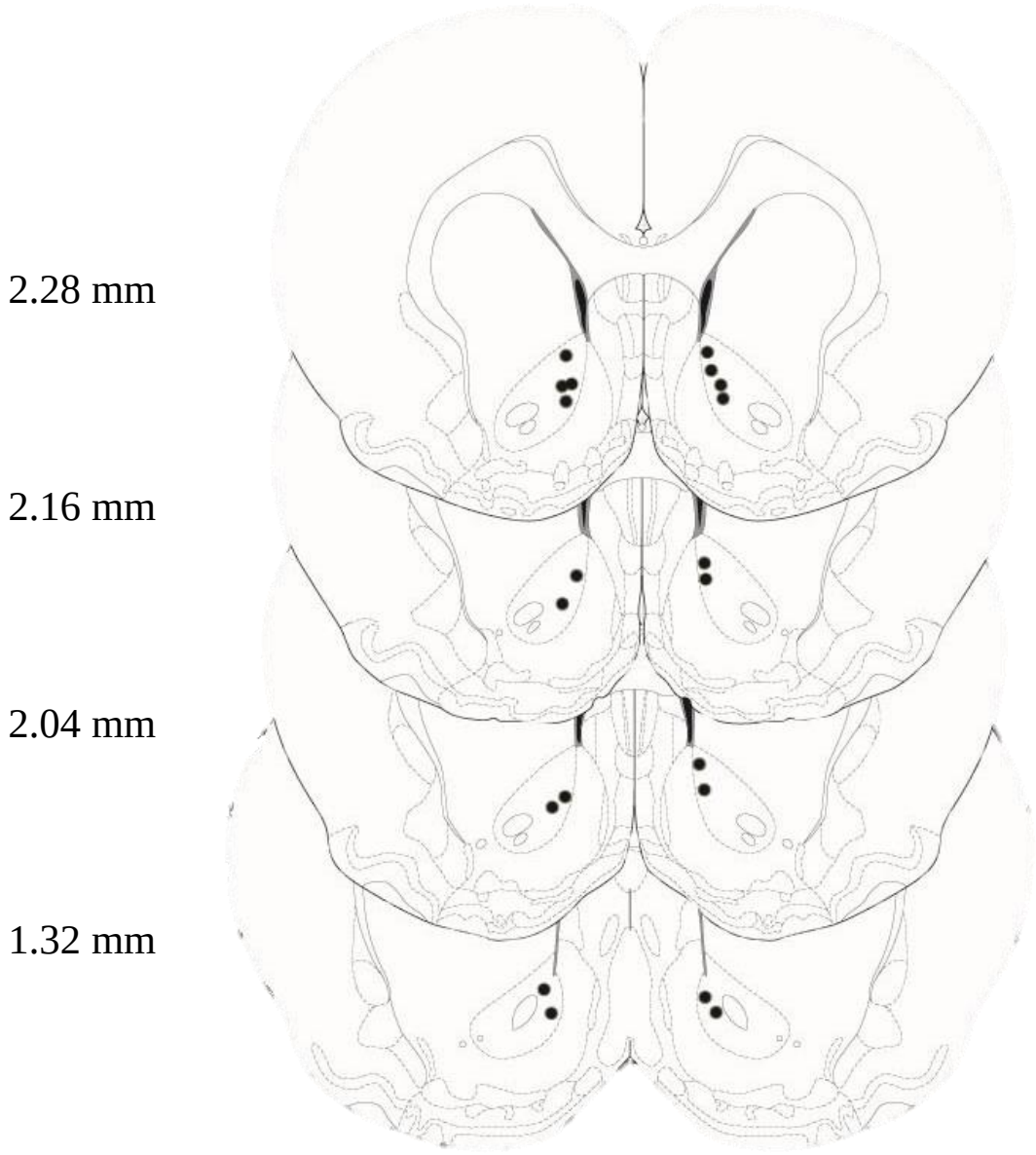

Figure 8 Anatomical coronal diagrams depicting the microinjection sites into the NAc core. The numbers to the left of the image depict the distance in mm from bregma.

Chapter Four: Discussion

The aim of the present thesis was to investigate OTR and V1a receptor mediation of OXY to inhibit METH-primed reinstatement to METH-seeking behaviour in rats, through the administration of OXY with selective receptor antagonists either peripherally or within the NAc core. Overall, this thesis provides the novel finding that peripherally administered OXY activates AVP V1a receptors to have its attenuating effects on METH-primed reinstatement, although this interaction within the NAc core remains undetermined. The minimal involvement of the OTR in OXY-METH interactions is further confirmed following peripheral OXY administration, or local infusion into the NAc core.

4.1 Findings

4.1.1 Experiment 1: *Effects of peripherally administered OXY on METH-primed reinstatement when pre-treated with either SR49059 or Compound 25*

To our knowledge, this experiment is the first to demonstrate that the V1a receptor plays an important role in mediating the effect of systemic administration of OXY to attenuate METH-primed reinstatement of drug-seeking behaviour and acute METH-hyperactivity.

Rats pre-treated with 1 mg/kg of OXY showed a complete suppression of METH-primed reinstatement of METH-seeking behaviours, equivalent to extinction levels of responding on the drug-paired lever. These results are largely in line with the similar, albeit lesser effect of OXY on METH-primed reinstatement demonstrated by Carson et al. (2010a). Both experiments trained rats to self-administer METH for a similar duration, however Carson et al. (2010a) utilised a progressive ratio schedule of reinforcement, which results in rats expending increasing amounts of energy to self-administer drugs (Winger & Woods, 1985). This increased motivation to seek METH may have resulted in a comparatively stronger reinstating effect of the METH-prime, and as such, may reflect the difference in the

magnitude of the effect of OXY between the two studies. More broadly, the current finding aligns with an increasing body of work demonstrating the effectiveness of exogenous OXY administration in modulating METH-related behaviours (Qi et al., 2008; 2009; Cox et al., 2013; Hicks et al., 2014; Baracz et al., 2012; 2015; in press-a).

Previous research has demonstrated that the effects of OXY administration to the NAc core may not be mediated by the OTR (Baracz et al., in press-a). However, the involvement of the OTR has not been investigated in the inhibitory effects of OXY on METH-primed reinstatement when peripherally administered. As such, rats were pre-treated with the selective non-peptide OTR antagonist Compound 25 prior to OXY administration, and then primed with METH. This OTR antagonist has previously been used to investigate OTR involvement in physiological function and social behaviour (Brown et al., 2010; Ramos et al., 2013; Hicks et al., 2014; 2015). In the present study, pre-treatment with Compound 25 did not prevent the inhibitory effect of OXY on METH-priming. Importantly, the OTR antagonist did not influence lever pressing activity when administered with either saline or a METH-prime, suggesting that OTRs are not involved in the maintenance of extinction or in mediating METH-seeking behaviour. These findings are in keeping with Baracz et al. (2015; in press-a), which demonstrated that OTR antagonism in the NAc core or STh regions did not alter METH-seeking behaviour, nor did they specifically mediate the attenuation of METH-seeking produced by exogenous OXY application to these brain regions.

In light of the modest, or even null involvement of the OTR, we investigated the role of the AVP V1a receptor in the inhibition of METH-seeking behaviours produced by the peripheral administration of OXY. Prior to receiving OXY, rats were peripherally injected with 1 mg/kg of the selective non-peptide AVP V1a receptor antagonist SR49059, a compound which has previously been used to explore OXY-V1a receptor interactions in physiological function and numerous social and cognitive behaviours (Hicks et al., 2014;

2015; Sala et al., 2011; Ramos et al., 2013; Bowen et al., 2014). Strikingly, rats engaged in substantially more METH-induced active lever pressing behaviour when pretreated with SR49059 prior to OXY than when given OXY alone. Furthermore, active lever pressing in this condition was not significantly different than in the vehicle session, indicating a complete reversal of the OXY effect on METH-primed reinstatement by V1a receptor blockade. Additionally, independent administration of the SR49059 had no effect on active lever pressing following either a METH or saline prime, suggesting that V1a receptors are not required for maintaining extinction or METH-seeking behaviour. It is of interest that Hicks et al. (2015) demonstrated that this same dose of SR49059 impaired social memory in rats, to suggest that the V1a receptor may mediate endogenous processes underlying social- but not drug-related memory. Together, these findings are broadly consistent with previous indications of OXY-driven behaviours occurring through V1a receptor modulation, although it is the first such demonstration of this interaction in a drug addiction paradigm.

The hyperactivity induced by METH administration was also substantially inhibited by systemic OXY treatment, an effect which has been consistently found across mice and rats, when delivered peripherally or ICV (Qi et al., 2008; Carson et al., 2010a;b). The present study did not investigate the sole effect of OXY administration on locomotor activity, and despite early suggestions of OXY administration to produce sedation at the present dose (Uvnas-Moberg et al., 1994), varying doses of IP OXY have shown no effect on the locomotor activity of rats that have been extensively handled and habituated to the test chamber (Carson et al., 2010a). Similar to METH-seeking behaviour, pre-treatment with an antagonist for the V1a receptor, but not the OTR, substantially inhibited the attenuating effect of OXY on METH-induced hyperactivity. In addition, administration of the V1a antagonist alone did not reduce METH-primed locomotor activity. These data suggest that the V1a receptor is not involved in the hyperactivity produced by METH administration, but plays an

important role in mediating the effect of OXY to suppress METH-induced locomotor activity. While these effects may simply reflect the locomotor activity required for drug-seeking behaviour, previous research has shown a deviation in the two behaviours, as OXY injected directly into the NAc core or STh reduced METH-seeking behaviour yet left METH-induced hyperactivity unaffected (Baracz et al., 2015; in press-a). In light of this, the present study suggests that systemically administered OXY may affect other neural substrates to attenuate the stimulant effects of acute METH, and that this attenuation is mediated by a V1a receptor mechanism.

The present experiment conducted nine reinstatement sessions, however, the inclusion of a final vehicle+METH session demonstrated that rats still reinstated to a METH prime to the same extent as at the start of reinstatement testing. Indeed, a study utilising the same amount of reinstatement test days to METH injection has been published recently (Cox et al., 2013). Furthermore, to control for the three IP injections rats received during conditions five and six (see Table 1), rats received an additional vehicle injection prior to testing. Neither lever pressing behaviour nor locomotor activity were affected by the addition of a third IP injection.

Overall, this experiment demonstrates that the V1a receptor but not the OTR mediates the attenuating effects of peripheral OXY administration on both METH-reward and locomotor stimulation. The following study aimed to address the central mechanisms that may produce these behavioural effects through manipulation of OTR and V1a receptors in a key brain region for drug relapse and locomotor stimulant effects, the NAc core.

4.1.2 Experiment 2: *Effects of OXY administration in the NAc core on METH-primed reinstatement when co-administered with either SR49059 or desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT*

Previous research has demonstrated that the NAc core is an important region for acute OXY and METH interactions (Carson et al., 2010b; Baracz et al., 2012; in press-a). In the present study, 3 pmol OXY microinjected into the NAc core moderately attenuated METH-primed reinstatement of METH-seeking. Although significant, this effect of OXY administration was approximately half the magnitude of the effect measured by Baracz et al (in press-a) with either a 1.5 or 4 pmol dose. However, rats in the present study acquired near-maximal METH self-administration faster, and received substantially more infusions of METH across the self-administration phase, potentially increasing the incentive motivational properties of the drug, and resulting in more pronounced neuroadaptation in addiction-related regions than in the aforementioned study. Indeed, Baracz et al. (in press-b) showed that following 20 days of METH-self administration, OTR-immunoreactive fibres in the NAc core are down-regulated compared to controls. As such, the increased METH exposure to rats in the present study, relative to Baracz et al. (in press-a), may have resulted in greater down-regulation of OTR expression, potentially reducing the efficacy of exogenous OXY application to the NAc core. Although, given the minimal involvement of the OTR relative to the V1a receptor in the effects of systemic OXY as demonstrated in the first experiment, OTR downregulation in the NAc core may actually result in increased OXY-V1a receptor binding. However, chronic METH self-administration may also dysregulate V1a receptor expression in the NAc core, although this has not yet been investigated.

In keeping with our previous findings in Baracz et al. (in press-a), co-administration of OXY with the selective OTR antagonist desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT into the

NAc core prior to the METH-prime did not significantly prevent the inhibitory effect of OXY on METH-primed reinstatement, although both studies suggest a trend towards reversal of the OXY effect. Given that the OTR has been located in the NAc core, albeit inconsistently reported, and in low densities (Stoop, 2012), particularly following chronic METH self-administration (Baracz et al., in press-b), this suggests that OXY administration may be working through other receptors within this region to reduce METH-seeking behaviour.

To further understand the modulating effect of OXY on METH-seeking behaviour in the NAc core, we co-administered OXY with the non-peptide selective AVP V1a receptor antagonist SR49059 into this region prior to the METH-prime. This condition resulted in similar METH-induced active lever pressing activity as in the OXY condition, suggesting that OXY maintained its inhibitory effect despite V1a receptor antagonism. However, this finding contradicts the recent literature describing OXY-V1a interactions (Ramos et al., 2013; Hicks et al., 2014; Bowen & McGregor, 2014), as well as the first experiment of the present thesis which demonstrated a strong involvement of the V1a receptor in mediating the effect of OXY to reduce drug-seeking, a behaviour known to be modulated by the NAc core (Ito et al., 2000). A synthesis of these two experiments may suggest that the effect of systemic administration of OXY is mediated by V1a receptors in regions other than the NAc core. For example, OXY administration to the STh reduced METH-seeking behaviour, which was independent from OTR activation (Baracz et al., 2015). Alternatively, δ-subunit-containing $GABA_A$ receptors, which have been shown to modulate OXY-ethanol interactions, may also be important for OXY-METH interactions and at the level of the NAc core (Bowen et al., 2015). Whilst these are worthy avenues for exploration, it is more likely that in the present study, the 3 pmol dose of the V1a antagonist SR49059 chosen to dose-match OXY was insufficient to prevent OXY-V1a binding to an extent relevant to behavioural function. Indeed, doses of SR49059 as high as 400 pmol have been microinjected

into medulla subregions to impair V1a dependent sympathetic nerve activity (Kc et al., 2009). This substantially larger dose may be necessary in regions with dense V1a receptor expression, such as the medulla, while regions such as the NAc core, with lower receptor number, should require a lesser dose for sufficient V1a receptor blockade (Ostrowski et al., 1994). In light of this, future investigations into OXY-V1a interactions within the NAc core should evaluate the dose-response of SR49059 to reverse the effect of OXY administration on METH-seeking behaviour.

Acute METH-induced hyperactivity was also demonstrated in this experiment, as expected. Importantly, neither OXY nor the OXY+antagonist treatments altered the acute stimulant effect of the METH-prime. This agrees with previous findings, whereby OXY microinjected into the NAc core had no effect on METH-hyperactivity (Baracz et al., 2012;in press-a). This suggests that the effect of peripherally administered OXY to reduce METH-induced hyperactivity is occurring in neural substrates other than the NAc core, highlighting the likely different brain regions involved in reward versus stimulant processes of METH (Gong, Justice & Neill, 1997).

Overall, the findings from this experiment provide further evidence for the modest, or even null role of the OTR in mediating the inhibitory effect of OXY in the NAc core on METH-primed reinstatement. However, the involvement of the V1a receptor in mediating acute OXY-METH interactions in this region remains undetermined.

4.2 Implications, limitations, and future directions

Consideration of the behavioural findings from the present studies suggest that OXY has a large inhibitory effect on METH-related processes throughout the brain, including a moderate effect within the NAc core, and that these effects may not be mediated by the OTR but rather by the AVP V1a receptor. These findings are particularly important for the

discovery of novel non-peptide ligands which aim to replicate the central effects that OXY has on behaviour, whilst improving brain penetration, oral bioavailability and receptor selectivity, compared to OXY itself (Manning et al., 2012). The development of these ligands has hitherto presumed that OXY primarily activates the OTR to elicit behavioural effects (Manning et al., 2012). One such compound, WAY 267,464 was shown to only partially mimic the behavioural and physiological effects, and brain region specific c-Fos expression of OXY administration (Ring et al., 2009; Hicks et al., 2012; 2014). Furthermore, treatment with WAY 267,464 resulted in impaired OXY-relevant social recognition in rats, which has since been attributed to the compound acting as a competitive antagonist at the AVP V1a receptor (Hicks et al., 2015). In conjunction with the findings from the present study, and the partial or opposing effects of WAY 267,464 administration on OXY-induced behaviours, this suggests that novel ligands which seek to mimic the effects of OXY administration should act as V1a receptor agonists.

The importance of an OXY-V1a interaction in the mediation of behaviour is further highlighted by the once prevalent use of Atosiban as a selective OTR antagonist. In animals, Atosiban has been widely used to explore a variety of presumably OTR-mediated effects including social behaviours, social memory and learning, and anxiolysis (Matthews, Abdelbaky and Pfaff, 2005; Amica et al., 2004; Wu and Yu, 2004). However, Manning et al. (2001) demonstrated that Atosiban is not a selective OTR antagonist, as it is only 8 times more potent at the OTR than the V1a receptor in rats. As such, many of these behaviours once thought to be OTR mediated have now been shown to also involve V1a receptor activation (for review see: Donaldson and Young, 2008; Insel 2008). The issue of receptor selectivity is even more pronounced in humans, in which Atosiban has 100 times greater affinity for V1a receptors than the OTR (Manning et al., 2001). The recent development of selective OTR and V1a receptor agonists and antagonists, including those used in the present

thesis, allow more accurate interpretation of the interaction between the two receptor families (Manning et al., 2012).

The findings of this thesis add to the broader body of work demonstrating the importance of understanding the relative mediation of OXY effects by OTR and V1a receptors. Furthermore, the distinctive strength of the effect on METH-primed reinstatement of OXY administered peripherally in experiment one, versus locally within the NAc core in experiment two, suggests the considerable involvement of other addiction-relevant brain regions. To develop a greater understanding of how OXY administration regulates METH-seeking behaviours, further delineation of the underpinning neural circuitry is needed.

4.2.1 Other neural substrates of interest

4.2.1.1 Subthalamic Nucleus

The subthalamic nucleus (STh) is basal ganglia structure classically associated with regulating motor function, but has more recently been implicated in drug reward and addiction (Baunez and Lardeux, 2011). It has been demonstrated that METH acutely administered to the STh resulted in pronounced hyperactivity (Pontieri et al., 1990), and that electrical stimulation of the STh reduced motivation to self-administer cocaine, and prevented the formation of a cocaine place preference in rats (Rouaud et al., 2009). Importantly, OXY administration has been shown to attenuate acute METH-induced c-Fos expression in the STh (Carson et al., 2010b), as well as the acute rewarding effects of METH when OXY is microinjected into the STh (Baracz et al., 2012). Although the STh appears to be an important site for METH and OXY interactions, recent findings suggest that whilst OXY in the STh can modestly attenuate METH-primed reinstatement, this effect is independent of the OTR (Baracz et al., 2015). A more complex OTR-STh-METH interaction has also been described, whereby chronic METH self-administration increased the density of OTR-immunoreactive

fibres in the STh, an effect which persisted even after extinction (Baracz et al., in press-b). In light of this potentially increased OTR availability following chronic METH exposure, it could be suggested that the dose of OTR antagonist used in Baracz et al. (2015) to block the action of OXY in the STh may not have been sufficient to entirely prevent the OXY-OTR binding. Whilst this warrants re-examination with more substantial pharmacological OTR blockade, the present thesis highlights the importance of investigating OXY-V1a receptor interactions in discrete brain regions. Investigation of the OXY-V1a interactions within the STh on METH-primed reinstatement would help to clarify recent findings, and advance the understanding of the circuitry underpinning OXY-METH interactions.

4.2.1.2 Central amygdala

The central nucleus of the amygdala (CEA) plays an important role in emotional learning, reinforcement and fear processes (Koob, 2009; Cardinal et al., 2002), and is functionally linked to the mesolimbic dopamine system (Everitt et al., 1999). Lesion studies in rats have demonstrated that the CEA regulates appetitive pavlovian conditioning and dopaminergic activity within the NAc core (Killcross et al., 1997; Everitt et al., 1999; Simon et al., 1988). More recently, 10ng but not 100ng of OXY microinjected into the CEA produced an OTR dependent effect to reduce anxiety, and form a conditioned place preference (László et al., in press). The latter finding may appear to conflict with previous research showing that 0.6ng OXY in the NAc core or STh was not acutely rewarding (Baracz et al., 2012), although unification of these studies more likely reflects the bell-shaped-curve of responding characteristic of neuropeptides (Oitzl, Hasenhörl & Huston, 1990; László et al., 2012). In regards to METH, the peripheral administration of OXY moderately attenuated METH-induced c-Fos expression in the CEA (Carson et al., 2010b). In addition, OTR expression was substantially up-regulated in the CEA following chronic cocaine or METH treatment in mice (Georgiou et al., 2015; Zanos et al., 2014), likely representing a

compensatory neuroadaptation in this region to the persistent reduction in circulating and central OXY which is associated with chronic psychostimulant exposure (Sarnyai et al., 1994). Human studies have also demonstrated an important action of OXY in the amygdala to recover deficits in facial recognition and to enhance trust (Domes et al., 2007; Baumgartner et al., 2008; Gorka et al., 2015).

Importantly, whilst the OTR has been functionally identified in the CEA (László et al., 2016), the AVP V1a receptor and the mRNA which encodes for its expression have also been localised in this region (Caffe, van Leeuwen, et al., Luiten, 1987; Ostrowski, 1992). The CEA also receives OXYergic projections from magnocellular SON and PVN cells (Knoblock et al., 2012). Taken together, this suggests that OXY may act in the CEA to modulate acute and chronic METH-related behaviours, which is potentially mediated by both the OTR and V1a receptors.

4.2.1.3 Prelimbic cortex

The prelimbic region of the medial prefrontal cortex (PLC) is a key component of the mesocorticolimbic dopaminergic pathway and primarily projects glutamatergic afferents to the VTA, NAc and CEA, among other regions (Vertes, 2004). The modulation of the PLC has been associated with the reinforcement of drug cues and operant responding, and the cue- and primed-reinstatement of drug-seeking behaviours (McBride, Murphy and Ikemoto, 1999; Peters, Kalivas and Quirk, 2009; Rocha & Kalivas, 2010). However, recent optogenetic experiments have demonstrated a more complex role for the PLC in acquisition and reinstatement of drug seeking. Martin-Garcia et al. (2014) demonstrated that in rats which self-administered cocaine at a high frequency and then underwent extinction, optogenetic inactivation of the PLC impaired the ability of a cocaine-prime to reinstate drug-seeking behaviour. Conversely, PLC silencing during acquisition facilitated cocaine self-

administration. Importantly, these effects were not found in low frequency cocaine self-administering rats, suggesting that the PLC only exerts control over drug seeking behaviours in high responders, potentially reflecting a neuroadaptive recruitment of the PLC following excessive psychostimulant exposure. A similar opposite role of PLC-mediated acquisition and reinstatement was also found in rats trained to self-administer alcohol (Willcocks and McNally, 2013). This is problematic, as pharmacotherapies which inhibit PLC activity to reduce the likelihood of drug-primed reinstatement of drug-seeking behaviours may simultaneously enhance operant responding if the behaviour is reinforced.

In terms of OXY interactions in the PLC, METH-induced c-Fos activation in this region was substantially attenuated by peripheral administration of OXY (Carson et al., 2010b), and when microinjected into the PLC, OXY attenuated amphetamine-induced dopamine elevations in the NAc core of rats (Young et al., 2014). Additionally, antagonism of the OTR in the PLC prevented the formation of a partner preference in female voles (Young et al., 2001). In light of these findings, the dissociation of the role of the PLC should be investigated with respect to the ability for OXY to act in this region to modulate acquisition and reinstatement of METH-seeking behaviours. Furthermore, as both OTR and V1a receptors have been localised to the PLC in rats and voles (Hernando et al., 2001; Ostrowski et al., 1994; Lim, Murphy and Young, 2003), both of these receptor systems should be explored for their mediation of the effects of OXY administration on behaviour.

4.2.1.4 Lateral orbitofrontal cortex

Activation of the lateral aspect of the orbitofrontal cortex (OFC) has also been implicated in OXY and addiction-related behaviours (Goldstein & Volkow, 2002; Hooker & Knight, 2006). Neuroimaging and lesion studies in humans have demonstrated that the lateral OFC is responsible for impulsive, compulsive and reward-related behaviours, as measured by

a reversal learning task (Kringelback, 2005; Hornak et al., 2004). Additionally, OXY driven behaviours such as maternal love are associated with increased activity in the lateral OFC, suggesting a behaviourally important role for endogenous OXY in this region (Bartels & Zeki, 2004). Furthermore, METH-induced c-Fos expression in the lateral OFC was substantially attenuated by exogenous OXY, suggesting that the lateral OFC may be an important site for acute OXY-METH interactions (Carson et al., 2010b). Indeed, neurons expressing OTR and V1a receptor mRNA have been located in the OFC, although at relatively low densities (Vaccari et al., 1998; Ostrowski et al., 1994). As such, the lateral OFC should be investigated as a potential site at which OXY acts on OTR or V1a receptors to attenuate METH-primed reinstatement.

Other worthwhile avenues of investigation may be discovered following recent demonstrations of OXY administration modulating METH-induced GABA and glutamate activity within the dorsal hippocampus (Qi et al., 2012), δ-subunit-containing $GABA_A$ receptors modulating OXY interactions with ethanol (Bowen et al., 2015), and social reward in mice requiring coordinated activation of NAc OXY and serotonin (Dölen, Darvishzadeh, Huang, & Malenka, 2013).

In summary, the role of V1a receptors in mediating the effect of OXY administration to alter METH-relevant behaviours still needs to be adequately assessed in the NAc core. Moving forward, investigations into this OTR or V1a receptor mediated OXY-METH interaction in other regions may prove fruitful for the development of a working model of how OXY administration can reduce drug seeking behaviours. However, limited understanding of the localisation of OTR and V1a receptors in the brain may hamper these investigations. Rats which express an OXY-fluorescent protein transgene have been recently used to visualise OXY neurons in the brain (Hashimoto, Matsuura & Ueta, 2014). The development of transgenic lines of rats which express fluorescent markers on OTR and V1a

receptors would enable the localisation and density analysis of these receptors in addiction circuitry. Overall, these pharmacology and transgenic experiments would help to characterise the neuroadaptive processes which follow chronic METH exposure, and advance the understanding of the receptor systems and brain regions which regulate the promising therapeutic effects of OXY in METH addiction.

4.2.2 Pharmacology and alternative research techniques

Pharmacology is a useful research tool for investigating behaviour and its underlying neurobiology. In the present thesis, pharmacological manipulation of receptor sites enabled the identification of the AVP V1a receptor as an important mediator of the inhibitory effects of systemic OXY on METH-induced relapse and hyperactivity. However, the investigation of this interaction within the NAc core was restricted by the inherent limitations of drug microinjection techniques. Repeated local injections into discrete brain regions causes tissue damage, potentially altering tonic activity, as well as responsiveness to subsequent drug infusions. As such, four microinjections is typically considered to be the maximum which can be reliably interpreted (Cornish et al., 2005). Therefore only one dose of the V1a receptor antagonist SR49059 could be evaluated in the second experiment, and was subsequently found to be ineffective at inhibiting the effects of OXY.

Furthermore, OXY diffuses widely throughout the brain and may activate off-target receptor sites for up to 20 minutes post infusion (Zoli et al., 1995; Landgraf & Neumann, 2004). Whilst microinjection sites were relatively homogenous in the present experiment (Figure 8), OXY delivered into the NAc core may diffuse into neighbouring brain regions, eliciting imprecise behavioural effects. Additionally, the lack of selective V1a receptor agonists restricts the bidirectional manipulation of this receptor system (Manning et al., 2012). Whilst future pharmacology studies will aim to elucidate the OXY-V1a receptor

interaction within the NAc core with higher SR49059 doses, the advent of novel neuroscience techniques with greater spatial and temporal resolution provide a means for overcoming many of these limitations.

Viral mediated or transgenic expression of channelrhodopsin-2 (ChR2) in targeted brain regions allows for temporally and spatially precise depolarisation of neurons (Zhang et al., 2006). If further combined with flp- or cre-recombinase techniques, ChR2 expression can be targeted to distinct neuronal populations. For example Fenno et al. (2014) intricately demonstrated that by combining anterograde and retrograde vectors and multiple recombinases in a line of transgenic mice, an optic light could be used to selectively target dopaminergic neurons projecting from the VTA and terminating in the NAc. This combination of genetic and neuroscience techniques allows for unparalleled control of OTR- or V1a-dependent neuronal activity between regions within the mesocorticolimbic circuit.

A similarly powerful technique involves the use of designer receptors exclusively activated by designer drugs (DREADD; Dong et al., 2010). A recent study by Williams et al. (2015) demonstrated that precise optical or clozapine-*N*-oxide activation of AVP V1b expressing neurons could be achieved by transfecting cre-dependent DREADDs into V1b-Cre transgenic mice. This technique is currently being used to understand the role of V1b in social memory and aggression, and could feasibly be adjusted to examine the involvement of V1a expressing neurons in OXY-relevant behaviours, including the attenuation of METH-induced relapse and hyperactivity observed in the present thesis.

In summary, the pharmacological techniques utilised in the current thesis were sufficient to elucidate a significant role of the V1a receptor in mediating the effects of systemically administered OXY on METH-related behaviours. However, the investigation into the NAc core in experiment 2 was restricted by the limitations of pharmacology as a

research technique. To this end, future designs should incorporate the previously discussed pharmacosynthetic techniques, as a greater understanding of the mechanisms which underpin the impressive effects of OXY will further progress its development as a pharmacotherapy for METH dependence.

4.2.3 Oxytocin as a pharmacotherapy

The use of OXY as a pharmacotherapy is facilitated through the ease of administration to humans in out-patient settings through nasal delivery techniques. Whilst there is some question as to whether peripherally administered OXY can penetrate the brain, isotopic labelling procedures have demonstrated that within 20 mins, intranasally administered OXY can reach diverse regions which are protected by the blood-brain-barrier (BBB) including the caudate, putamen, parietal cortex and septal nucleus of rats (Carson et al., 2015). Furthermore, nasal delivery of OXY in rats resulted in increased OXY levels in blood plasma, as well as in extracellular fluid within the amygdala and dorsal hippocampus, as measured by *in vivo* microdialysis (Neumann et al., 2013). These findings provide convincing evidence for the efficacy of intranasally delivered OXY in rats to reach behaviourally-relevant structures in the brain. Additionally, as intranasal drug delivery technologies evolve, the viability of intranasal administration should improve (see review: Guastella et al., 2013).

Numerous clinical trials have demonstrated the therapeutic potential of OXY treatment for disorders characterised by social deficits (see review: Anagnostou et al., 2014). Acute administration of intranasal OXY improved affective speech comprehension and reduced repetitive behaviours in adults diagnosed with autism spectrum disorders (ASD; Hollander et al., 2007; 2003), as well as improving emotion recognition in youth with ASD diagnoses (Guastella et al., 2010). Intranasal OXY has also been shown to increase eye gaze

(Guastella, Mitchell and Dadds, 2008), facilitate the extinction of a conditioned fear response in healthy adult males (Eckstein et al., 2015), and increase eye contact in both healthy and ASD diagnosed adult males (Auyeung et al., 2015). However, few studies have explored the effects of chronic OXY administration in humans. Two studies have demonstrated that long term (1 to 6 months) daily intranasal OXY treatment is safe and may improve social cognition, although results are mixed between trials (Tachibara et al., 2008; Anagnostou et al., 2012). This literature suggests that OXY is worthy of ongoing clinical trials for its pharmacotherapeutic potential, and more broadly indicates that in-patient treatment with intranasal OXY may be a viable means for treating METH dependent individuals, perhaps in rehabilitation programs or even in needle-syringe exchange centres.

4.2.3.1 Adjunctive effects of OXY in treating METH dependence

Previous research has demonstrated that OXY administration can interact with the psychological symptoms prevalent during METH withdrawal which may increase the propensity to relapse. In humans, traditional antidepressants have failed to treat these abstinence-induced anxiety and depression-like symptoms (Galloway et al., 1996; Kongsakon et al., 2005), however pre-clinical studies in animals have shown OXY administration to have powerful anti-depressant (Neumann & Landgraf, 2012) and anxiolytic effects (Slattery and Neumann, 2010). Indeed, acute intranasal OXY reduced marijuana-cravings and anxiety in cannabis-dependent humans (McRae-Clark et al., 2013), and blocked a range of withdrawal symptoms in people dependent upon alcohol (Pedersen et al., 2013). As such, OXY administered during abstinence from METH may ameliorate withdrawal symptoms, further reducing the risk of relapse beyond its interactions with METH, although this is yet to be determined in a clinical human trial.

Psychological interventions such as CBT and contingency management are widely used therapeutic techniques for addressing METH dependence (Lee et al., 2008). However, these strategies are most effective in reducing patient symptomology when adherence is high, and a patient-practitioner bond is formed (Conboy et al., 2010). Given the facilitating role OXY has on partner-preferences in voles (Young et al., 2001) and the positive effect it has on trust in humans (Kosfeld et al., 2005), it is conceivable that concurrent OXY treatment during psychosocial therapies may enhance the formation of a strong patient-practitioner relationship to facilitate favourable treatment outcomes.

With the promise of OXY as useful pharmacotherapy, not all reported effects of exogenous OXY treatments have been so positive. For example, OXY administration improved facial recognition memory in healthy adults, however, this effect was not found for happy faces, but rather, recognition of angry faces was enhanced (Savaskan et al., 2008). Hypersensitivity to angry faces over to happy faces is associated with anxiety disorders (Phan et al., 2006) and given the over-representation of comorbid anxiety disorders in METH dependent populations (Zweben et al., 2004), OXY-heightened recognition of angry relative to happy faces may be detrimental for these individuals. In addition, numerous studies across diverse populations have found an association between the gene encoding for the OTR and increased risk for ASD (Jacob et al., 2007; Lerer et al., 2008; Loparo et al., 2015). The reasons for this association are poorly understood, but given the discussed involvement of the V1a receptor in mediating social behaviour, future studies should investigate the association between ASD risk and the V1a receptor gene. Furthermore, OXY has also been shown to promote anti-social behaviours in healthy adults, with acute intranasal administration increasing envy and gloating (Shamay-Tsoory et al., 2009). Overall, these adverse effects and potentially negative associations with ASD are poorly understood and clearly warrant further examination. Most importantly, these results emphasise the need for determining the

mechanisms by which OXY treatment changes specific behaviours so that selective treatments may be developed. Careful consideration of individual circumstances is also of utmost importance to ensure that OXY-based therapies are only administered to individuals who are likely to benefit.

4.2.3.2 Issues with exogenous OXY treatment

Clinical trials are providing preliminary evidence for short-term effects of OXY, however, the long-term consequences of exogenous OXY administration have not been adequately explored in animals or humans. There are early indications that exogenous OXY may induce endogenous release of OXY in a feed-forward manner (Rossoni et al., 2008), with even small doses causing enduring morphological changes to hypothalamic OXY neurons (Catheline et al., 2006). Acute OXY has also been shown to induce a near fivefold increase in c-Fos expression in OXYergic neurons in the PVN, suggesting intense activation of the endogenous OXY system (Carson et al., 2010b). Chronic METH exposure has also shown a region-dependent alteration in the expression of OTR-immunoreactive fibres, suggesting substantial disruption to the endogenous OXY system, which may persist despite abstinence (Baracz et al., in press-b).

The poor BBB penetration and metabolic instability of OXY in the blood stream necessitates large doses to be administered when OXY is delivered peripherally. This is problematic, as OXY has relatively poor selectivity for its cognate receptor over AVP receptor subtypes (Chini and Manning, 2007), meaning that large doses may induce physiological and behavioural effects associated with peripheral and central V1a, V1b and V2 activation. This is especially concerning given the often oppositional role of OTR/V1a and V1b-mediated effects. For example, findings by Subiah et al. (2012) suggest that activation of V1b receptors could facilitate METH conditioned place preferences in rats,

however, activation of the OTR impaired a dopamine place preferences (Baracz et al., 2013), and low doses of OXY into the NAc core and STh, likely only able to activate local OTR and V1a receptors, attenuated a METH conditioned place preference (Baracz et al., 2012). Other studies have also implicated V1b activation in increased social anxiety and social defeat, and impaired social cognition (Litvin, Murakami and Pfaff, 2011; Winslow and Insel, 2004). Clearly then, strategies are needed to more potently and selectivity stimulate OTR and/or V1a receptors in brain addiction-related brain regions.

4.2.3.3 Targeting the endogenous OXY system

Pharmacotherapies which target the endogenous OXY system may overcome many of the limitations of exogenous OXY treatments; including low receptor selectivity, poor pharmacokinetic and BBB penetrant properties. One recently proposed target for stimulating endogenous OXY is the use of pharmacological agonists of melanocortin receptors. These receptors, of which there are four subtypes, have classically been investigated for their effects on pigmentation, sexual function, and obesity (see review: Todorovic and Haskell-Luevano, 2005). However, the melanocortin-4 receptor (MC4R) is primarily located on the OXY synthesising magnocellular neurons within the PVN which project out to numerous brain regions important for emotion, reward, and memory (Swanson & Sawchenko, 1983). As such, selective activation of the MC4R should result in localised excitation of OXYergic neurons in the PVN, resulting in increased endogenous OXY signalling to its afferent regions.

The acute administration of PF446687, a highly selective MC4R agonist with favourable pharmacokinetic and BBB penetrative properties (Lansdell et al., 2010), induced a partner preference in voles which was mediated by the OTR (Modi et al., 2015). Other melanocortin receptor agonists less selective for the MC4R were also shown to enhance the

formation of a partner preference, and resulted in similar activation of hypothalamic OXY neurons as exogenous OXY itself (Modi et al., 2015). Additionally, daily neonatal treatment with PF446687 in voles facilitated partner preferences in adulthood (Barrett et al., 2014). However, it is not currently known whether chronic activation of the MC4R results in neuroadaptive changes to the expression of melanocortin receptors, or to the morphology of the magnocellular neurons. Furthermore, understanding the effects of long-term MC4R stimulation on the OTR and AVP V1a receptor systems is an important next step for understanding the pharmacotherapeutic potential of MC4R agonists. Overall, this preliminary data suggests that targeting the MC4R may overcome many of the problems facing exogenous OXY administration, and warrants exploration into MC4R agonists as potential regulators of OXY for the treatment of METH dependence.

4.2.4 Intravenous drug self-administration paradigm

The IVSA paradigm utilised in the present study has consistently provided valuable insights into the role of the OXY system in METH-seeking behaviours (Baracz et al., 2015; in press-a,b), and was an effective model for exploring the receptor mechanisms by which OXY inhibits METH-primed reinstatement. This 20 day, 2 hr daily, FR1 iteration of METH self-administration has also been shown to result in substantial changes to OTR-fibre density within reward-relevant brain regions (Baracz et al., 2015; in press-b). It is suggested that the current paradigm may model both the behavioural and neurobiological effects of chronic METH exposure in humans, although perhaps not addiction *per se*.

Drug addiction in humans is characterised by the inability to limit drug consumption, increased motivation to seek the drug, and persistent drug intake despite adverse effects (APA, 2013). Whilst the present methodology does not adequately measure comparable behaviours in rats, the IVSA paradigm is highly amenable to model such a diagnosis

(Deroche-Gamonet et al., 2004). Indeed, by varying the frequency and duration of drug self-administration, the ratio of responding required for drug delivery, and the suppressing effect of concurrent punishment, Deroche-Gamonet et al (2004) intricately identified a subgroup of rats which met all three diagnostic criteria, making up 17% of the entire cohort. This is strikingly similar to the percentage of human cocaine users who go on to develop an addiction to cocaine (13%), suggesting an underlying addiction-vulnerable phenotype which pervades both humans and rats, and possibly the numerous other species of animals which readily self-administer rewarding drugs (Anthony, Warner & Kessler, 1994; Deroche-Gamonet et al., 2004). Whilst the identification of this vulnerability is important, vulnerability alone does not appear to be sufficient for addiction to form (Deroche-Gamonet et al., 2004).

The engagement in drug-seeking behaviour and propensity to relapse is directly related to the degree of exposure to the drug (Panlilo & Goldberg, 2007). For example, prolonged access to METH (e.g. 6 hours) resulted in escalated drug seeking behaviours in rats (Kitamura et al., 2006). Additionally, rats which initially acquired a stable level of cocaine self-administration during short access sessions rapidly increased drug intake when given extended access to cocaine (Knackstedt & Kalivas, 2007). The magnitude of drug-primed reinstatement was also enhanced in rats which had been given extended access to the drug during self-administration. Furthermore, primer doses typically too low to cause reinstatement were able to induce drug-seeking behaviour in extended access rats (Mantsch et al., 2004; Knackedstedt & Kalivas, 2007). In light of these findings, future investigations into OXY-METH interactions in self-administration should incorporate extended access to METH, to better model the pattern of use typical of drug addiction in humans.

Importantly, the effects of extended access to the drug were not associated with increased sensitisation, suggesting that the neural substrates underpinning the differences

between the extended and short access groups are likely reward-related, not stimulant-related brain regions (Knackedstedt & Kalivas, 2007). Indeed, Mantsch et al (2004) found that levels of mRNA encoding for the dopamine-2 receptor in the NAc were higher in extended access rats, which was positively correlated with the magnitude of drug-primed reinstatement. Taken together, these findings suggest that dysregulation of dopaminergic systems within the NAc may mediate the heightened risk of relapse associated with extended access to drugs, and further highlights the importance of the NAc in human-like addictive behaviours.

Furthermore, there is substantial evidence for sex differences in METH addiction in humans (Dluzen & Liu, 2008), as well as differential efficacy of therapies for drug addiction (Ashley, Marsden & Brady, 2009). Whilst a few pre-clinical studies have compared METH-related behaviours in male and female animals (Roth & Carroll, 2004; Reichel, Chan, Ghee & See, 2012; Cox et al., 2013), the vast majority of self-administration studies are conducted in males. This is problematic when generalising the findings of the present thesis beyond males, as exogenous OXY (Bales & Carter, 2002; Rilling et al., 2014; Feng et al., 2014) as well as the endogenous OXY system are mediated by sex (Carter, 2007; Dumais & Veenema, 2015). Importantly, female rats have less OTR binding in the NAc core and medial amygdala, potentially limiting the effectiveness of exogenous OXY administration to attenuate the rewarding action of METH in these regions (Dumais et al., 2013). Sex-dependent regional differences in V1a receptor binding have also been shown in rats, hamsters and voles (Dumais & Veenema, 2015). Future drug self-administration studies investigating the efficacy of OXY as a therapy for METH-dependence need to incorporate female subjects to address this gender-skew, and to further the development of sex-tailored treatments for humans addicted to METH.

Overall, the IVSA paradigm was suitably adapted to address the research question in the present thesis. Although pre-clinical findings strongly suggest that OXY has therapeutic

potential for METH and other drug addictions, this has not yet been adequately assessed in self-administration paradigms which more precisely measure addiction like behaviours. Furthermore, the comparative efficacy of OXY in low- and high-responders, and males and females has not been explored. Future experiments will investigate whether the efficacy of OXY-based therapies for METH-dependence varies according to sex, duration of access to drug, or vulnerability to addiction.

4.3 Conclusions

Overall, there is considerable therapeutic potential for the use of OXY in the treatment of addiction to METH. However, the full complement of receptor systems and brain regions which mediate these promising effects of OXY still need to be investigated and identified. Our incomplete understanding of the mechanisms behind the effects of administered OXY limits the advancement towards a working model of the diverse behavioural and neurobiological effects of OXY, as well as the development of novel OXY-based therapies. The current thesis extends recent observations of OXY-METH interactions, and represents the first demonstration of systemic OXY reducing METH-related behaviours by activating a receptor other than its own: the AVP V1a receptor. Further confirmation of the importance of OXY acting in the NAc core to inhibit relapse to METH was demonstrated, although the role of the V1a receptor within this region remains undetermined. These findings prompt further investigation into OXY and V1a receptor interactions within addiction-relevant brain regions, and provide direction for the improvement of pre-clinical methodologies for understanding OXY-based pharmacotherapies for METH addiction.

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Appendix A: Animal Research Authority 2013/018 (Experiment 1)

MACQUARIE University

ANIMAL RESEARCH AUTHORITY (ARA)

AEC Reference No.: 2013/018-8 **Date of Expiry:** 31 August 2015

Full Approval Duration: 1 September 2013 to 31 August 2015 (24 Months)

This ARA remains in force until the Date of Expiry (unless suspended, cancelled or surrendered) **and will only be renewed upon receipt of a satisfactory Progress Report before expiry (see Approval email for submission details).**

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the Principal Investigator / Associate Investigator named above
or Manager, CAF: 9850 7780 / 0428 861 163 and Animal Welfare Officer: 9850 7758 / 0439 497 383

The above-named are authorised by MACQUARIE UNIVERSITY ANIMAL ETHICS COMMITTEE to conduct the following research:

Title of the project: A comparison of the acute and long-term effects of WAY 267, 464, oxytocin and vasopressin on intravenous self-administration of methamphetamine in female rats

Purpose: 4 - Research: Human or Animal Biology

Aims: To compare the acute and long-term effects of OXY, WAY 267, 464, AVP and the SOC-1 and SOC-2 compounds on intravenous self-administration of METH in female rats.

Surgical Procedures category: 4 - Minor Surgery With Recovery

All procedures must be performed as per the AEC-approved protocol, unless stated otherwise by the AEC and/or AWO.

Maximum numbers approved (for the Full Approval Duration):

Species	Strain	Age/Sex/Weight	Total	Supplier/Source
02-Rat	Sprague-Dawley	3 weeks / any	96	ARC Perth
02-Rat	Sprague-Dawley	3 weeks/any	48	ARC Perth
02-Rat	Sprague-Dawley	3 weeks/any	16	ARC Perth
		TOTAL	160	

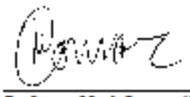
Location of research:

Location	*Full street address*
Central Animal Facility	Building F9A, Research Park Drive, Macquarie University, NSW 2109

Amendments approved by the AEC since initial approval:
1. *Amendment #1: Change of experimental design for the initial cohort of 32 animals (Exec approved 12 Dec 2013, ratified by AEC 20 February 2014)*
2. *Amendment #2: Extension request of 12 months & addition of 64 animals to fulfil grouping experiment (Approved at AEC 18 September 2014)*
3. *Amendment #3: Request addition of a test for cannabidiol in group 7 of protocol (Executive approved, ratified by AEC 19 February 2015)*
4. *Amendment # 4: Request to use 16 male rats in group 9 using the vasopression anagonist SR 49059(AEC approved 19 February 2015)*
5. *Amendment #5: Addition of Nicholas Everett as Associate Investigator (Approved by AEC 19 February 2015)*
6. *Amendment #6: Approval of male/female rats across all groups ((approved by AEC 19 March 2015)*

Conditions of Approval: N/A

Being animal research carried out in accordance with the Code of Practice for a recognised research purpose and in connection with animals (other than exempt animals) that have been obtained from the holder of an animal suppliers licence.



Professor Mark Connor (Chair, Animal Ethics Committee) **Approval Date:** 19 March 2015

Adapted from Form C (issued under part IV of the Animal Research Act, 1985)

Appendix B: Animal Research Authority 2015/009 (Experiment 2)

MACQUARIE University

ANIMAL RESEARCH AUTHORITY (ARA)

AEC Reference No.: 2015/009 **Date of Expiry:** 20 April 2016

Full Approval Duration: 20 April 2015 to 19 April 2017 (24 months)

This ARA remains in force until the Date of Expiry (unless suspended, cancelled or surrendered) **and will only be renewed upon receipt of a satisfactory Progress Report before expiry (see Approval email for submission details).**

Principal Investigator:
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0404 807 175

Associate Investigators:
Nicholas Everett 0447 285 037
Michael Hunter 0408 420 117

In case of emergency, please contact:
the Principal Investigator / Associate Investigator named above
or Manager, CAF: 9850 7780 / 0428 861 163 and Animal Welfare Officer: 9850 7758 / 0439 497 383

The above-named are authorised by MACQUARIE UNIVERSITY ANIMAL ETHICS COMMITTEE to conduct the following research:

Title of the project: **The effect of Vasopressin V1A receptor antagonism in the nucleus accumbens on relapse to methamphetamine seeking-behaviour**

Purpose: **4 - Research: Human or Animal Biology**

Aims: **To investigate the effects of oxytocin and SR49059, a V1a receptor antagonist, on relapse to METH-seeking behaviour, using the self-administration paradigm.**

Surgical Procedures category: 5 - Major Surgery with Recovery
All procedures must be performed as per the AEC-approved protocol, unless stated otherwise by the AEC and/or AWO.

Maximum numbers approved (for the Full Approval Duration):

Species	Strain	Age/Weight/Sex	Total	Supplier/Source
02 Rattus	Sprague Dawley	3 months/250g/Male	16	ARC Perth
			16	

Location of research:

Location	*Full street address*
Central Animal Facility	Building F9A, Research Park Drive, Macquarie University, NSW 2109

Amendments approved by the AEC since initial approval: N/A

Conditions of Approval: N/A

Being animal research carried out in accordance with the Code of Practice for a recognised research purpose and in connection with animals (other than exempt animals) that have been obtained from the holder of an animal suppliers licence.

Professor Mark Connor (Chair, Animal Ethics Committee) **Approval Date: 16 April 2015**

Adapted from Form C (issued under part IV of the Animal Research Act, 1985)